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Life History of *Paracoccus marginatus* (Hemiptera: Pseudococcidae) on Four Host Plant Species Under Laboratory Conditions

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ABSTRACT Life history of the mealybug, *Paracoccus marginatus* Williams and Granara de Willink, on three ornamental plants [*Hibiscus rosa-sinensis* L., *Acalypha wilkesiana* (Muell.-Arg.), and *Plumeria rubra* L.] and one weed species (*Parthenium hysterophorus* L.) was studied under laboratory conditions. Mealybugs were able to develop, survive, and reproduce on all four hosts; however, there were differences in the life history parameters. Adult females that developed on acalypha and parthenium emerged ≈ 1 d earlier than those that developed on hibiscus and plumeria. Adult males had a longer developmental time on plumeria than on the other hosts. Survival of first- and second-instar nymphs and cumulative adult survival were lowest on plumeria. Longevity was not affected by hosts for males and females and averaged 2.3 ± 0.1 and 21.2 ± 0.1 d, respectively. On plumeria, $58.9 \pm 1.7\%$ of the adults were females, which was a higher female percentage than on the other hosts. No egg production occurred in virgin females. Prereproductive and reproductive periods of the females were not affected by hosts and averaged 6.3 ± 0.1 and 11.2 ± 0.1 d, respectively. Mean fecundity of 186.3 ± 1.8 eggs on plumeria was lower than on the other three plant species. Life history parameters of *P. marginatus* on hibiscus, acalypha, plumeria, and parthenium show its ability to develop, survive, and reproduce on a wide variety of plant species.

KEY WORDS development, hibiscus, plumeria, acalypha, parthenium

The mealybug, *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae), is a polyphagous insect and a pest of various tropical fruits, vegetables, and ornamental plants (Miller and Miller 2002). Its host range includes *Carica papaya* L. (papaya), *Citrus* spp. L. (citrus), *Persea americana* P. Mill. (avocado), *Solanum melongena* L. (eggplant), *Hibiscus* spp. L. (hibiscus), *Plumeria* spp. L. (plumeria), and *Acalypha* spp. L. (acalypha) (Miller and Miller 2002). *P. marginatus* was first described by Williams and Granara de Willink (1992) and redescribed by Miller and Miller (2002). *P. marginatus* was originally reported from the neotropical regions in Belize, Costa Rica, Guatemala, and Mexico (Williams and Granara de Willink 1992). This species was introduced to the Caribbean in the early 1990's and spread among many of the Caribbean islands by 1994 (Walker et al. 2006). In 1998, *P. marginatus* was first reported in the United States in Florida, in Palm Beach, Manatee, and Broward counties, on hibiscus (Miller et al. 1999). There-

after, it was recorded in several other counties in Florida from >25 genera of plants (Walker et al. 2006). Heavy infestations of *P. marginatus* on papaya were recorded in Guam in 2002 (Meyerdirk et al. 2004, Walker et al. 2006) and in the Republic of Palau in 2003 (Muniappan et al. 2006, Walker et al. 2006). In 2004, *P. marginatus* was reported in Hawaii on papaya, plumeria, hibiscus, and *Jatropha* spp. L. (Heu et al. 2007).

Since its introduction to the Caribbean, the United States, and the Pacific islands, *P. marginatus* has established in most of the Caribbean islands, Florida, Guam, the Republic of Palau, and Hawaii. *P. marginatus* potentially poses a threat to numerous agricultural products in the United States, especially in Florida, and states such as California and Hawaii, which produce similar crops. The southern part of Texas, where the country's third largest citrus production exists (CNAS 2007), is also susceptible to invasion by *P. marginatus*.

The life history of *P. marginatus* has not been studied. Understanding the life history of a pest insect is important in predicting its development, emergence, distribution, and abundance. Life history information also plays an important role in pest management, especially when applying chemical and biological control methods. Because there is a high possibility of *P. marginatus* invading other areas in the United States, it is important to study its life history using host plant

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species that are either widely grown in the susceptible areas or container-grown ornamental plant species that are commonly transported to these areas. In this study, three ornamental plants *Hibiscus rosa-sinensis* L. (hibiscus), *Plumeria rubra* L. (plumeria), *Acalypha amentacea* Roxb. ssp. *wilkesiana* (Muell.-Arg.) cultivar *Marginata* (acalypha), and one weed species, *Parthenium hysterophorus* L. (parthenium), were selected to study the life history of *P. marginatus*. These four plant species were previously recorded as host plants of *P. marginatus* (Miller and Miller 2002) and are widely grown in many parts of the United States.

