# Developmental Time, Longevity, and Lifetime Fertility of Three Introduced Parasitoids of the Mealybug *Paracoccus marginatus* (Hemiptera: Pseudococcidae)

KAUSHALYA G. AMARASEKARE,[1,2,4] CATHARINE M. MANNION,[1] AND NANCY D. EPSKY[3]

Environ. Entomol. 41(5): 1184–1189 (2012); DOI: http://dx.doi.org/10.1603/EN11061

**ABSTRACT** Developmental time, longevity, and lifetime fertility of three previously introduced parasitoids (*Acerophagus papayae* Noyes and Schauff, *Anagyrus loecki* Noyes and Menezes, and *Pseudleptomastix mexicana* Noyes and Schauff) (Hymenoptera: Encyrtidae) of the mealybug *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae) were studied in the laboratory to understand the outcome of their recovery in field studies conducted in the United States. The developmental time of both male and female *A. papayae* and *A. loecki* was shorter than the developmental time of male and female *P. mexicana*. Male parasitoids of all three species had a shorter developmental time than their females. All parasitoids had a shorter developmental time in adult-female mealybugs than in second instars. Mating status (unmated and mated) had no effect on the male longevity. Unmated and mated females that were not allowed to oviposit had similar longevity and lived longer than those that were allowed to oviposit. Virgin females produced male only progeny with higher number of males from *A. loecki* or *P. mexicana* than from *A. papayae*. The number of females and the cumulative progeny was smaller for *A. papayae* than for *A. loecki* or *P. mexicana*. The progeny sex ratio (proportion of females) was not different among the parasitoids. *A. papayae* had the shortest reproductive period followed by *A. loecki* and *P. mexicana*, respectively. This information is important in evaluating the efficiency, recovery and establishment of *A. papayae*, *A. loecki*, and *P. mexicana*.

**KEY WORDS** biological control, parasitoids, mealybug, parasitism

*Acerophagus papayae* Noyes and Schauff, *Anagyrus loecki* Noyes and Menezes, and *Pseudleptomastix mexicana* Noyes and Schauff (Hymenoptera: Encyrtidae) are three previously introduced solitary koinobiont endoparasitoids of the mealybug *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae). *P. marginatus* is a polyphagus mealybug species that was first detected in the United States in Florida in 1998 (Miller and Miller 2002). This mealybug is believed to be native to Mexico and Central America (Noyes and Schauff 2003). It is a pest of many tropical and subtropical fruits, vegetables, and ornamental plants such as *Carica papaya* L. (papaya), *Hibiscus* spp. (hibiscus), *Citrus* spp. (citrus), and *Persea americana* Millar (avocado) (Miller and Miller 2002). Before invading the United States, *P. marginatus* had been established in the Caribbean since 1994 (Miller et al. 1999). After detected in Florida, *P. marginatus* was identified in Guam (Meyerdirk et al. 2004), the Republic of Palau (Muniappan et al. 2006), and Hawaiian islands (Heu et al. 2007). *P. marginatus* potentially poses a threat to many agricultural products in Florida and other states such as California, Hawaii, and Texas that produce similar crops.

Classical biological control was identified as an important pest management practice for *P. marginatus* by the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) (Meyerdirk et al. 2004). Currently, *A. papayae*, *A. loecki*, and *P. mexicana* are used in the control of *P. marginatus* in the United States, the Caribbean, and the Pacific (Meyerdirk et al. 2004). These three species of parasitoids were discovered in Mexico (Noyes and Schauff 2003). With the joint efforts of the Dominican Republic, Puerto Rico, and the United States, these parasitoids are mass-reared in Puerto Rico and released in mealybug-affected areas by the USDA–APHIS (Meyerdirk et al. 2004, Walker et al. 2006). *A. papayae* and *A. loecki* were released with two other parasitoids (*Anagyrus californicus* Compere and *Pseudaphycus* sp.) in Bradenton (Manatee County), FL, in 2000 (Ngyuen 2000) but the outcome of releases of these parasitoids is yet to be determined (Walker et al. 2006). *A. papayae*, *A. loecki*, and *P. mexicana* have been released in Miami-Dade and Broward counties (Flor-

[1] University of Florida, Institute of Food and Agricultural Sciences, Tropical Research and Education Center, 18905 SW 280th Street, Homestead, FL 33031.

