Analyzing the Mass-Rearing System of the California Red Scale Parasitoid *Aphytis melinus* (Hymenoptera: Aphelinidae)

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Analyzing the mass-rearing system of the California red scale parasitoid *Aphytis melinus* (Hymenoptera: Aphelinidae)

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Abstract

Results from studies to improve mass rearing production of the parasitoid *Aphytis melinus* De Bach (Hymenoptera: Aphelinidae) are presented. Parasitoid production was carried out following standard commercial procedures using an alternative host, *Aspidiotus nerii* Bouché (Hemiptera: Diaspididae), infesting *Cucurbita moschata* (Duchesne) (Cucurbitaceae), butternut squash. We found that the initial number of *A. melinus* adults introduced into rearing cages to start production and the scale/parasitoid ratio in those cages profoundly influenced future parasitoid production. We also observed that scale parasitism was positively correlated with the production of parasitoid adults, but this relationship was negatively correlated if > 2.6 parasitoids per d, per cm^2, were used in the cages to start parasitism. Supplemental honey (provided on the squash surface) had no clear impact on parasitoid production or survival, but improved host parasitism. Approximately 47% of the host scale population on squash was parasitized, with another 43.1% of the population recorded as dead. We found that ≤ 10 host scales per cm^2 on squash was an adequate density for mass production purposes.

Key Words: scale-parasitoid ratio; parasitism; *Cucurbita moschata*; *Aonidiella aurantii*; *Aspidiotus nerii*

Resumen

En el presente trabajo se presentan resultados de estudios dirigidos a mejorar la cría en masa del parasitoide *Aphytis melinus* De Bach (Hymenoptera: Aphelinidae). La producción del parasitoide se llevó a cabo según los procedimientos habituales de una producción comercial utilizando un huésped alternativo, *Aspidiotus nerii* Bouché (Hemiptera: Diaspididae) criado sobre la calabaza *Cucurbita moschata* (Duchesne ex Lamarck) (Cucurbitaceae). Se encontró que el número inicial de adultos de *A. melinus* introducidos en las cajas de cría para comenzar la producción y la razón cóccido/parasitoide en dichas cajas afectaron de forma significativa la producción final de parasitoides. También se observó que el parasitismo en el cóccido huésped estuvo correlacionada de forma positiva con la producción final de parasitoides, pero esta relación estuvo afectada negativamente cuando se utilizaron más de 2,6 parasitoides por día y cm^2 para iniciar el parasitismo en las cajas de cría. El uso de un suplemento de miel (colocada en la superficie de las calabazas) no tuvo un efecto significativo en la producción de parasitoides ni en la supervivencia de los adultos, pero mejoró significativamente el parasitismo en el huésped. Aproximadamente el 47% de la población del cóccido huésped que había sobre las calabazas fue parasitado, mientras que el 43,1% de la población estaba muerta. Una densidad de ≤ 10 cóccidos huésped por cm^2 de calabaza fue adecuada para la producción del parasitoide.

Palabras Clave: razón cóccido-parasitoide; parasitismo; *Cucurbita moschata*; *Aonidiella aurantii*; *Aspidiotus nerii*

In several citrus-growing regions of the world, the parasitoid *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae) has been used for many years to control California red scale, *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae). Also, this parasitoid has been commercially mass produced previously for augmentive release to control this scale pest in southern California lemon orchards (Moreno & Luck 1992) timed in the spring to coincide with the presence of virgin adult female California red scale, *A. aurantii*. Moreover, *A. melinus* also has been used to control another citrus pest, *Aspidiotus nerii* Bouché (Hemiptera: Diaspididae), in California and Spain (Grafton-Cardwell et al. 2011; Olivas et al. 2011).