Materials and Methods

Rearing Mealybugs. Red potatoes (*Solanum tuberosum* L.) (Ryan Potato Company, East Grand Forks, MN) were allowed to sprout and were used in rearing a colony of *P. marginatus*. Potatoes were soaked in a 1% solution of bleach (Clorox; The Clorox Co., Oakland, CA; 6% sodium hypochlorite) for 15 min and rinsed with water, air-dried, and placed in bags made from black cotton cloth to encourage sprouting. Bags were kept inside a dark room at $27 \pm 1^\circ\text{C}$ and $65 \pm 2\%$ RH. Each week, 30 newly sprouted potatoes were infested with ovisacs of *P. marginatus* to maintain the colony. Each sprouted potato was infested with three to five ovisacs, depending on the size of the potato and ovisacs. Infested potatoes were kept in 3.8-liter plastic containers at the rate of 10 per container (Rubbermaid; Newell Rubbermaid, Atlanta, GA). Air circulated in the container through screen material (Amber Lumite; Bio Quip, Gardena, CA) glued to a hole in the lid. The mealybug colony was kept at $25 \pm 1^\circ\text{C}$, $65 \pm 2\%$ RH, and a photoperiod of 12:12 (L:D) in an environmental growth chamber (Percival I-36LL; Percival Scientific, Perry, NC).

Eggs to be used in the studies were obtained from gravid females identified by a body length (2–2.5 mm), which is approximately twice the size of newly emerged virgin females (1.1–1.3 mm). To obtain eggs, gravid females from the colony (each from a different infested potato) were placed individually on newly sprouted potatoes.

Development and Survival. Preliminary studies showed that it takes approximately 1 mo for eggs of *P. marginatus* to hatch and develop into adults. Therefore, it was necessary that each host plant maintain leaves for this time period. Use of tender leaves could avoid leaf senescence during this time. Hibiscus cuttings can root within 2–3 wk time in water, maintaining tender leaves. Even after 30 d, acalypha cuttings were not rooted. The use of rooting hormones could have accelerated the process of rooting. However, because the impact of rooting hormones on the development of insects is not known, fresh cuttings were used. Cuttings obtained from parthenium, a soft herbaceous plant, were unable to survive 30 d in water. When parthenium plants with intact root system were used, the leaves were able to withstand this period. Plumeria cuttings were able to survive >30 d without leaf senescence with the provision of daily hydration

through a ball of cotton tied around the cut end of the plumeria terminal. During this time, new leaves grew from the shoots indicating that these shoots were continuously growing and alive.

All plant material was collected and prepared 24 h before the experiment. Hibiscus cuttings were obtained from 1-yr-old container-grown hibiscus maintained in a shadehouse. Acalypha and plumeria cuttings were obtained from plants in the landscape on TREC premises. Parthenium seedlings were collected from the field. A fully expanded young leaf with a stem 4 cm long was used for each replicate of hibiscus and acalypha. For parthenium, a whole plant ≈ 8 cm in height with an intact root system was used as each replicate. A tender leaf was selected from each parthenium plant, and the remaining leaves were removed. For plumeria, a 5-cm-long terminal shoot with one tender leaf was selected as each replicate.

Host tissue was placed in arenas (9-cm-diameter petri dish with a small hole in the bottom for hibiscus, acalypha, and parthenium; 18-cm-diameter petri dish for plumeria). The stem of each leaf of hibiscus and acalypha was inserted through the hole, and the lid was placed on the petri dish. For parthenium, the main stem of the plant was inserted through the hole in the petri dish until the leaf was completely placed inside the petri dish. Each petri dish was kept on a 162-ml translucent plastic soufflé cup (Georgia Pacific Dixie, Atlanta, GA) filled with distilled water into which the stem was submerged. For plumeria, each terminal shoot was hydrated using a ball of cotton tied to the cut end of the shoot and moistened daily with distilled water.

Eggs collected from a single female were placed on the leaves of all four hosts with 10 eggs per leaf using a paintbrush (No. 000; American Painter 4000, Loew-Cornell, Englewood Cliffs, NJ). Eggs were collected within 24 h of oviposition. Petri dishes were kept in an environmental growth chamber as above. Dishes were checked daily for egg hatch and shed exuviae. The number of days to egg hatch and emergence, survival of each instar, and number of emerging adult males and females were recorded. Developmental time and the survival of eggs and first instars were not separated by sex. The sex of each individual mealybug was determined during the latter part of the second instar when males change their color from yellow to pink. At this point, the developmental times of males and females were recorded separately. For each plant species, 35 petri dishes (replicates) each with 10 eggs were used. This experiment was repeated twice for a total of 105 replicates.

