Age-specific life tables of *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae) and its parasitoid *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae)

Khalid MOHAMMED1,2, İsmail KARACA3*,4, Manjree AGARWAL3, James NEWMAN1, YongLin REN1
1 School of Veterinary and Life Sciences, College of Science, Health, Engineering and Education, Murdoch University, Murdoch, Western Australia
2 Department of Plant Protection, College of Agriculture and Forestry, University of Mosul, Mosul, Nineveh, Iraq
3 Department of Plant Protection, Faculty of Agricultural, Isparta University of Applied Sciences, Isparta, Turkey

Abstract: Biological parameters of the California red scale (*Aonidiella aurantii* [Maskell] [Hemiptera: Diaspidae]) were determined under laboratory conditions at 3 different temperatures (20, 23, and 27 °C) on butternut squash (*Cucurbita moschata* Duchesne ex Lamarc) (Cucurbitaceae), while the biological parameters of its parasitoid *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae) were conducted at 27 °C. The survival of *A. aurantii* ranged between 80.0% and 88.3%. The highest mortality was recorded during the adult stage, with mortalities ranging between 12% and 20%. On *C. moschata*, the total development time was 93.1 ± 9.73, 81.8 ± 7.13, and 65.7 ± 6.37 days, while the adult longevity was 54.65 ± 0.71, 47.05 ± 0.97, and 39.35 ± 1.07 days at 20, 23, and 27 °C, respectively. The oviposition period of *A. aurantii* was 44.3 ± 0.51, 40.65 ± 0.41, and 34.5 ± 0.45 days at 20, 23, and 27 °C, respectively. Average fecundity was 73.25 ± 1.827, 109.7 ± 3.569, and 129.35 ± 4.564 individuals at 20, 23, and 27 °C, respectively. For *A. melinus*, adult longevity was 19.24 ± 0.73 days, and average fecundity 62.7 ± 2.81 eggs at 27 °C. The preoviposition period was 0.82 ± 0.05 days, oviposition period was 15.7 ± 0.52 days, and postoviposition period was 2.21 ± 0.09 days. The intrinsic rate of increase (*r*) of *A. melinus* (0.188 ♀/♀/day) was significantly greater than that of *A. aurantii* (0.080) at 27 °C. These laboratory results demonstrated that *A. melinus* is an effective parasitoid for decreasing *A. aurantii* populations. Fecundity of *A. aurantii* and *A. melinus* was determined with the Enkegaard equation. The best-fit parameters of fecundity were calculated as \(a = 0.410, b = 0.099; a = 0.624, b = 0.098; a = 0.661, b = 0.091; a = 1.190, b = 0.179\) for *A. aurantii* at 20, 23, and 27 °C, and *A. melinus* at 27 °C, respectively.

Key words: *Aphytis melinus*, biological control, California red scale, life table, population ecology

1. Introduction

Australia has an important role in citrus (*Citrus* spp., Rutaceae) production in the world, and this production has increased (FAO, 2017). Many pest species such as *Ceratitis capiata* (Wiedemann) (Diptera: Tephritidae), *Aonidiella aurantii*, *Tessaritula oleae* (Gomez-Menor Ortega) (Hemiptera: Coccidae), *Toxoptera citricada* (Kirkaldy) (Hemiptera: Aphidiidae), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), et al. were observed in WA (Western Australia) citrus orchards (Sonia, 2006). Growers have followed various methods to avoid damage caused by these pests. Biological control is one of these methods (FAO, 2017; Pekas, 2011a; Uygun and Satar, 2008). There are 32 species in the genus *Aonidiella* Berlese & de Leoni, which is a genus of scale insects in the family of Diaspididae (Hemiptera), the armoured scale insects (Ben-Dov, 2006). California red scale *Aonidiella aurantii* (Maskell) occurs on numerous host plants throughout the world. They attack different crops such as fruit trees and ornamental plants all over the world, and cause heavy damage to the plants. Individual species infest leaves, fruits, branches, main stems, trunks, and roots. They are distributed throughout the world except in the cold extremes of the Arctic and Antarctic regions (Miller, 2005). California red scale *Aonidiella aurantii* is one of the most important pests infesting citrus trees in different parts of the world (Garcera et al., 2008; Badary and Abd-Rabou, 2010; Hill, 2008; Karaca and Uygun, 1992; Uygun et al., 1995). California red scale feeds on various parts of host plants, such as twigs, leaves, and fruit (Beardsley and Gonzalez, 1975), harming them by inserting its mouthparts deep into plant tissue and sucking sap from parenchyma cells and injecting toxic saliva into the plants during the feeding process. Severe infestations of the scale cause leaf drop, defoliation, and dieback of twigs and branches. Young trees may die out in the absence of effective control (Hely et al., 1982; Smith et al., 1997).