[2] Current affiliation: Oregon State University, Mid-Columbia Agricultural Research and Extension Center, 3005 Experiment Station Drive, Hood River, OR 97031.

[3] USDA–ARS Subtropical Horticulture Research Station, 13601 Old Cutler Road, Miami, FL 33158.

[4] Corresponding author, e-mail: kaushalya2641@yahoo.com.

ida) for the first time in 2003 (D. M. Amalin, personal communication). In July 2003, 1,400 *A. papayae*, 1,200 *A. loecki* and 3,400 *P. mexicana* were released in Miami–Dade and Broward counties as a one-time release in 21 locations (D. M. Amalin, personal communication).

Field efficiency studies conducted in Florida showed a better adaptability of *A. papayae* over the heterospecific *A. loecki* and a lower efficiency and/or inability of field recovery of *P. mexicana* (Amarasekare et al. 2009). Understanding these life history parameters of *A. papayae*, *A. loecki*, and *P. mexicana* is important in explaining the results obtained in the field efficiency studies. There is no information available on the biology or life history parameters of these three parasitoids. This study focused on the developmental time, longevity, and lifetime fertility of three previously introduced parasitoids of *P. marginatus* under laboratory conditions to understand the outcome of their recovery in field studies conducted in Florida.

## Materials and Methods

**Mealybugs.** A colony of *P. marginatus* was maintained on red potatoes (sprouted) (*Solanum tuberosum* L.) (Ryan Potato Company, East Grand Forks, MN) in an environmental growth chamber (Percival I-36LL, Percival Scientific Inc., Perry, NC) set at 25 ± 1°C, 65 ± 2% RH, and a photoperiod of 12:12 (L:D) h. Initially, *P. marginatus* was collected from a papaya field in Homestead, FL. Sprouted potatoes were infested weekly with *P. marginatus* ovisacs (3–5 ovisacs per potato) collected from colony mealybugs (Amarasekare et al. 2008). Sprouted potatoes were infested for 8 wk before they were used in the experiment.

To obtain mealybugs for experiments, ovisacs that were <24-h old were placed on a leaf in an arena. Leaves were obtained from containerized hibiscus (*Hibiscus rosasinensis* L.) plants that maintained in a shadehouse. Arenas were prepared from a 9-cm diameter petri dish with a 0.6-cm diameter hole at the bottom. The stem (5 cm long) of a tender hibiscus leaf was inserted through the hole in the petri dish and each dish was kept on a 162 ml translucent plastic cup (Georgia Pacific Dixie, Atlanta, GA) filled with water, which allowed the stem below the petiole to be in water. These arenas were used for all studies, with ovisacs or mealybugs placed onto the hibiscus leaf in the arena. The gender of mealybugs was determined during the latter part of the second instar when males change their color from yellow to pink. Therefore, the gender was not determined for the first and second instars, but the third instars and adults used were females. Newly molted mealybugs, which were recognized by the size and presence of shed exuviae, were selected for all the experiments to reduce the variation in host quality. (Preliminary studies showed that the third [prepupa] and fourth instar [pupa] males were not selected for oviposition by the parasitoids, therefore these stages were not used in this study. Adult male mealybugs were not tested for parasitoid development because of their winged nature and shorter life span [Amarasekare et al. 2008]).

**Rearing Parasitoids.** Sprouted red potatoes with second and third-instar colony mealybugs were used for parasitoid rearing. Colonies of *A. papayae*, *A. loecki*, and *P. mexicana* were maintained in an insectary at 25 ± 2°C temperature, a photoperiod of 12:12 (L:D) h, and 65 ± 2% RH. Colonies were initiated with parasitoids obtained from the Biological Control Laboratory of Puerto Rico Department of Agriculture through USDA–APHIS and were maintained in Plexiglas cages (30× 30 × 30 cm) (each with two cloth sleeves). Potatoes with parasitized mealybugs were moved to a new cage and new mealybug infested potatoes were supplied once a week. A solution of honey and water (1:1) was streaked on four pieces (5 × 5 cm) of Benchkote surface protector paper (Fisherbrand, Fisher, Pittsburgh, PA) attached to the cage using labeling tape (Fisherbrand, Fisher). Water was provided in two clear plastic 73.9-ml containers (Tristate Molded Plastic Inc., North Dixon, KY) per cage. In each container, a 1-cm diameter hole was made in the center of the lid and a 7.6 cm long piece of cotton roll (TIDI Products, Neenah, WI) was inserted through the hole to allow parasitoids to access water.

