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ORIGINAL PAPER

Some biological interactions between the parasitoid *Anagyrus pseudococci* (Girault) (Hymenoptera: Encyrtidae) and its host *Planococcus ficus* (Signoret) (Hemiptera: Coccoidea: Pseudococcidae)

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Abstract The development, longevity, fecundity and life-table parameters of the endoparasitoid *Anagyrus pseudococci* (Girault) (Hymenoptera: Encyrtidae), 15 d.o. (3rd-instar nymphs) and 21 d.o. (young adult females) of the vine mealybug, *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) at 28 ± 1°C, 65 ± 10%RH and 16:8h L:D under laboratory conditions. The developmental time of female parasitoids within the host was 17.7 ± 0.39 days in 15 d.o. and 16.65 ± 0.25 days in 21 d.o. hosts; for males, development time was 16.85 ± 0.29 and 15.25 ± 0.09 days, respectively. The average number of offspring per female was 22.35 ± 1.68 in 15 d.o. and 34.8 ± 2.56 in 21 d.o. vine mealybugs. The longevity of female parasitoids was 14.8 ± 0.98 days in 15 d.o. and 15.65 ± 0.92 days in 21 d.o. mealybugs, respectively; for males, longevity was determined as 7.3 ± 0.43 and 6.7 ± 0.54 days, respectively. The mean time of pupation was 7.85 ± 0.003 days in 15 d.o. mealybugs and 8.65 ± 0.003 days in 21 d.o. mealybugs. The aggregate encapsulation rate in the parasitized 15 d.o. mealybugs was 49.73 and 60.36% in 21 d.o. mealybugs. Furthermore, effective encapsulation was 24.82% in 15 d.o. mealybugs and 37.50% in 21 d.o. mealybugs. Population growth rate (r_m) for *A. pseudococci* was 0.0999 female/female/days in 15 d.o. mealybugs and 0.1269 female/female/days in 21 d.o. mealybugs. The mean population generation time was 23.49 days for parasitoids reared in 15-days-old and 22.39 days when reared in 21 d.o. mealybugs.

Keywords *Anagyrus pseudococci* · *Planococcus ficus* · Encapsulation · Host age · Life-table parameters · Turkey

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Introduction

Anagyrus pseudococci (Girault) (Hymenoptera: Encyrtidae) is well known as a parasitoid of the vine mealybug, *Planococcus ficus* Signoret and a polyphagous, cosmopolitan, solitory, koinobiont and endoparasitoid (Noyes and Hayat 1994). It is also attacks distantly related species such as *Dysmicoccus brevipes* (Cockerell), *Maconellicoccus hirsutus* Green, *Planococcus citri* (Risso), *Pseudococcus comstocki* (Kuwana) and *Phenacoccus herreni* Cox and Williams (Noyes and Hayat 1994; Daane et al. 2004a). Due to its' wide host and geographic range, *A. pseudococci* is also one of the most commonly commercially reared parasitoids and has often been used for biological control of pseudococcids in several countries.

The vine mealybug, *P. ficus*, is a cosmopolitan species and a important pest of grapes, figs and ornamental plants in greenhouses (Düzgüneş 1982; Ben-Dov 1994). Vine mealybug was first recorded on *Vitis* spp. in Turkey by Düzgüneş (1982), but later researchers (Anonymous 1999) suggested that the

mealybug on grapes was *P. citri.* However, Kaydan et al. (2004) showed that this mealybug on grapes was *P. ficus*. The vine mealybug feeds on the roots, trunks, cordons, canes, leaves and fruit of its host plants and this can result in leaf drop, and to weakened plants, as with many homopteran species. The honeydew secretion of mealybugs is a medium for saprophytic fungi (the fumagine) and results in both a reduced quality and quantity of the crop. Furthermore, *P. ficus* is a virus vector (Engelbrecht and Kasdorf 1990), and, therefore, is considered economically important even at low densities.