Previously, several classic studies have examined the biology and host relationships of *A. melinus* under laboratory conditions (Collier 1995; Heimpel & Rosenheim 1995; Hare 1996; Heimpel et al. 1997), whereas other studies have examined the efficacy of this species under field conditions(Murdoch et al. 2006; Sorribas et al. 2012; Zappalà et al. 2012). Initial bionomic and behavioral data from several of these studies provided the framework for mass- rearing procedures based on the work by DeBach and White (1960) with few studies on *A. melinus* production published since (Raciti et al. 2003; Zappalà et al. 2006).

In this paper we present large scale mass-rearing production data of *A. melinus* during a 3-yr cooperative augmentive release program with a citrus-producing company to control California red scale in Spain. Identification and analysis of production parameters, as they influenced parasitoid abundance, focused on: (a) variables affecting physical production of parasitoids in rearing units, (b) effects of supplemental honey sources on parasitoid survival, and (c) variables affecting the parasitism of the host scale *A. nerii*.

Materials and Methods

REARING PROCEDURES

The *A. melinus* colony used in these studies was obtained from Koppert-España (La Mojonera, Almería, Spain), which produced this

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parasitoid in their facilities at Aguilas (Murcia, Spain). In addition, we supplemented our colony with individuals collected in citrus groves along the Guadalquivir Valley in the province of Cordoba (Spain). *Aphytis melinus* was reared at the University of Seville, following the method developed for rearing *Aphytis lingnanensis* DeBach (DeBach & White 1960; Rose 1990; Raciti et al. 2003). This rearing method is used commonly in commercial insectaries for *A. melinus* production.

A parthenogenetic strain of the scale *A. nerii* reared on butternut squash (*Cucurbita moschata* Duchesne ex Lamarck [Cucurbitaceae]) (average size: 18.5 ± 0.3 cm L, 8.7 ± 0.2 cm W) served as a substrate host for *A. melinus* production. Infested squash, with third instar scales, was exposed to 2- to 3-d-old adult parasitoids (male and female) in a screened cage (41 cm W × 32 cm H × 52 cm D) provided with honey, ad libitum, in plastic dishes. Third instar *A. nerii* is the preferred host age for *A. melinus* to oviposit and maximize progeny production (Forster et al. 1995). One to 2 parasitoid releases were conducted in each cage. Parasitoids were removed using CO_2 after about 3 d and survival determined. Hosts and parasitoids were maintained in the laboratory at 25 °C, 60.0 ± 5% RH under continuous light.

At the end of 14 d, newly emerged adult *A. melinus* were collected every 2 to 3 d until production ceased in each rearing cage. During collection, adults were anaesthetized with CO_2 (the cage was first sealed and then the gas introduced through an inlet valve for 1 min) and dislodged from the squash surface and interior of the cage with an air blast. Adults then were collected from the white paper lining the bottom of each cage onto a piece of black cardboard (29.5 cm × 21 cm). A photograph was taken while adult parasitoids remained anaesthetized and total abundance recorded. Also, adult parasitoid survival and progeny production were evaluated with or without a supplemental carbohydrate source of honey in rearing cages. The supplemental source consisted of 5 to 10 drops of "rosemary honey" (Ynsadiet, Leganés, Madrid, Spain) smeared on the surface of squash.

HOST PARASITISM ON SQUASH

On average, 1 parasitoid production cycle (generation) was completed in about 20 d. At that time, scale-infested squashes were brought to the laboratory where 100 randomly selected adult scales were examined using a binocular stereomicroscope to record if they were dead, alive, or parasitized. A total of 136 squashes were examined from 72 rearing cages during the 5 mo study.