Parasitoids were removed from the rearing cage immediately after their emergence. Each parasitoid was kept individually in a glass vial and sexed. Females were separated from males by presence of ovipositor, body size, and variation in antennae (Noyes 2000, Noyes and Schauff 2003). To obtain mated-female parasitoids, newly emerged females of each species were placed singly in disposable glass culture tubes (1.2 × 7.5 cm) (Fisherbrand, Fisher) and closed with two-ply tissue (Kimwipes EX-L, Kimberly-Clerk Global Sales Inc., Roswell, GA) secured with a piece of rubber tubing (0.95 × 2.5 cm) (Fisherbrand, Fisher). Five newly emerged males were placed in each tube with a female and were allowed to mate for 24 h. (Preliminary studies conducted to obtain mated-female parasitoids have shown that by using the above method could obtain mated parasitoids. A progeny produced by a mated parasitoid consisted of both males and females while an unmated female can produce male-only progeny.) A streak of honey and water (1:1) was provided for each tube. After 24 h, males were removed and females were used in experiments. All experiments were carried out at 25 ± 2°C temperature, a photoperiod of 12:12 (L:D), and 65 ± 2% RH.

**Developmental Time.** To evaluate the developmental time of each parasitoid species in different mealybug instars, 10 individuals (comprising one replicate) each from second instars, third instar females, and adult females were selected and placed separately on hibiscus leaf arenas (prepared 48 h before the experiment) using a paintbrush (No. 000; American Painter 4000, Loew-Cornell Inc., Englewood Cliffs, NJ). Mealybugs were placed on the leaves 24 h before the experiment to allow them to settle on the leaves. A mated female parasitoid was placed in each arena and covered with a piece (15 × 15 cm) of chiffon cloth

material (Jo-Ann Fabrics and Crafts, Miami, FL) and secured with a rubber band to avoid parasitoid escape. Parasitoids were allowed to oviposit for 24 h and were then removed. Mummified mealybugs were individually placed in disposable glass culture tubes and were secured as above. Time for adult emergence, their gender and number were determined for the developmental time, sex ratio, and proportion of parasitism. The mean of the 10 individuals on each hibiscus leaf (replicate) was used in analyses. This procedure was followed for all three parasitoid species with 50 replicates for each species.

**Longevity.** Longevity of all three species was studied using four mating conditions for female parasitoids (unmated and mated with or without oviposition), and two mating conditions for male parasitoids (unmated and mated). To collect both males and female parasitoids for each species, third-instar mealybugs on sprouted potatoes were placed in Plexiglas cages and mated female parasitoids were released to each cage. After 24 h, females were removed and mealybugs were allowed to mummify. Mummified mealybugs were collected and were placed individually in glass culture tubes as above.

When parasitoids started to emerge from the mummified mealybugs, 50 newly emerged virgin males and females were separately placed in glass culture tubes for unmated status, and each tube was provided with a streak of honey and water, and secured with two-ply tissue. For unmated females with oviposition, 50 newly emerged females were transferred individually to clear plastic 500 ml deli cups (Georgia Pacific Dixie, Atlanta, GA) and provided with mealybugs on potatoes to oviposit. Each cup was covered with a piece of chiffon cloth material. (A circular area of 8.5-cm-diameter was removed from the 12-cm-diameter lid to facilitate air circulation.) For mated males, one male was placed in a glass culture tube with a streak of honey and five females were provided for mating for 24 h, and then the females were removed. The mated males were retained in the culture tubes. For mated females (with or without-oviposition), one female was placed in a glass culture tube with a streak of honey, and five males were provided for 24 h and then the males were removed. The females were retained in the tubes (without oviposition) or transferred individually to clear plastic 500 ml deli cups and provided mealybugs on potatoes (with oviposition). The number of days each parasitoid lived was counted for both males and females in all conditions. There were 50 replicates for each gender and mating condition. These procedures were repeated for all three parasitoid species.