* Correspondence: ikaraca98@gmail.com

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The control of *A. aurantii* has encountered many difficulties, which has raised interest in alternative control methods (Vacas et al., 2010). *Aonidiella aurantii* has numerous predators and parasitoids (Hely et al., 1982; Forster et al., 1995). Among the parasitoids that feed on *A. aurantii*, *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae) is known to be relatively important (Erler and Tunç, 2001). It was introduced into Australia in 1961 from the University of California, Riverside (Furness et al., 1983; Malipatil et al., 2000). It is now widely distributed in the citrus orchards of Victoria, South Australia, Western Australia, and the inland New South Wales citrus districts, and in Alice Springs in the Northern Territory (Smith et al., 1997). *Aphytis melinus* is commercially reared for release in citrus orchards to control *A. aurantii* (Grafton-Cardwell et al., 2006). Regarding parasitoids, females are more important to produce, because they are responsible for parasitising the host (via oviposition and host-feeding) and, thus, keep the pest's population under an economic threshold. A plethora of experiments have been conducted to determine the environmental conditions which best contribute to increases in the production of females (Ode and Hardy, 2008).

For the success of crop protection programs, it is essential to know some biological information about the pest. This information can be obtained by constructing a life table providing how different mortality factors act in sequence on the successive developmental stages. The intrinsic rate of increase (*r*$_{in}$) is of value as the mean of describing the potential growth of population under given climatic and food conditions; it is an important parameter in inductive strategic and management models of pest populations (Abou Hatab, 1999; Bayoumy et al., 2009). Few studies have been conducted on the biological parameters of the California red scale and its parasitoid *A. melinus* (Heimpel, 1997; Badary and Abd-Rabou, 2010). Therefore, the aims of the present work were to simultaneously study the life history and behaviour of *A. aurantii* and its parasitoid *A. melinus* at 3 constant temperatures (20, 23, and 27 °C) under laboratory conditions.

2. Materials and methods

2.1. Rearing of *Aonidiella aurantii* and *Aphytis melinus*

Specimens of *A. aurantii* were initially collected in the field in 2016 from a population of *A. aurantii* in a citrus orchard located in WA (32.30°S, 116.01°E; 69 m AMSL), Australia. Stock cultures of *A. aurantii* were maintained on butternut squash (*Cucurbita moschata* Duchesne ex Lamarck), in climate chamber laboratory conditions [27 °C, 65 ± 5% RH, Light 16 h (L16) : Dark 8 h (D8) photoperiod] in the Murdoch University insect culture room.

The adult wasps of *A. melinus* used to start the colony were provided by Biological Services commercial insectary (Adelaide, Australia). *Aphytis melinus* adults used in the experiments were reared following the method developed by Opp and Luck (1986) for rearing *A. melinus* in the climate chamber (26 °C, 40%–60% RH, L16 : D8 photoperiod). This method is commonly used to produce these insects in commercial insectaries. The production method is based on rearing the pest *A. aurantii* on butternut squash. When the host reached the third instar, which is the preferred age for the parasitoid to lay eggs and maximise progeny production, the infested squash with third instar scales was exposed to releases of 2-day-old adult parasitoids (male and female) in a ventilated cage (30 cm W × 30 cm H × cm D) with honey distributed on plastic trays. Adult parasitoids emerged 11–14 days later. Parasitoids thus produced participated in the experiments 1 day after emergence.