VARIABLES QUANTIFIED IN *APHYTIS MELINUS* PRODUCTION

A description of the associated variables that were measured for influence on *A. melinus* production are summarized in Table 1. Specifically, 8 variables were selected to predict total parasitoid production; these were: (1) number of adult *A. melinus* parasitoids added to a cage, (2) number of host *A. nerii* present on squash in each cage, (3) total squash surface available in each cage, (4) initial number of adult *A. melinus* added to each cage combined with amount of time to complete parasitism of hosts (referred to as "Momentum of *A. melinus*"), (5) ratio of third instar *A. nerii* presented in the cage to *A. melinus* introduced into each cage, (6) number of parasitoid-host exposure days, (7) number of parasitoid introductions into each cage to parasitize hosts, and (8) presence or absence of a supplemental source of carbohydrate (honey). For all variables where adult parasitoids were used, both sexes were represented. It was assumed that the sex ratio of *A. melinus* was around 3:2 (female:male) based on work by González-Zamora et al. (2015).

The squash surface was estimated using the surface of a cylinder or the surface of a truncated cone, depending of the shape of the par-

Table 1. Variables used in the analysis of *Aphytis melinus* production, with their average values and standard errors (SE).

Variable	Description	Average values (SE)	Units	Statistical transformation
A. melinus obtained	per cage, or per cm² squash per cage	1122 (93)	*A. melinus* produced per cage	Square root
A. melinus added	per cage, or per cm² squash per cage	1279 (84)	*A. melinus* added per cage	Square root
A. nerii presented	per cage, or per cm² squash per cage	9.4 (0.8)	*A. nerii* per cm² per cage	Logarithm
Squash surface	Total surface per cage (cm²)	1208 (34)	cm² of squash per cage	Logarithm
Momentum of *A. melinus*	per cage, or per cm² squash per cage	3588 (307)	Total *A. melinus* × days in the cage to parasitize the host	Logarithm
Ratio Scale to Parasitoid	Ratio between both populations in the cage	9.8 (0.7)	Ratio Scale to Parasitoid in the cage	Logarithm
Days with *A. melinus*	Number of days that *A. melinus* were in the cage parasitizing the host	3.0 (0.1)	Number of days of *A. melinus* parasitizing in the cage	
Number of inoculations	Times that *A. melinus* was introduced in the cage	1.4 (0.1)	Times that *A. melinus* were introduced in the cage	
Honey on squash	Presence or absence of extra honey on the squash surface	33 (with honey) 95 (without honey)	Cages with or without honey on the squash surface	

n = 128 cages and 252 squash, average of 2.0 ± 0.1 squash per cage.

ticular squash. Length and diam of the squash were the parameters used to estimate squash surface. The density of *A. nerii* on squash was estimated with the assistance of a piece of cardboard with a 1 cm^2 hole cut into it. The hole was placed randomly on at least 9 places on the surface of each squash and the average number of *A. nerii* per cm^2 was calculated. Using the estimated squash surface and the density of the parasitoid, the estimated total host population on the surface was determined.

STATISTICAL ANALYSIS

Production variables were analyzed first to meet the requirements of normality, and transformed if necessary (Table 1). There were no correlations between the explicative variables, except for "Momentum of *A. melinus*" and "*A. melinus* added" (r varied between 0.82 and 0.90, with $P < 0.01$). "Momentum" was therefore excluded from further analysis.

The General Linear Model procedure was applied to total individuals (hosts and parasitoids) per cage as well as individuals (hosts and parasitoids) per cm^2 of squash per cage. The analyses initially included 8 independent (or explicative) variables in the model. Those variables that were not significant ($P > 0.05$) were eliminated in subsequent steps from the regression model. Comparison of adult *A. melinus* mean survival with or without a supplementary source of carbohydrate (honey) on squash was analyzed with Student's t test, $P < 0.05$.

Parasitism of *A. nerii* on squash was analyzed using a logistic regression in which the dependent variable, frequency of parasitism recorded, was expressed as a percentage. Average cage data from 64 cages were used initially for this analysis. Total parasitism summed values of (1) scales with emerged parasitoids, (2) scales containing parasitoid larvae or pupae, and (3) scales with dead parasitoids. These values were expressed as a percentage of all scales present. One of the response variables ("*A. nerii* presented") (see Table 1) was converted from a continuous distribution to a categorical one with 5 divisions or groups. Data from cages with studentized residuals > 3 were not included in the analysis; the final number of cages included in the analysis was 58. Two logistic regression analyses were performed: (1) applied to the independent variables expressed as total number of host or parasitoid per cage, and (2) applied to the independent variables expressed as host or parasitoid per cm^2 of squash per cage. The second analysis above is presented in results because it contained fewer significant variables (4 total) compared with the first analysis on host or parasitoid per cage. All statistical analyses were performed with Statgraphics Centurion XVI v16.1.07 (Stat Point Technologies 2010).