**Lifetime Fertility.** Lifetime fertility of each parasitoid species was studied using two mating conditions (mated and unmated). A newly emerged virgin female was either held alone or allowed to mate with five newly emerged males for 24 h in a glass culture tube provided with a streak of honey and water. After removing the males, the females were transferred individually to clear plastic 3.8 liter round jars (Rubbermaid, Newell Rubbermaid Inc., Atlanta, GA). Each jar was covered with a piece of chiffon cloth material. A 9-cm-diameter area was removed from the lid to allow air circulation. Unmated females were also transferred individually to clear plastic jars as mated females. Each mated or unmated female was provided ≈300 third-instar female mealybugs on 1–2 infested potatoes daily for oviposition until the death of the females. The potatoes with parasitized mealybugs were placed in clear plastic 500 ml deli cups as above to allow mummification. When the parasitoids started to emerge, the number of males and females were counted. For each parasitoid species, 25 replicates were used for each mating condition.

**Statistical Analyses.** A completely randomized design (CRD) was used for all experiments. A two-way analysis of variance (ANOVA) was performed using a general linear model (PROC GLM; SAS Institute 1999) to find interaction between parasitoids and mealybug instar for developmental time, and parasitoids and mating conditions for the longevity, the lifetime fertility (male and cumulative progeny) and the reproductive period. Means were compared at $P = 0.05$ significance level using least square means (PROC LSMEANS; SAS Institute 1999). One-way ANOVA was performed using a general linear model (GLM) for the number of female progeny and the sex ratio. Means were compared at $P = 0.05$ significance level using the Tukey's honestly significant difference (HSD) test. The proportion of female (sex ratio) was square-root arcsine-transformed by using

$$p' = \arcsin \sqrt{p} \qquad [1]$$

where, $p$ = proportion of female, to adjust the variances (Zar 1984) before ANOVA.

**Voucher Specimens.** Voucher specimens of P. *marginatus*, *A. papayae*, *A. loecki*, and *P. mexicana* were deposited in the Entomology and Nematology Department insect collection at Tropical Research and Education Center (TREC), University of Florida, Homestead, FL.

## Results

**Developmental Time.** There was no significant interaction between parasitoid and host stage ($F = 0.04$; df 10, 882; $P = 1.0000$). The developmental time of both male and female *A. papayae* (13.1–13.8 and 14.1–14.8 d) and *A. loecki* (13.1–13.7 and 14.1–14.7 d) was shorter than the developmental time of male and female *P. mexicana* (21.0–21.8 and 22.1–22.9 d), respectively (Table 1). All three parasitoids had a shorter developmental time in the adult-female mealybugs than in the second instars. Male parasitoids had a shorter developmental time than their females (Table 1).

**Longevity.** There was significant interaction between parasitoid and mating status for longevity ($F = 5.15$; df 10, 882; $P = < 0.0001$). Both male (45.9–47.5 d) and female (40.1–63.1 d) *P. mexicana* lived longer than the male (22.0–23.3 and 36.6–37.3 d) and female (13.9–33.1 and 23.0–48.9 d) *A. papayae* or *A. loecki*, respectively (Table 2). *A. papayae* had the shortest

**Table 1. Mean developmental time (egg to adult eclosion) in days (±SEM) for male and female *A. papayae*, *A. loecki*, and *P. mexicana* reared in second instar, third-instar female, and adult-female *P. marginatus***

| Parasitoid | Stage of *P. marginatus* | | |
|---|---|---|---|
| | Second instar | Third-instar female | Adult female |
| *A. papayae* (male) | 13.8 ± 0.2dA[a] | 13.5 ± 0.2dAB | 13.1 ± 0.2dB |
| *A. loecki* (male) | 13.7 ± 0.2dA | 13.4 ± 0.2dAB | 13.1 ± 0.3dB |
| *P. mexicana* (male) | 21.8 ± 0.2bA | 21.5 ± 0.2bAB | 21.0 ± 0.2bB |
| *A. papayae* (female) | 14.8 ± 0.2cA | 14.5 ± 0.2cAB | 14.1 ± 0.2cB |
| *A. loecki* (female) | 14.7 ± 0.2cA | 14.4 ± 0.2cAB | 14.0 ± 0.2cB |
| *P. mexicana* (female) | 22.9 ± 0.2aA | 22.7 ± 0.2aAB | 22.1 ± 0.3aB |
| ANOVA results | | | |
| Source | *F* | df | *P* |
| Model | 319.84 | 17, 882 | <0.0001 |
| Parasitoid | 1080.58 | 5, 882 | <0.0001 |
| Stage | 17.01 | 2, 882 | <0.0001 |
| Parasitoid*stage | 0.04 | 10, 882 | 1.0000 |