2.2. Survival, longevity, and fecundity of *Aonidiella aurantii*

Uniform groups of *A. aurantii* were obtained by leaving butternut squash undisturbed for 24 h in the *A. aurantii* colony maintained in the insect culture room at Murdoch University. These squashes were then removed from the colony, and each 10 randomly selected settled crawlers (those with the stylet inserted into the fruit and already forming the waxy cover) were enclosed by a micro plastic cage (small plastic thimble, 3 cm diameter with fine holes on its top) affixed with the help of modelling clay to the surface of the butternut squash. The squash fruit and nymphs were marked to track them throughout the experiment.

An age-specific life table of *A. aurantii* was constructed at 3 different constant temperatures (20, 23, and 27 °C). The infested fruits were kept in a ventilated polystyrene box (30 cm × 30 cm × 30 cm). Each treatment of 2 squash fruits (6 replicates) were kept on a plastic tray in identical climate cabinets (HW'S Ningbu Southeast Equipment Co. Ltd., China) in which the experiment was conducted under controlled climatic conditions (20, 23, and 27 °C, 65 ± 5% RH, and L16 : D8 photoperiod). The development of the individuals of *A. aurantii* was observed daily using a stereomicroscope (×20) until the death of the adult females, and the following observations were recorded.

2.3. Survival, longevity, and fecundity of *Aphytis melinus*

Newly emerged adult parasitoids were shifted to an Agilent glass vial (1.5 mL). The vial was then plugged with a cotton bung. A drop of honey was supplied for each vial. After 24 h, each female parasitoid was confined under a micro plastic cage (small plastic thimble, 3 cm diameter with fine holes on its top) affixed with the help of modelling clay to the surface of butternut squash containing 10 female individuals female of the 3rd instar stage of *A. aurantii*. A virgin female and male of *A. melinus* were confined into each of these cages, as the parasitoid prefers to oviposit on initial stages of this host (Heimpel, 1997).
stereoscopic magnifying glass was used to observe mating. The parasitoids were transferred every 24 h to a new cage and observations continued until the female parasitoid died (Qiu et al., 2007). Determining the number of eggs laid without removing the armour scale of the nymphs is impossible, as the process leads to the death of the nymph (Foldi, 1990). A parallel experiment, identical to that used in the longevity and oviposition experiment, was therefore undertaken. Nonparasitised individuals were inverted to check for egg remains or unhatched parasitoid eggs, indicating unsuccessful parasitism or infertile eggs, respectively (Urbaneja et al., 2007). Observations were recorded to get the information on preoviposition, oviposition, and postoviposition periods and the number of eggs laid each day. Parasitised nymphs were kept to determine when the adult parasitoid emerged in order to estimate developmental time and survivorship (a number determined from the number emerged divided by total number parasitised). The experimental unit was a group of 10 female parasitoids. Each experimental unit was replicated 3 times.

2.4. Design and statistical analysis
A completely randomised design was used in all of the experiments. An analysis of variance (ANOVA) was subsequently performed, and means of survivorship, developmental times, longevity, and fecundity were compared using Tukey’s multiple comparison tests (P < 0.05). Subsequently, all data were analysed using Levene’s test to ensure homogeneity of variance.