Reproduction. Newly emerged virgin females obtained from the development study on each plant species were used to assess reproduction. Virgin females were placed individually in petri dishes with either a leaf or a terminal shoot of each plant species prepared as mentioned above. Females were held alone to assess asexual reproduction or were provided with three newly emerged males from same plant species for sexual reproduction. The date oviposition began, the number of eggs laid, and adult mortality

Table 1. Mean no. of days (\pm SEM) for each developmental stadium of *P. marginatus* reared on four host species

Host	Stadia								Cumulative	
	Egg	First	Second		Third		Fourth	Male	Female	
			Male	Female	Male	Female	Male			
Acalypha	8.6 \pm 0.1b	5.9 \pm 0.1c	6.5 \pm 0.1b	3.8 \pm 0.1c	2.8 \pm 0.1b	6.3 \pm 0.1a	4.5 \pm 0.1a	28.4 \pm 0.1bA	24.5 \pm 0.1bB	
Hibiscus	8.4 \pm 0.1c	6.2 \pm 0.1b	6.8 \pm 0.1b	5.0 \pm 0.1b	2.3 \pm 0.1c	5.9 \pm 0.1b	3.9 \pm 0.1b	27.6 \pm 0.1cA	25.5 \pm 0.1aB	
Parthenium	8.8 \pm 0.1a	5.8 \pm 0.1c	5.6 \pm 0.1c	5.2 \pm 0.1ab	3.4 \pm 0.1a	4.7 \pm 0.1d	4.1 \pm 0.1b	27.7 \pm 0.1cA	24.4 \pm 0.1bB	
Plumeria	8.5 \pm 0.1b	6.6 \pm 0.1a	9.6 \pm 0.1a	5.3 \pm 0.1a	2.7 \pm 0.1b	5.1 \pm 0.1c	2.6 \pm 0.1c	30.0 \pm 0.1aA	25.5 \pm 0.1aB	
<i>F</i>	25.44	63.78	358.88	122.56	32.37	109.51	128.68	239.96	74.78	
<i>df</i>	3,416	3,416	3,413	3,416	3,415	3,416	3,415	3,415	3,416	
<i>P</i>	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	

n = 105.

Sex could not be determined before the second instar.

Means within a column followed by the same letters are not significantly different at α = 0.05 (Tukey's HSD test).

Means within a row followed by the same letters are not significantly different at α = 0.05 (Least Square Means (LSMEANS) Test) for cumulative males and females.

were recorded. For each of the two treatments (sexual and asexual), 35 females were used, and each female was considered a replicate. This experiment was repeated twice using newly emerged males and females collected from developmental time experiments for a total of 105 replicates.

Statistical Analysis. The experimental design was completely random for all experiments. The 10 eggs or mealybugs in each petri dish were considered as a single unit/replicate, and the mean of the response variable was calculated and used in subsequent analyses in all experiments. One-way analysis of variance (ANOVA) was performed using a general linear model (GLM) for all experiments (SAS Institute 1999). Means were compared at the P = 0.05 significance level using the Tukey's honestly significant difference (HSD) test. Mean developmental times for cumulative male and female were compared using least square means (LSMEANS) test. Data for proportions of females (sex ratio) and survival were arcin square-root transformed, when necessary before ANOVA (Zar 1984).

Voucher Specimens. Voucher specimens of *P. marginatus* were deposited in the Entomology and Nematology Department insect collection, at the Tropical Research and Education Center, University of Florida.

Results

Development. There were differences in the developmental times of *P. marginatus* reared on four host species (Table 1). Adult females emerged \approx 1 d earlier on acalypha and parthenium than on hibiscus and plumeria. Adult males had longer developmental time on acalypha and plumeria than on parthenium and hibiscus (Table 1). Males had longer cumulative developmental time than females (F = 771.6; *df* = 7,832; P < 0.0001) on all host plants.

Survival. Eggs survived similarly on all four plants (Table 2). The lower survival of the first and second instars on plumeria was reflected in the cumulative adult survival on plumeria. Survival for the third-instar males and females and the fourth-instar males was not affected by the host species (Table 2).