Results

ANALYSIS OF PRODUCTION

Results from statistical analyses of *A. melinus* production data are provided in Table 1. The last column includes the transformation applied to variables that did not meet statistical requirements. Coefficients of variables "*A. melinus* added" and "scale to parasitoid" ratio significantly affected production positively (Table 2). These results indicated that adding additional *A. melinus* adults to each cage and increasing the number of available host scales per adult parasitoid (ratio of scales per parasitoid) led to higher production of the parasitoid.

Aphytis melinus production varied from cage to cage with an average of 1,122 ± 93 (SE) individuals per cage (Fig. 2b). Emergence of adult *A. melinus* from their scale hosts started about 14 d after initial introduction of the parental generation, and reached its peak within the first 3 d of parasitoid emergence (Fig. 1a). Fifty percent of the total population emerged by the d 4, and 95% reached by the d 12 (Fig. 1b). The application of honey to the squash surface did not significantly increase adult *A. melinus* survival compared with no honey application, and combined survival averaged about 25.4 ± 1.1% ($t = -0.84$, $P = 0.40$).

HOST PARASITISM ON SQUASH

We found that as the variable "*A. melinus* obtained" significantly increased, the probability of obtaining higher parasitism on host *A. nerii* also increased (Table 3). The variable "*A. nerii* on squash" indicated that the optimum density of host scale available for parasitism was ≤ 10 scales per cm^2. The other 2 significant variables from the logistic analysis were "Momentum of *A. melinus*" and "Honey on the squash" where both odds ratio were < 1. As the variable "Momentum of *A. melinus*" (as defined previously) increased, the probability of *A. nerii* parasitism significantly decreased. However, adding 2.6 parasitoids per d per cm^2 squash to cages was the optimum density to maintain efficient parasitism of hosts. Also, there was a lower probability of *A. nerii* parasitism in the absence of honey on the surface of squash compared with its presence (Table 3). We observed that approximately 47% of the *A. nerii* population on squash was parasitized, whereas 43% of the host population was dead.

Discussion

Mass production of beneficial insects is an important aspect of biological control implementation, especially for augmentative release

Table 2. Significant variables with their coefficients and standard errors (SE) from the General Linear Model analysis, of *Aphytis melinus* production per cage and per cm^2 squash per cage.

Production of *A. melinus* per cage[a]			Production of *A. melinus* per cm^2 squash per cage[b]		
Variable	Coefficient	SE	Variable	Coefficient	SE
Constant	−26.93	4.04	Constant	−0.74	0.12
√ (*A. melinus* added)	1.09	0.08	√ (*A. melinus* added per cm^2)	1.04	0.08
Log (Scale/Parasitoid)	10.62	1.19	Log (Scale/Parasitoid)	0.31	0.04
$F_{2,114} = 116.40$ $p < 0.001$ $R^2 = 0.67$ SE = 8.71			$F_{2,114} = 106.90$ $p < 0.001$ $R^2 = 0.65$ SE = 0.26		

[a]Dependent variable is √ (A. melinus obtained per cage).
[b]Dependent variable is √ (A. melinus obtained per cm^2 of squash per cage).