Chemical control of vine mealybug can be difficult since a portion of the pest population often resides in protected locations on the grape, such as under the bark of trunk or cordon. The thick layers of protective wax it secretes may also be a factor leading to difficulties in their control. Therefore, because chemical control can be ineffective, biological controls may offer a possible alternative method to suppress vine mealybug populations. One of the most important biological control agents of this mealybug is *A. pseudococci* (Noyes and Hayat 1994; Daane et al. 2004a, b). *A. pseudococci* is a native parasitoid of vine mealybug in many countries including Argentina, Israel, Italy, South Africa and Turkey (Berlinger 1977; Öncüer 1991; Trjapitzin and Trjapitzin 2002; Daane et al. 2004a).

Encyrtid parasitoids of mealybugs are mostly solitary parasitoids (Noyes and Hayat 1994). Only one individual can develop in a single host while some of the eggs can become encapsulated in parasitized hosts (Götz 1986). Encapsulation is a common defense mechanism exerted by a host insect in response to invasion by metazoan parasitoid or other foreign organisms (Blumberg 1997). It can adversely affect biological control by reducing parasitoid efficacy and thus hinders the establishment of introduced parasitoids and increasing the frequency of pest outbreaks. High level encapsulation may cause difficulties in mass production programs of parasitoids (Blumberg et al. 2001). On the other hand encapsulation rates can be affected by host ages, host variety, environmental factors (temperature and humidity) (Blumberg and Van Driesche 2001; Blumberg et al. 2001).

In Turkey, *A. pseudococci* reduces the population of the citrus mealybug (*P. citri*) to 36% (Anonymous 1997), but interactions between and vine mealybug have not yet been studied depthly. The goal of this study was to determine some life-table parameters and encapsulation rates for *A. pseudococci* on different ages of *P. ficus* collected from vineyards in Ankara-Turkey.

Materials and methods

Mealybug source

Vine mealybugs were collected from the vineyard of Ankara University, Faculty of Agriculture (Turkey) and then reared on sprouted potatoes in plastic jars (44 × 47 × 67 cm in dimensions) which had ventilation holes covered in muslin (40 meshes). The cultures were maintained in a climatic chamber at 28 ± 1°C and 60 ± 5%RH, under a photoperiod of 16:8 L:D. Adult female mealybugs with an ovisac become available after 4–6 weeks from egglaying. Four weeks after the initial infestation, 3rd instar nymphs (15-days-old) and young adult female (21-days-old) of *P. ficus* (following to emergence of crawlers) were collected and used for the experiments.

Parasitoid source

A. pseudococci were obtained from *P. ficus* in the same vineyard at Ankara University. The parasitoids were cultured on *P. ficus* on sprouted potatoes. Adult parasitoids (30♀, 30♂) were placed into plastic jars each containing 20 infested sprouted potatoes supporting 3rd instar nymphs and young females of *P. ficus*. Emerged parasitoids were collected 20–25 days after parasitism, and transferred to new plastic jars with infested sprouted potatoes. Colonies were maintained in a climatic chamber at 28 ± 1°C, 60 ± 5% RH, under a photoperiod of 16:8 L:D.

Experimental procedure

Newly emerged female and male parasitoids were reared on 15 or 21-days-old mealybugs after egg hatch were used for experiments. One female and one male parasitoid were transferred into the cage in which artificially infested potatoes with 15 d. o. or 21 d.o. After 24 h the parasitoids couples were transferred to a new cage. This procedure was repeated every 24 h until the death of all parasitoid couples. These observations replicated 20 times (n = 20 parasitoid couples/treatment). All experiments were maintained in a climatic chamber at 28 ± 1°C, 60 ± 5%RH, under a photoperiod of 16:8 L:D. After parasitization, the time to emergence of the new parasitoid (development time), the number emerging of each sex, the total number of offspring for each parasitoid female, preoviposition, oviposition and postoviposition times, times to mummification, longevity of females and males were recorded.