2.5. Life table parameters
The main purpose of age-specific fecundity table studies is to predict the rate of population growth of A. aurantii and A. melinus. The fertility life table was constructed by making a list of the data collected on the developmental and reproductive biology of the species as per formulae provided by Birch (1948). Daily adult numbers from observation from the first day of female exclusion until complete female death, daily survival rate (l_x), and female fecundity (m_x) were calculated according to the equation of the life tables to predict the growth rate of the population under laboratory conditions at 27 °C. From the fertility and survival rate, several population growth parameters including the net reproductive rate (R_0), mean generation time (T_o), and intrinsic rate of natural increase (r_m) were calculated using the formulas suggested by Carey (1993), where x is the age of individuals in days, l_x is the age-specific survival, and m_x is the age-specific number of female offspring.

Age-specific life table parameters of the insects were calculated according to the Euler–Lotka equation (Birch, 1948). These parameters are:

Net reproductive rate \( R_0 = \sum l_x m_x \);
Intrinsic rate of natural increase \( r_m \);

\[ l = \sum \frac{f(x)}{l_x} e^{-b x} \]
Mean generation time, \( T_o = \frac{l n R_0}{r_m} \)
Gross reproduction rate, \( GRR = \sum m_x \)
Finite rate of increase, \( \lambda = e^{r_m} \)
Age-specific eggs laid by a female were described by the Enkegaard equation:

\[ F(x) = a x e^{b x} \]

Where \( F(x) \) is the daily age-specific fecundity rate (eggs/female/day), x is the female's age in days, and a and b are constants. Day 1 is the first day of the oviposition period. Analyses were done by using JMP (v. 5), MS Excel (v. 2003), SPSS (v. 24.0), and CurveExpert pro (v. 1.6.7) software.

3. Results

3.1. Survival, development, and oviposition of Aonidiella aurantii
Table 1 shows the survival rates of the immature state of A. aurantii at different temperatures (20, 23, and 27 °C). The rates were similar but varied between 80.0% and 88.0% for these 3 temperatures. The average mortality recorded at the 3 different temperatures was 0.16%. The highest mortality occurred during the preadult stage, with values ranging between 0.12% and 0.20% (depending on the temperature). Based on these results, we assume that there were no significant differences in the survival of A. aurantii reared on C. moschata at these different temperatures.

The obtained data in Table (2) indicated that the duration of each developmental stage was affected by temperature. These results indicated that 27 °C was an adequate tested temperature for A. aurantii, resulting in the highest oviposition (129.35 ± 4.56 eggs/female), the shortest incubation period (4.45 ± 0.14 days), and greatest adult longevity (39.35 ± 1.07 days).

3.2. Survival, development, and oviposition of Aphytis melinus
A. melinus mortality was concentrated in older individuals. The adult A. melinus individuals started to die from day 10 until day 18 and showed a sharp drop on days 13 and 16 (Figure 1).

The oviposition period of A. melinus lasted an average of 15.7 ± 0.52 days (Table 3). The average oviposition rate was 3.95 ± 0.22 eggs female\(^{-1}\) day\(^{-1}\). Each female laid an average total of 62.7 ± 2.81 eggs. The preoviposition period was 0.82 ± 0.05 day; adult longevity was an average of 19.24 ± 0.73 days, while the egg–pupal stage was 11.25 ± 0.12 day.

3.3. Life table parameters
Age-specific fecundity rates (m_x) of A. aurantii reared on fruits of C. moschata at 3 constant temperatures (20, 23, and 27 °C) and Aphytis melinus (27 °C) are given in Table 4 and Figure 2.
It was observed that *A. aurantii* had a higher net reproduction rate ($R_0$) and mean generation time ($T_0$), and conversely, intrinsic rate of increase ($r_m$) than *A. melinus* (Tables 4). Parameters show that the *A. aurantii* population grows 61.02 times at 27 °C in one generation. The net reproduction rate for each female of a generation was considerably higher for *A. aurantii* than *A. melinus*, reaching 28.14 at 27 °C (Table 4), which indicates its high reproductive capacity when fed butternut squash.

Moreover, the net reproduction rate obtained for *A. melinus* indicates a low substitution potential for each female having *A. aurantii* as a host under laboratory conditions. The intrinsic rate of increase ($r_m$), defined as a
population’s capacity to multiply, was higher for *A. melinus* (0.188) than *A. aurantii* which was 0.080 at 27 °C (Table 4).