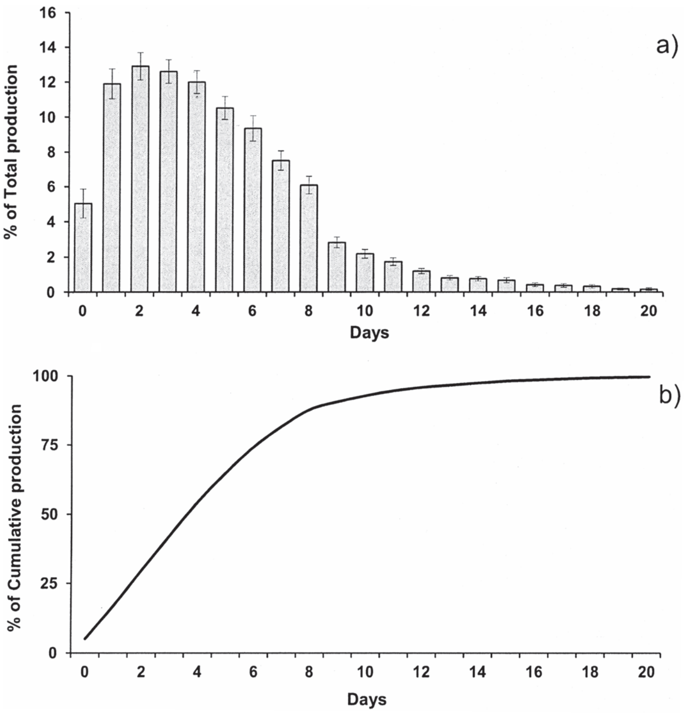

Fig. 1. Parasitoid (*Aphytis melinus*) production (as percentage of the total production given as mean ± SE) in the production cages: (a) daily emergence; (b) cumulative emergence.

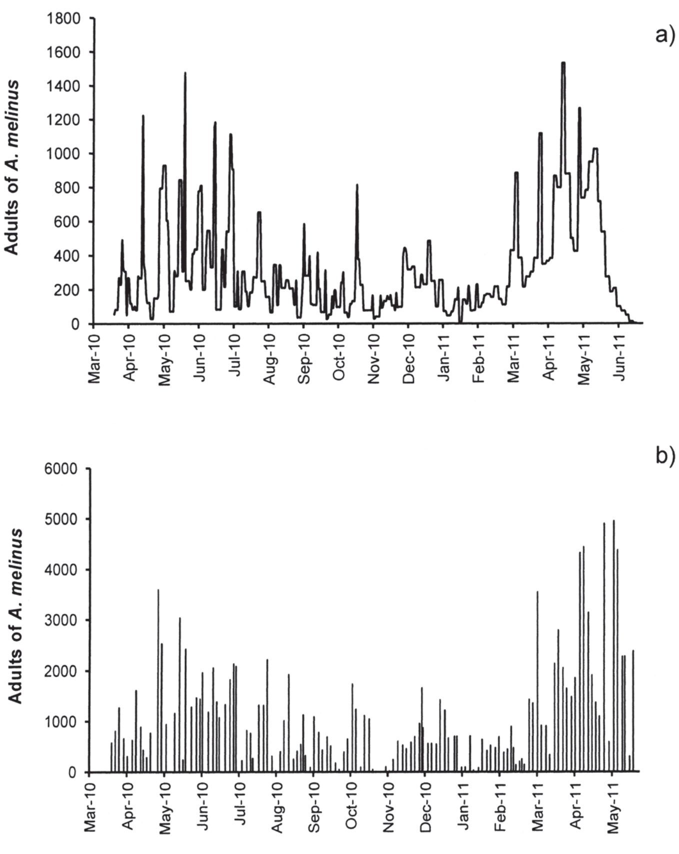

Fig. 2. Production of *Aphytis melinus* over the period in which the pilot system was working, from 18 March 2010 to 20 Jun 2011: (a) Total production per day, when different cages were in production at the same time and collection was every 2 to 3 days; (b) Total production per cage. Each bar in (b) represents the production of an individual cage, and is displayed as the date when parasitoid emergence began.