Determining encapsulation rates

To determine encapsulation reactions of 15 and 21 d.o. vine mealybug, ten male and ten female parasitoids, each 2–3-days-old, were put with 50 individuals of each age of mealybugs on potato sprouts a plastic vials ($4 \times 10 \times 11$ cm diameter). Each experiment had five replicates. The parasitoids were removed from the vials after 24 h. Götz (1986) described the sequence of events during cellular encapsulation and tentatively divided the process into ten consecutive steps, most of which are completed within 15 min, the entire reactions lasting between 1 and 3 days. Therefore the vine mealybugs were put in the vials contain saline solution 3–4 days after parasitism, then dissected to determine encapsulation rates of the parasitoids larvae and eggs for each age of vine mealybug. Two parameters were used to measure encapsulation rates: aggregate encapsulation (AE) and effective encapsulation (EE) (Blumberg and Van Driesche 2001). These are defined as:

Aggregate encapsulation (AE)

The percentage of encapsulated eggs observed in the parasitized mealybugs

$$\text{AE} = \frac{\text{No of encapsulated eggs}}{\text{Total egg number}} \times 100$$

Efficient encapsulation (EE)

Percentage of parasitized mealybugs which completely prevented parasitoid development.

$$\text{EE} = \frac{\text{Mealybugs with encapsulated eggs only}}{\text{Total parasitized mealybug number}} \times 100$$

Statistical analyses

Data on developmental time, longevity and fecundity of the parasitoid and encapsulation rates were analyzed by one-way ANOVA followed by t test ($P \leq 0.05$) between different ages.

Population growth rates on different age intervals of the vine mealybug were calculated by using life tables according to Andrewartha and Birch (1970):

$$1 = \sum e^{-rx} 1_x m_x$$

For building up the life tables, l_x (age-specific survival rates) and m_x (number of female offspring) for each age interval (x) per day were used. From these data, net reproductive rate (R_0 = female/female/generation), intrinsic rate of increase (r = female/female/day), and mean generation time ($T_0 = \ln(R_0/r)$, in days) were calculated.

It was calculated from the original data (r_{a11}), the differences of r_m- values for each age of vine mealybug were tested for significance by estimating the variance using the jack-knife method (Meyer et al. 1986; Sokal and Rohlf 1981). The jack-knife pseudo-value r_j was calculated for the each sample (n) using

$$r_j = nr_{a11} - (n-1)r_i$$

The mean values of (n–1) for mean growth rate in each treatment were analyzed by t Test ($P \leq 0.05$) to determine the differences between parasitoids reared on each age of the vine mealybug. All statistical analyses were made by using STATISTICA (StatSoft Inc. Tulsa, OK).

Results

The development time for *A. pseudococci* reared on 15 and on 21 d.o. mealybugs ranged from 15.25 ± 0.09 to 17.7 ± 0.39. For both male and female *A. pseudococci*, development was significantly faster in the 21-day-old melybug treatment (Table 1). There was no different in development time between female and male parasitoids in the 15 d.o. mealybug treatment, while development was significantly faster for males in the 21 d.o. treatments (t test = 2.25; df = 38; P = 0.03) (Table 1).

Preoviposition, oviposition and postoviposition periods of *A. pseudococci* were not statistically different between these parameters in either 15 d.o. or 21 d.o. mealybugs (preoviposition = t test = 1.28; df = 38; P = 0.206; oviposition: t = 0.15; df = 38; P = 0.877; postoviposition: t test = 0.399; df = 38; P = 0.691) (Table 1).

The longevity of female and male were not significantly different in the 15 and 21 d.o. mealybugs treatments (t test = 0.11; df = 38; P = 0.912). Longevity of male parasitoids was shorter than that for female parasitoids for both ages of mealybugs (t = 0.87; df = 38; P = 0.39) (Table 1).