$n = 50$ for each parasitoid species and stage of *P. marginatus*.
[a] Means within a column followed by the same lowercase letters, and means within a row followed by the same uppercase letters are not significantly different at $\alpha = 0.05$ (Least Square Means Test).

longevity. Mating status had no effect on the longevity of males. Both mated and unmated females that were not allowed to oviposit had similar longevity and lived longer than the females that were allowed to oviposit. Both unmated and mated females that were allowed to oviposit also had similar longevity.

**Lifetime Fertility.** Unmated females produced male only progeny in all three parasitoids ($F = 73.4$; df 5, 144; $P = < 0.0001$) (Table 3). For mated females, the progeny comprised of both males and females ($F = 199.88$; df 2, 72; $P = < 0.0001$). *A. loecki* (173.2–197.6) and *P. mexicana* (159.5–208.9) produced more progeny than *A. papayae* (88.0–92.8) for both mating conditions ($F = 71.17$; df 5, 144; $P = < 0.0001$). All species had similar progeny sex ratios with ≈50% of males and females. The reproductive period was the longest for *P. mexicana* (30.8–31.6 d), and the shortest was for *A. papayae* (11.9–13.9 d) ($F = 118.28$; df 5, 144; $P = < 0.0001$).

**Table 2. Mean longevity in days (±SEM) for male (unmated and mated), and female (unmated and mated with or without oviposition) *A. papayae*, *A. loecki*, and *P. mexicana***

| Mating status of male and female parasitoids | Longevity (days) | | |
|---|---|---|---|
| | Parasitoid | | |
| | *A. papayae* | *A. loecki* | *P. mexicana* |
| Unmated male | 23.3 ± 0.4bC[a] | 37.3 ± 0.7bB | 47.5 ± 1.8cA |
| Mated male | 22.0 ± 0.4bC | 36.6 ± 0.5bB | 45.9 ± 0.9cA |
| Unmated female without oviposition | 33.1 ± 0.6aC | 48.9 ± 1.0aB | 63.1 ± 1.8aA |
| Unmated female with oviposition | 13.8 ± 0.2cC | 23.9 ± 0.5cB | 41.1 ± 0.7dA |
| Mated female without oviposition | 32.3 ± 1.0aC | 47.6 ± 1.2aB | 58.4 ± 1.2bA |
| Mated female with oviposition | 13.9 ± 0.3cC | 23.0 ± 0.4cB | 40.1 ± 0.7dA |
| ANOVA results | | | |
| Source | *F* | df | *P* |
| Model | 250.54 | 17, 882 | <0.0001 |
| Parasitoid | 1264.68 | 2, 882 | <0.0001 |
| Mating status | 335.66 | 5, 882 | <0.0001 |
| Parasitoid* mating status | 5.15 | 10, 882 | <0.0001 |

$n = 50$ for each parasitoid species and mating status.
[a] Means within a column followed by the same lowercase letters, and means within a row followed by the same uppercase letters are not significantly different at $\alpha = 0.05$ (Least Square Means Test).

## Discussion

Information on biology and life history of a parasitoid is important when evaluating its efficiency and understanding its long-term effects in a system (Hemerik and Harvey 1999). Life history parameters are also important when comparing establishment of several parasitoid species that have been released in a classical biological control program to manage a single host (Hemerik and Harvey 1999). There were differences in the developmental time, longevity, and lifetime fertility of *A. papayae*, *A. loecki*, and *P. mexicana.* The differences in these fitness parameters are important when evaluating the efficiency of these three introduced parasitoids as classical biological control agents of *P. marginatus* and explaining the results obtained in the field efficiency and establishment studies carried out in the United States (Amarasekare et al. 2009).