Proportion of Females and Adult Longevity. The sex ratio of adults emerging on plumeria was more female-biased than on the other three hosts (F = 8.15; *df* = 3,416; P < 0.0001). The mean proportion of adult females ranged from 53 to 59% (acalypha: 53.9 \pm 1.3, hibiscus: 53.7 \pm 1.1, parthenium: 53.4 \pm 1.0, plumeria: 58.9 \pm 1.7). No difference in adult longevity of males (F = 0.69; *df* = 3,416; P = 0.5562) and females (F = 0.52; *df* = 3,416; P = 0.6659) occurred among the hosts. Mean longevity of adult males and females was 2.3 \pm 0.1 and 21.2 \pm 0.1 d, respectively.

Table 2. Mean \pm SEM percent survival (%) for each developmental stadium of *P. marginatus* reared on four host species

Host	Survival (%)						
	Egg	First	Second	Third		Fourth	Cumulative
				Male	Female	Male	
Acalypha	82.8 \pm 0.7	83.2 \pm 0.9a	89.4 \pm 1.1a	89.6 \pm 1.4	89.3 \pm 1.4	89.7 \pm 1.6	49.9 \pm 0.8a
Hibiscus	83.3 \pm 0.6	82.7 \pm 0.9a	89.4 \pm 1.0a	89.8 \pm 1.5	89.0 \pm 1.5	89.7 \pm 1.6	50.4 \pm 0.8a
Parthenium	83.5 \pm 0.6	82.7 \pm 0.9a	89.1 \pm 0.9a	89.5 \pm 1.4	89.7 \pm 1.4	89.6 \pm 1.5	50.1 \pm 0.7a
Plumeria	82.2 \pm 0.7	58.4 \pm 1.4b	64.9 \pm 1.7b	84.5 \pm 2.3	81.8 \pm 2.4	81.4 \pm 3.5	20.0 \pm 0.5b
<i>F</i>	0.89	73.44	58.67	0.42	1.55	0.48	379.44
<i>df</i>	3,416	3,416	3,416	3,416	3,416	3,416	3,416
<i>P</i>	0.4475	<0.0001	<0.0001	0.7398	0.1998	0.6955	<0.0001

n = 105.

Means within a column followed by the same letters are not significantly different at α = 0.05 (Tukey's HSD test).

Reproduction. Virgin females did not lay eggs on any of the four plant species. The females reared on plumeria laid a lower number of eggs (186.3 ± 1.8) than did females reared on hibiscus (244.4 ± 6.8), acalypha (235.2 ± 3.5), and parthenium (230.2 ± 5.3 ; $F = 29.9$; $df = 3,416$; $P = <0.0001$). The mean pre-oviposition (6.3 ± 0.1 d) and oviposition periods (11.2 ± 0.1 d) were not affected by the host species ($F = 0.23$; $df = 3,416$; $P = 0.8739$ and $F = 0.12$; $df = 3,416$; $P = 0.9496$, respectively).

Discussion

Determining the life history of an insect is important to understand its development, distribution, and abundance. In polyphagous insects, life history can vary with the plant species it feeds on. There were differences in the life history parameters of *P. marginatus* reared on four plant species; however, *P. marginatus* was able to develop, survive, and reproduce on all four plants. Different plant species provide different nutritional quality and chemical constituents, which can affect the development, reproduction, and survival of an insect. The differences observed in the life history of *P. marginatus* may be caused by nutritive factors, allelochemical compounds, and physical differences in leaf structures, although none of these factors were studied for *P. marginatus* in this study. Use of different host plant growing conditions may have confounded the results but preliminary studies found that these were the best ways to maintain each host in a condition suitable for the tests.

Different host plant species have been shown to affect the life history parameters of other mealybug species. Longer prereproductive period and a higher progeny production were observed for *Rastrococcus invadens* Williams reared on different varieties of *Mangifera indica* L. (Boavida and Neuenschwander 1995). Mortality of the of citrus mealybug *Planococcus citri* (Risso) was higher on green than on red or yellow variegated *Coleus blumei* 'Bellevue' (Bentham) plants, and development was faster and fecundity higher on red variegated plants (Yang and Sadof 1995). The developmental time of female *Planococcus kraunhiae* (Kuwana) was shorter when reared on germinated *Vicia faba* L. seeds than on leaves of a *Citrus* sp. L. and on *Cucurbita maxima* Duchesne, and it survived better when reared on germinated *V. faba* seeds than on citrus leaves (Narai and Murai 2002). The pink hibiscus mealybug, *Macronellacoccus hirsutus* (Green), was able to develop equally well on *Cucurbita pepo* L. as on *C. maxima* (Serrano and Lapointe 2002). There was no difference in survival, development, and fecundity of cohorts of the mealybug, *Phenacoccus parvus* Morrison, when reared on *Lantana camara* L., *Lycopersicon esculentum* Miller, and *Solanum melongena* L. (Marohasy 1997). However, *Gossypium hirsutum* L., *Ageratum houstonianum* Miller, and *Clerodendrum cunninghamii* Benth were identified as less suitable host plants for the development of *P. parvus* compared with *L. camara* (Marohasy 1997).