Table 3. Significant variables with their odds ratio and confidence limits (at 95%) in the logistic regression analysis of total parasitized *Aspidiotus nerii* (expressed as percentage) on squash used in the rearing of *Aphytis melinus*. Variables used in the analysis are expressed per cm^2 squash per cage (when pertinent).

Variables	Odds ratio	95% Lower limit	95% Upper limit
A. melinus obtained (per cm^2)	1.399	1.287	1.522
A. nerii on the squash (per cm^2)			
Level=A[a]	1.803	1.506	2.158
Level=B	1.489	1.278	1.733
Level=C	1.012	0.868	1.180
Level=D	1.095	0.943	1.271
Momentum[b] of *A. melinus* (per cm^2)	0.882	0.853	0.912
Honey on squash			
Level = 0[c]	0.785	0.709	0.869

The model was significant ($P < 0.0001$), and explained 0.65 of the variability of the data (n = 58 cages from the original 64 cages used to analyze the parasitism observed on *Aspidiotus nerii*).

[a]*A. nerii* per cm^2: A ≤ 3.0, B = 3.1 to 7.0, C = 7.1 to 11.5, D = 11.6 to 15.0, E ≥ 15.1 (used as reference level).

[b]It is the initial parasitoid number by duration of parasitism.

[c]Level = 0 (no honey on the squash), Level = 1 (honey on the squash, used as reference level).

programs. Most of the work devoted to the mass-rearing of beneficial insects is related to their biology or their relationship with the host (Ghimire & Phillips 2010; Saleh et al. 2010; Whistlecraft et al. 2010), as well as the analysis of production mechanics for improved efficiency (Canale & Benelli 2012; Vacari et al. 2012; Tormos et al. 2014). Another facet of beneficial insect mass production commonly analyzed is the general quality of the product produced (Vasquez & Morse 2012; Veiga et al. 2013). Our results suggested that production of *A. melinus* was affected primarily by the number of adult parasitoids introduced in cages to initiate production and scale to parasitoid ratio; as the number of hosts available per parasitoid increased, so did the productivity of parasitoids in the rearing cages. The importance of keeping an adequate scale to parasitoid ratio has been suggested to be about 5 to 10 scales per parasitoid on a daily basis (González-Zamora et al. 2015).

We routinely provided honey, in plastic dishes, when mass-rearing *A. melinus* because a sugar source is considered critical to parasite survival (Heimpel et al. 1997; Wäckers 2003). Interestingly, supplemental application of honey to the surface of squash did not improve adult parasitoid survival, but did increase the percentage of parasitized scales. We also observed a high percentage (43.1%) of scales on squash were either dead, most probably due the host feeding activity of *A. melinus* adults, or parasitized (46.9%), with only a small percentage surviving (9.9%). Many authors have observed host feeding behavior of *A. melinus* in laboratory and field studies (Collier 1995; Heimpel et al. 1997; Sorribas & García-Marí 2010). This behavior appears to be fundamental in their biology because it increases longevity and lifetime fecundity. This is especially true for those that are idiobiont parasitoids with a synovigenic egg load (as *A. melinus*) (Thompson 1999; Wäckers 2003; Strand & Casas 2008; Tena et al. 2015).

Additionally we found that the lower host density appeared to promote a higher probability of being parasitized compared with higher host density. One possible explanation is that as host density increased, the capacity of parasitoids to parasitize them was not linear. We also observed that increasing the initial number of parasitoids in a cage or the exposure time of *A. melinus* to hosts (referred to as "Momentum of *A. melinus*") decreased the probability of parasitism. It is possible that as more parasitoids are introduced, or exposure time to hosts lengthened, more hosts can be killed by host feeding. Similar results have been obtained in previous laboratory tests by González-Zamora et al. (2015). In summary, we found the number of *A. melinus* adults initially introduced into rearing cages, and the host scale to parasitoid ratio during the rearing process profoundly affected subsequent parasitoid production. Optimizing the density of hosts can improve parasitism levels and hence the productivity of the system.

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