Short generation time is one of the desirable characters of a parasitoid (Greathead 1986). Generally, the developmental time of a biological control agent should be shorter than the developmental time of the host (Greathead 1986). Then the parasitoid can produce its progeny at a faster rate than the host and can parasitize the host population in a shorter time (Greathead 1986). The adult female *P. marginatus* can develop on hibiscus in 25.9 d, and the second instars and the third-instar females can emerge within 15.2 and 20.8 d, respectively (Amarasekare et al. 2008). Shorter developmental times of *A. papayae* and *A. loecki* (compared with their host's longer developmental time) may have contributed to their recovery and establishment in the field. Although, *A. loecki* was recovered from *P. marginatus* and was established in release areas, its multiple host preference (Noyes 2000) may have caused the lower efficiency (Amarasekare et al. 2009). Development time of *P. mexicana* is longer than the developmental time of the other two parasitoids and is similar to the development time of the host. This makes *P. mexicana* less efficient in controlling *P. marginatus* than the other two parasitoid species and may have contributed to its unsuccessful recovery in the field.

Lifetime fertility or progeny production of a parasitoid is important in its long-term establishment as a biological control agent (King 1987). A parasitoid with higher lifetime fertility with female-biased progeny can parasitize a higher number of hosts (King 1987). In this study, the smallest of the three species (Noyes 2000, Noyes and Schauff 2003), *A. papayae* produced the least progeny compared with *A. loecki* and *P. mexicana*, both of which produced twice the progeny of *A. papayae*, however, all three parasitoids had progeny sex ratios with approximately similar proportions of males and females.

**Table 3. Mean (±SEM) no. of male and female progeny, cumulative progeny, sex ratio, and reproductive period of mated and unmated *A. papayae*, *A. loecki*, and *P. mexicana***

| Mating status | Parasitoid | Number of male progeny | Number of female progeny | Cumulative progeny | Sex ratio (proportion of females) | Reproductive period (days) |
|---|---|---|---|---|---|---|
| Mated | *A. papayae* | 44.5 ± 1.0b[a] | 48.3 ± 1.2b[b] | 92.8 ± 1.9b | 0.52 ± 0.006 | 13.9 ± 0.7c |
| | *A. loecki* | 97.8 ± 1.3a | 99.8 ± 1.6a | 197.6 ± 2.5a | 0.51 ± 0.004 | 20.1 ± 0.7b |
| | *P. mexicana* | 103.0 ± 3.4a | 105.9 ± 3.3a | 208.9 ± 6.6a | 0.50 ± 0.013 | 30.8 ± 0.9a |
| Unmated | *A. papayae* | 88.0 ± 2.97b | — | 88.0 ± 2.97b | — | 11.9 ± 0.6c |
| | *A. loecki* | 173.2 ± 10.2a | — | 173.2 ± 10.2a | — | 18.3 ± 0.7b |
| | *P. mexicana* | 159.5 ± 7.7a | — | 159.5 ± 7.7a | — | 31.6 ± 0.9a |
| | *F* | 73.40 | 199.88 | 71.17 | 2.99 | 118.28 |
| | df | 5, 144 | 2, 72 | 5, 144 | 2, 72 | 5, 144 |
| | *P* | <0.0001 | <0.0001 | <0.0001 | 0.0566 | <0.0001 |

$n = 25$ for each parasitoid species and mating status.

[a] Means within a column followed by the same lowercase letters for no. of male progeny, cumulative progeny, and reproductive period are not significantly different at $\alpha = 0.05$ (Least Square Means Test).

[b] Means within a column followed by the same lowercase letters for no. of female progeny and sex ratio are not significantly different at $\alpha = 0.05$ (Tukey honestly significance difference Test).

Body size of a parasitoid is frequently related to fecundity, longevity, and host finding ability (Hemerik and Harvey 1999). Results observed for longevity in this study showed that (irrespective of mating status) the smallest parasitoid (*A. papayae*) had the shortest longevity for both males and females compared with longevity of *A. loecki* and *P. mexicana.* Females of all three parasitoid species outlived males. This has been recorded in other parasitoids species such as *Anagyrus kamali* Moursi (Sagarra et al. 2000). In this study, smaller body size, lower progeny production, and shorter longevity may not have contributed to the field efficiency of *A. papayae* although there may be a relationship between body size and fitness parameters for parasitoid efficiency in other species. A significant relationship between size and both longevity and lifetime fecundity was found in fitness parameter studies in *Trichogramma evanescens*, a gregarious, polyphagus egg parasitoid (Doyon and Boivin 2005).