The parameters (a, b, and R²) of the Enkegaard regression model applied on the age-specific fecundity rate (m_x) of *A. aurantii* and *A. melinus* are given in Figure 3. The relationship between days and fecundity was higher at all temperatures.

### 4. Discussion

Since the 1960s, there have been intensive studies on mortality and growth of *A. aurantii* conducted worldwide (DeBach et al., 1971; Abdelrahman, 1974; Karaca et al., 1987; Badary and Abd-Rabou, 2010). Information about the biology of *A. aurantii* is important for improving the mass production of certain parasitoids used in biological control, particularly *A. melinus*, a species that is used to control *A. aurantii* (Olivas et al., 2011). The use of constant temperature provides a useful reference point for the performance of *A. aurantii*. The survival values of *A. aurantii* varied between 80% and 88%. These values were approximately similar to those obtained by Karaca (1990) at 26 °C on lemon, orange, and grapefruit, and higher than the survival of *A. aurantii* on mandarin, in a study on the development, survival, and longevity of *A. aurantii* on different citrus species, in which survival was only 0.90, 0.81, 0.85, and 0.61 on lemon, orange, grapefruit and mandarin, respectively. This could be due to the variety of the fruit species and laboratory conditions being different from those used in this study. The average mortality is approximately similar to that obtained by Vanaclocha

![Figure 2. Age-specific fecundity (m_x) of Aonidiella aurantii reared on fruits of Cucurbita moschata at 3 constant temperatures (20, 23, and 27 °C) and Aphytis melinus (A.m) (27 °C).]
Adult females of many species obtain materials required for egg maturation by feeding upon host insects (host-feeding) (Yu and Luck, 1988). The duration of development in A. aurantii usually short and depends on climatic conditions, principally temperature and humidity (Yu and Luck, 1988). For example, at 26.7 °C, A. aurantii completes its development in almost a fortnight, whereas it takes 1 month to complete development at 17 °C. Nutrition also contributed to longevity in the ectoparasitoid species A. melinus. Higher longevity was observed in females that were fed than those not allowed to host-feed (Heimpel et al., 1997). As a result, greater availability of females could occur over time when the temperature is appropriate and there is food, but this is not necessarily associated with an increase in the parasitoidism rate. Aphytis melinus has been found to have 2–3 generations to 1 generation of its host A. aurantii (Yu and Luck, 1988). The duration of the longevity period of A. melinus is approximately consistent with that obtained by Vanaclocha et al. (2012), who determined that at 26.7 ± 1.5 °C the duration of the longevity period of A. melinus was 76 days, while the total number of eggs per female was about 11.7 eggs fewer than the number produced by A. melinus at 26.7 ± 1.5 °C [39]. The diet of adult parasitoids has an effect on lifetime reproductive success (Flaih, 2007). Adult females of many species obtain materials required for egg maturation by feeding upon host insects (host-feeding).

The analysis of variance in Table 2 revealed that the total life cycle and development rates of the female of A. aurantii were significantly different depending on the difference in the temperature used, except for the duration of the third instar which did not differ significantly between 20 °C and 23 °C. In addition, the preoviposition period, oviposition period, longevity, and the mean number of eggs per female of A. aurantii differed significantly depending on the difference in temperature. In general, the duration of each developmental stage recorded in this study was consistent with those determined for A. aurantii by Badary and Abd-Rabou (2010), and less than those determined by Karaca (1990). In other species of Aonidiella, the average developmental time ranged between 45 days and 94 days depending on the temperature used for A. orientalis (Flaih, 2007), 65 days for A. citrini in California; the reproductive period lasts 60 days under a constant temperature of 27.8 °C (Nel, 1933). Temperature is the most important abiotic factor affecting insect growth, development rate, oviposition, and survival of A. aurantii. With high temperatures within optimal limits, all processes occur significantly faster, which results in rapid ageing of the females with a significant increase in the total number of eggs laid and reduced longevity. Likewise, the number of eggs laid per day increases (Pekas, 2011b). This last parameter, together with the increased rate of oviposition and nymphal development, increases the potential pest status of A. aurantii in warm citrus-growing areas.