The fecundity of *A. pseudococci* was affected by the age of the host in which it matured. The number of offspring was 34.80 ± 0.16 when reared in 21 d.o. mealybugs, significantly greater than in 15 d.o. mealybugs (t test = 4.06; df = 38; P = 0.0002) (Table 2). Significantly more males than females emerged from both 15 and 21 d.o. mealybugs (t test = 5.44; df = 38;

Table 1 Development time, longevity and oviposition time of female *Anagyrus pseudococci* on the different age *Planococcus ficus*

Host age (day)	*n*	Duration (day)						
		Development time		Longevity		Oviposition		
		♀	♂	♀	♂	Preoviposition	Oviposition	Postoviposition
15-days-old	20	17.7 ± 0.39^{aA}	16.85 ± 0.29^{aA}	14.8 ± 0.98^{aA}	7.3 ± 0.43^{aB}	0.35 ± 0.18^{a}	13.95 ± 0.91^{a}	0.5 ± 0.15^{a}
21-days-old	20	16.65 ± 0.25^{bA}	15.25 ± 0.09^{bB}	14.65 ± 0.92^{aA}	6.7 ± 0.54^{aB}	0.10 ± 0.07^{a}	14.15 ± 0.91^{a}	0.4 ± 0.19^{a}

Within the lines, pairs of means followed by the same letters are not significantly different ($P < 0.05$)

Table 2 Pupation time and fecundity of the female *Anagyrus pseudococci* on the different age *Planococcus ficus*

Host age (day)	*n*	Pupation time (day)	♀/♀ parasitoid number	♂/♀ parasitoid number	Total offspring number/♀
15-days-old	20	7.85 ± 0.003^{b}	10.1 ± 0.71^{bA}	12.25 ± 0.99^{bA}	22.35 ± 0.13^{b}
21-days-old	20	8.65 ± 0.003^{a}	17.45 ± 1.27^{aA}	17.45 ± 1.31^{aA}	34.80 ± 0.16^{a}

Within the lines, pairs of means followed by the same letters are not significantly different ($P < 0.05$)

$P = 0.0001$ for females; t test = 3.10; $df = 38$; $P = 0.003$ for males). Furthermore there were no significant difference between male and female offspring number for both ages (t test = 1.75; $df = 38$; $P = 0.08$ for 15-days-old, t test = 0.054; $df = 38$; $P = 0.95$ for 21-days-old) (Table 2).

The life-table parameters for *A. pseudococci* reared in different ages of vine mealybug are shown in Table 3. The intrinsic rate of increase (r_m) was estimated for the population reared in 21 d.o. mealybugs as 0.129 female/female/day; significantly greater than that obtained in 15 d.o. mealybugs ($t = 7.11$; $df = 38$; $P = 0.000$). The net reproduction rate (R_0) was also greater from 21 d.o. mealybugs (17.35 female/female/generation) than that from 15 d.o. mealybugs (10.1 female/female/generation). The mean generation time (T_0) for females reared in 21 d.o. mealybugs was shorter than that from 15 d.o. mealybugs (Fig. 1).

The sex ratio of the *A. pseudococci* population reared in 21 d.o. mealybugs was determined as 1:0.82 (♀:♂) while that for the population reared on 15 d.o. mealybugs was 1:1 (♀:♂).

Female *P. ficus* lived for 4 days after parasitization by *A. pseudococci*. During this time, there was no significant change in color but the black spot caused by oviposition by the parasitoid was visible. Movement of the parasitized mealybug stopped after 5 days and it changed into a mummy after 8 days. During the 9th and 10th days, the mummy became brownish and transparent. The mean time to mummification was 7.85 ± 0.003 days in 15d.o. mealybugs and 8.65 ± 0.003 days in 21 d.o. mealybugs (t test = 5.169; $df = 38$; $P = 0.000$) (Table 2). The adult parasitoid emerged from the mummy through a hole in the host abdomen.

The aggregate encapsulation rate in the parasitized 15 d.o. mealybugs was 49.73 and 60.36% in 21 d.o. mealybugs. Furthermore, effective encapsulation was 24.82% in 15 d.o. mealybugs and 37.50% in 21 d.o. mealybugs.