In this study, the progeny produced by all three mated parasitoids consisted with both males and females while unmated females produced male only progeny. Mating is required to achieve their full reproductive potential in some parasitoids and it is energy and time-consuming activity in insects, which can affect the outcome of the longevity, lifetime fecundity, and progeny production of hymenopteran parasitoids (Ridley 1988). The progeny sex ratio is the main fitness parameter that can be affected by mating. The majority of parasitoids need to mate once to attain their optimal sex ratio (Ridley 1993).

Increasing host age had affected the developmental time of *A. papayae*, *A. loecki*, and *P. mexicana.* All three parasitoids had a shorter developmental time in adult females than in the second instar mealybugs. In koinobiont parasitoids that consume the entire host before pupation, adult parasitoid size and developmental time are often strongly correlated with host size at the time when the host is developmentally arrested through destructive feeding by the parasitoid larva (Hemerik and Harvey 1999). Although a specific stage or stages of a mealybug are preferred by a parasitoid for oviposition, all or most of its stages can be susceptible to oviposition and subsequent parasitoid development. Parasitoids that develop in early instar mealybugs have a tendency to produce male progeny compared with those that develop in the late instars, in which they can produce more female progeny (Charnov et al. 1981, Sagarra and Vincent 1999). When developed in second-instar *P. marginatus*, *A. loecki*, and *P. mexicana* produce male-biased progenies and *A. papayae* produces female-biased progeny (Amarasekare et al. 2010). In addition to its shorter developmental time, the ability of *A. papayae* to produce a female-biased progeny in second instar hosts may have contributed to its superior characteristics shown in efficiency and establishment studies in the field.

Differences in the developmental time, longevity, and lifetime fertility of *A. papayae*, *A. loecki*, and *P. mexicana* are important in evaluating their efficiency as introduced parasitoids of *P. marginatus.* The shorter developmental time of *A. papayae* and *A. loecki* compared with the developmental time of *P. mexicana* makes *A. papayae* and *A. loecki* more suitable for *P. marginatus* control than *P. mexicana.* In this study, we did not see any outstanding contribution from the fitness parameters such as life time fertility or longevity of females to the field recovery and establishment of *A. papayae*, *A. loecki*, and *P. mexicana.* This information provides the insight needed to clarify the efficiency of *A. papayae* in controlling *P. marginatus* as well as to explain the lower efficiency of *A. loecki.* This information is important in understanding the inability of field recovery of *P. mexicana.*

## Acknowledgments

This study was conducted in partial fulfillment of the requirement for a Ph.D. at the University of Florida, by K. G. Amarasekare. We thank R. McSorley and L. Osborne of the University of Florida for reviewing an early draft of this paper; M. Brennan, IFAS Statistics, University of Florida, for her help with statistical analyses; D. M. Amalin of the University of Florida for providing the mealybug and parasitoid

information; M. Gates (Encyrtidae) and G. Evans (Pseudococcidae) of Systematic Entomology Laboratory, USDA–ARS for insect identification; A. Roda of USDA–APHIS and the Biological Control Laboratory, the Department of Agriculture, Puerto Rico for providing the initial parasitoid colonies; and the USDA–ARS for funding for this experiment.

## References Cited

**Amarasekare, K. G., C. M. Mannion, L. S. Osborne, and N. D. Epsky. 2008.** Life history of *Paracoccus marginatus* (Hemiptera: Pseudococcidae) on four host plant species under laboratory conditions. Environ. Entomol. 37: 630–635.

**Amarasekare, K. G., C. M. Mannion, and N. D. Epsky. 2009.** Efficiency and establishment of three introduced parasitoids of the mealybug *Paracoccus marginatus* (Hemiptera: Pseudococcidae). Biol. Control 51: 91–95.

**Amarasekare, K. G., C. M. Mannion, and N. D. Epsky. 2010.** Host instar susceptibility and selection and interspecific competition of three introduced parasitoids of the mealybug *Paracoccus marginatus* (Hemiptera: Pseudococcidae). Environ. Entomol. 39: 1506–1512.

**Charnov, E. L., R.L.L. Hartogh, W. T. Jones, and J. van den Assem. 1981.** Sex ratio evolution in a variable environment. Nature 289: 27–33.

**Doyon, J., and G. Boivin. 2005.** The effect of development time on the fitness of female *Trichogramma evanescens.* J. Insect Sci. 5: 1–5.