Aphytis melinus females showed gradual mortality over time, which can be attributed to death by natural ageing of the individuals. The developmental period of Aphytis spp. is usually short and depends on climatic conditions, principally temperature and humidity (Yu and Luck, 1988). For example, at 26.7 °C, A. aurantii completes its development in almost a fortnight, whereas it takes 1 month to complete development at 17 °C. Nutrition also contributed to longevity in the ectoparasitoid species A. melinus. Higher longevity was observed in females that were fed than those not allowed to host-feed (Heimpel et al., 1997). As a result, greater availability of females could occur over time when the temperature is appropriate and there is food, but this is not necessarily associated with an increase in the parasitoidism rate. Aphytis melinus has been found to have 2–3 generations to 1 generation of its host A. aurantii (Yu and Luck, 1988). The duration of the longevity period of A. melinus is approximately consistent with that obtained by Vanaclocha et al. (2012), who determined that at 26.7 ± 1.5 °C the duration of the longevity period of A. melinus was 76 days, while the total number of eggs per female was about 11.7 eggs fewer than the number produced by A. melinus at 26.7 ± 1.5 °C [39]. The diet of adult parasitoids has an effect on lifetime reproductive success (Flaih, 2007). Adult females of many species obtain materials required for egg maturation by feeding upon host insects (host-feeding).

Figure 3. Enkegaard distribution of Aonidiella aurantii and Aphytis melinus.
feeding), and materials necessary for adult maintenance and upon any of a number of sugar sources (Jervis, 1996; Heimpel and Collier, 1996).

These results confirm the potential of *A. melinus* to control *A. aurantii*. However, this characteristic must be compared with that of other species, such as other *Aphytis* spp., which can use the same field substrate. Studies carried out by Orphanides (1984) point out that there is interspecific competition and competitive displacement between *Aphytis* spp. which are *A. aurantii* parasitoids; thus, other species or subspecies could affect biological control success in the field. Comparing the life table parameters of *A. aurantii* and *A. melinus*, it can be seen that there are significant differences in all 5 of the parameters studied (Table 4). The net reproductive rate (number of females for each female of a generation) is significantly higher for *A. aurantii* (61.02) than that of *A. melinus* (28.14). Generation time, the mean time between 2 successive generations, is significantly longer in *A. aurantii* (51.39 days) than in *A. melinus* (19.06 days) (Table 4); this is interpreted as favourable for increasing the numbers and efficiency of the parasitoid (La Rossa et al., 2002). The intrinsic rate of increase (*r*'), which indicates the ability of a population to increase in abundance from generation to generation, is an essential indicator of the potential of a parasitoid to control its host (Mercado et al., 2014; Persad and Khan, 2002). The *r*’ parameter of *A. melinus* (0.188) is significantly greater than that of *A. aurantii* (0.080) at 27 °C (Table 4).

The relationship between days and fecundity was demonstrated well by using the Enkegaard regression model; at 20, 23, and 27 °C for *A. aurantii* (*R* 2 = 0.833, a = 0.410, b = 0.099; *R* 2 = 0.799, a = 0.624, b = 0.098; *R* 2 = 0.715, a = 0.661, b = 0.091, respectively) and at 27 °C for *A. melinus* (*R* 2 = 0.841, a = 1.190, b = 0.179). Most of the eggs were laid within the first half of the oviposition period (Figure 3). The influence of increased temperature was clearly seen for *A. aurantii* by increased the fecundity peak at an earlier age. Thus, the fecundity curve turns to the left at 27 °C earlier than at other temperatures; this is clearly seen for *A. melinus* as well (Figure 3).

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