Table 3 Net reproductive rate (R_0). generation time (T_0). and intrinsic rate of increase (r_m) of *Anagyrus pseudococci* on the different age *Planococcus ficus*

Life table parameters				
Host age	*n*	r_m	R_0	T_0
15-days-old	20	0.099 ± 0.006^{b}	10.1	23.260
21-days-old	20	0.129 ± 0.005^{a}	17.35	22.103

Within the lines, pairs of means followed by the same letters are not significantly different ($P < 0.05$)

Discussion

Development time of a parasitoid is affected by host age, host species and temperature (Islam and Copland 1997; Bokonon-Ganta et al. 1995; Çalişir et al. 2005). In our experiments at 28°C, development times for female and male *A. pseudococci* were influenced by host age. Daane et al. (2004b) found that *A. pseudococci* could complete development (egg to adult eclosion) in the temperature range 14–34°C in *P. ficus*, where development times ranged from 79.1 ± 1.0 days at 14°C to 10.2 ± 0.3 days at 34°C, with an optimal temperature of 24.7°C. On the other hand, Çalişir et al. (2005) found that the development times of *A. pseudococci* in *P. citri* were not affected by host age (13.2 days for females, 12.4 days for male on 12 and 18 d.o. *P. citri* at 28°C, respectively) and were shorter than those recorded herein *P. ficus*. Islam and Copland (1997) found that the male development time of *A. pseudococci* was

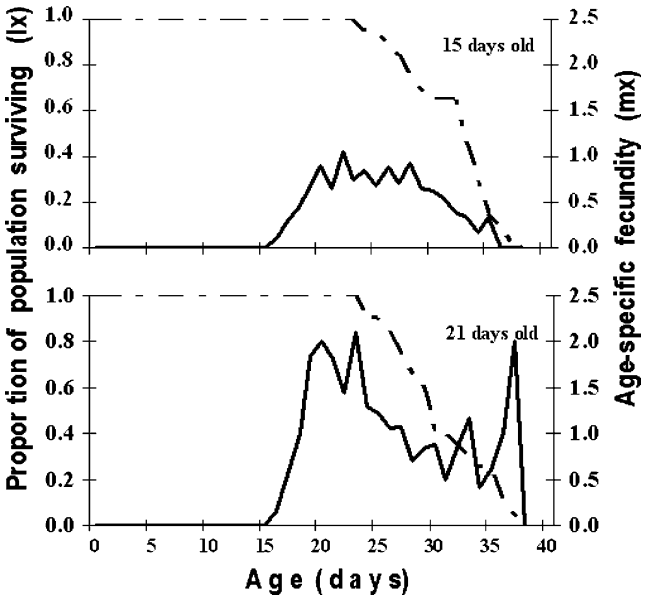

Fig. 1 Life table of *Anagyrus pseudococci* on the 15 and 21-days-old *Planococcus ficus*

delayed in young host stages of citrus mealybug, while the females developing in third instars and adult hosts were unaffected.

Preoviposition, oviposition and postoviposition periods for *A. pseudococci* were not significantly affected by the age of *P. ficus.* However, Çalişir et al. (2005) reported that the oviposition periods for *A. pseudococci* were 11.5 days in 12 d.o. and 13.35 days in 18 d.o. *P. citri*.

The longevity of both sexes of *A. pseudococci* was not affected by the age of *P. ficus*. Female parasitoids also lived longer than males in the experiments of Çalişir et al. (2005) found the longevity of female *A. pseudococci* was 14.3 days in 12 d.o., and 14.6 days in the 18 d.o. *P. citri*, while that for males was 6.7 and 7 days, respectively. Tingle and Copland (1989) stated that the longevity of *A. pseudococci* became shorter with increasing temperature (18–30°C).

In our experiments, the sex ratio of *A. pseudococci* was male biased in 15 d.o. mealybugs but was equal in 21 d.o. mealybugs. Daane et al. (2004a, b) found that most females were reared from the larger mealybugs. Similar results were obtained by Çalişir et al. (2005) in *P. citri* (0.75:1 (♀:♂) in 12 d.o. and 1:0.88 (♀:♂) in 18 d.o. mealybugs). Islam and Copland (1997) reported that *A. pseudococci* exhibited maternal adjustment of sex ratio as a function of host size with an increased proportion of females with increasing host size.