**Greathead, D. J. 1986.** Parasitoids in classical biological control, pp. 290–315. *In* J. Waage and D. Greathead (eds.), Insect Parasitoids. Academic Inc., Orlando, FL.

**Heu, R. A., M. T. Fukada, and P. Conant. 2007.** Papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae). New Pest Advisory. 4(3). Department of Agriculture, Honolulu, HI.

**Hemerik, L., and J. A. Harvey. 1999.** Flexible larval development and the timing of destructive feeding by a solitary endoparasitoid: an optimal foraging problem in evolutionary perspective. Ecol. Entomol. 24: 308–315.

**King, B. H. 1987.** Offspring sex ratios in parasitoid wasps. Q. Rev. Biol. 62: 367–396.

**Meyerdirk, D. E., R. Muniappan, R. Warkentin, J. Bamba, and G.V.P. Reddy. 2004.** Biological control of the papaya mealybug, *Paracoccus marginatus* (Hemiptera: Pseudococcidae) in Guam. Plant Prot. Quart. 19: 110–114.

**Miller, D. R., and G. L. Miller. 2002.** Re-description of *Paracoccus marginatus* Williams Granara de Willink (Hemiptera: Pseudococcidae) including descriptions of the immature stages and adult male. Proc. Entomol. Soc. Wash. 104: 1–23.

**Miller, D. R., D. J. Williams, and A. B. Hamon. 1999.** Notes on a new mealybug (Hemiptera: Coccoidea: Pseudococcidae) pest in Florida and the Caribbean: the papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink. Insecta Mundi. 13: 179–181.

**Muniappan, R., D. E. Meyerdirk, F. M. Sengebau, D. D. Berringer, and G.V.P. Reddy. 2006.** Classical biological control of the papaya mealybug, *Paracoccus marginatus* (Hemiptera: Pseudococcidae) in the republic of Palau. Fla. Entomol. 89: 212–217.

**Ngyuen, R. 2000.** Crawford announces new biological control of plant pest. Department Press Release 10–10-2000. Division of Plant Industry. Florida Department of Agriculture and Consumer Services, Gainesville, FL.

**Noyes, J. S. 2000.** Encyrtidae of Costa Rica, I. The subfamily Tetracneminae (Hymenoptera: Chalcidoidea), parasitoids of mealybugs (Homoptera: Pseudococcidae). Mem. Am. Entomol. Inst. (Gainesville) 62: 101–103.

**Noyes, J. H., and M. E. Schauff. 2003.** New Encyrtidae (Hymenoptera) from papaya mealybug (*Paracoccus marginatus* Williams and Granara de Willink) (Hemiptera: Sternorrhyncha: Pseudococcidae). Proc. Entomol. Soc. Wash. 105: 180–185.

**Ridley, M. 1988.** Mating frequency and fecundity in insects. Biol. Rew. 63: 509–549.

**Ridley, M. 1993.** A sib competitive relation between clutch size and mating frequency in parasitic Hymenoptera. Am. Nat. 142: 893–910.

**Sagarra, L. A., and C. Vincent. 1999.** Influence of host stage on oviposition, development, sex ratio, and survival of *Anagyrus kamali* Moursi (Hymenoptera: Encyrtidae), a parasitoid of the hibiscus mealybug, *Maconellicoccus hirsutus* Green (Homoptera: Pseudococcidae). Biol. Control 15: 51–56.

**Sagarra, L. A., C. Vincent, and R. K. Stewart. 2000.** Fecundity and survival of *Anagyrus kamali* (Hymenoptera: Encyrtidae) under different feeding and storage temperature. Eur. J. Entomol. 97: 177–181.

**SAS Institute. 1999.** SAS user's guide, version 9.1. SAS Institute. Cary, NC.

**Walker, A., M. Hoy, and D. Meyerdirk. 2006.** Papaya mealybug (*Paracoccus marginatus* Williams and Granara de Willink (Insecta: Hemiptera:Pseudococcidae)). EENY-302. Featured Creatures. Entomology and Nematology Department. Florida Cooperative Extension Service. Institute of Food and agricultural Sciences. University of Florida, Gainesville, FL.

**Zar, J. H. 1984.** Biostatistical analysis. Prentice Hall, Englewood Cliffs, NJ.

*Received 1 March 2011; accepted 17 May 2012.*