The age of the host had a significant effect on fecundity, with more offspring from 21 d.o. mealybugs than from 15 d.o. mealybugs. Daane et al. (2004a, b) observed that while there was an over-abundance of second, third and preovipositional adult mealybugs were available during the ovipositional period, there was a clear preference for third and adult stage mealybugs. For *A. pseudococci*, host size may correspond to higher adult fecundity. Islam and Copland (1997) observed that higher percentage emergence of parasitoid from older citrus mealybug.

The time to pupation in our observations was 7–11 days. Islam and Copland (1997) observed that *A. pseudococci* parasitized 1st instar *P. citri* were not mummified, but 2nd and 3rd instar mealybugs did become mummified after 8–11 days.

In the life table of *A. pseudococci*, the net reproductive rate (R_0) and the intrinsic rate of population increase (r_m) were higher from older than from young female mealybugs. Previous research (Avidov et al. 1967, Islam and Copland 1997) has suggested that *A. pseudococci* prefers to lay eggs in the larger stages of *P. citri*, which could encourage higher fecundity due to greater food quality. This was also noted with *A. indicus* Subba Rao in the spherical mealybug (*Nipaecoccus viridis* (Maskell)) (Bokonon-Ganta et al. 1995), *Anagyrus kamali* Moursi in the pink hibiscus mealybug (*M. hirsutus*) (Nechols and Kikuchi 1985), and *A. mangicola* Noyes in the mango mealybug *Rastrococcus invadens* Williams (Sagarra and Vincent 1999).

Parasitism can be affected by many factors, such as host and parasitoid species, age, sex, viability of host, climatic conditions and host plants. Host age is one of important factors affecting parasitism success (Blumberg 1997, Islam and Copland 1997, Sagarra and Vincent 1999, Daane et al. 2004a, b). Our results suggest that host age of the vine mealybug did affect encapsulation rates and the aggregate encapsulation and effective encapsulation rates of parasitoid eggs were higher on younger host stage than older. Similar results were stated by Çalişir et al. (2005) that the encapsulation rate of the *P. citri* parasitized by *A. pseudococci* changed with host age (AE = 31.8% and EE = 70.46% in 12 d.o. citrus mealybugs; AE = 28.30% and EE = 59.11% in 18 d.o. citrus mealybugs). Blumberg (1997) reported that larger hosts were more successful at encapsulation of eggs and larvae because they have more blood cells in their haemocoel. Sagarra et al. (2000) found that under laboratory conditions, adult pink hibiscus mealybugs required 30 h to encapsulate 50% of eggs (*A. kamali)* whereas in second and third instars, 50% level encapsulation was never reached. The incidence of encapsulation reflects the degree of host-parasitoids adaptability, and it is believed that absence or low rate of encapsulations is the result of co-evolution of host and its parasitoids (Bartlett and

Ball 1966). Blumberg et al. (2001) reported that AE and EE values of *P. ficus* (from vineyards in Israel) were 57 and 11%, respectively when exposed to the Israeli ecotype of *A. pseudococci.* Both *P. citri* and *P. ficus* are most suitable for development of *A. pseudococci* due to the low EE values, although *A. pseudococci* is probably not principal parasitoid of these two mealybugs.

Turkey is the fifth largest grape producer in the world, with 3.5 million tons of grapes from about 525 ha vineyard, of which 226.000 tons were raisins and 80,000 tons were table grapes were exported. This is valued at 196 million US $ (Anonymous 2003). Therefore, *P. ficus*, which occasionally causes damage in vineyards, is of great potential importance. Although *A. pseudococci* has been found parasitizing the vine mealybug in our natural ecosystem, there is a need to increase its effectiveness. Because of this, *A. pseudococci* is mass cultured and released into vineyards. The results of this study show that 21 d.o. vine mealybugs are the most suitable than 15 d.o. for mass culture of *A. pseudococci* even though it has a greater encapsulation rate. On the other hand, the results suggest that most suitable time to release the parasitoid into the field is at young adult instar stages of vine mealybug.

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