Although, the eggs of *P. marginatus* on plumeria hatched in a similar manner to the eggs on other three plant species, survival of the first- and second-instar nymphs was reduced on plumeria. Stickiness observed on plumeria leaves may have contributed to this low survival. This stickiness may have resulted from the experimental conditions and hydration methods used in this experiment. In the Republic of Palau, *P. marginatus* has caused serious damage to plumeria (Muniappan et al. 2006), and it is found to be the most common homopteran found on *P. alba* L. in Puerto Rico (Sloan et al. 2007), indicating its ability to develop well on this plant species. A loss of 17–18% of the first instars was also observed on hibiscus, acalypha, and parthenium. A lower survival rate of first-instar *P. kraunhiae* was observed when reared on *V. faba* than on citrus leaves (Narai and Murai 2002). The loss of first-instar *P. marginatus* may be caused by the movement of crawlers (first instars) away from the leaf tissues and falling off the plants. This movement was observed on all host species. Crawlers have a tendency to move toward light so the 12-h photoperiod used in this experiment may have caused them to move toward light and dislodge from the leaves. Preliminary studies showed that the crawlers of *P. marginatus*, when dislodged from the leaf, were not be able to survive, unless they moved back or were placed back on the leaf. Ultimately, the low percentage survival of eggs and first-instar nymphs was reflected in the low cumulative percentage survival of adult *P. marginatus*.

Insects may settle, lay eggs, and severely damage plant species that are unsuitable for development of immatures (Harris 1990). However, males and females that emerged from hibiscus, acalypha, plumeria, and parthenium were able to mate and reproduce successfully. Under experimental conditions, the mean number of eggs produced by an insect could be lower than its actual capacity because of restricted conditions and the experimental arena used. With a female developmental time of 24–25 d, even with the lowest fecundity observed from the females reared on plumeria, the number of eggs obtained here are large enough to build a substantial population in the field in a short time.

Although some mealybugs such as the cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero, can reproduce by thelytokous parthenogenesis (Calatayud et al. 1998, Le Ru and Mitsipa 2000), no virgin females produced eggs in this study. The sex ratio was slightly female biased; thus, we have no evidence for parthenogenetic reproduction in this species.

The ability of *P. marginatus* to develop on these plant species shows the possibility of movement, distribution, and establishment of *P. marginatus* into new areas in the United States. Hibiscus, acalypha, and plumeria are popular ornamental plants widely grown in Florida, California, and Hawaii (Criley 1998, Gilman 2003, USDA–NRCS 2007). Different hibiscus species are grown in many U.S. states (Gilman 1999, USDA–NRCS 2007), and potted hibiscus plants are transported to other parts of the United States and Canada. Parthenium is a noxious annual weed com-

monly found among the ornamental plants in the landscape of urban areas, agricultural lands, and in disturbed soil in >17 states in the eastern, southern, and south central United States (USDA–NRCS 2007). There is the possibility that *P. marginatus* can spread from weeds such as parthenium to economically important fruits, vegetables, and ornamental plants. However, the ultimate movement, distribution, and establishment of *P. marginatus* into other areas in the United States could be greatly influenced by other abiotic and biotic factors, such as temperature, availability of host plants, and the rules and regulations governing the movement of plant material from one state to the other.

Understanding the life history of *P. marginatus* is important in predicting its development, emergence, distribution, and abundance. With rapid development, high survival rates, and enormous reproductive capacity, a *P. marginatus* population could potentially reach a high level and cause significant damage to a large number of economically important crops unless suitable management practices are implemented in a timely manner. The life history information of *P. marginatus* plays an important role in the management of this pest, especially when applying chemical and biological control methods. Early-instar mealybugs are easier to control than late instars (Townsend et al. 2000). Timing the emergence of early instars using their developmental times will be helpful in implementing a suitable integrated pest management (IPM) program for *P. marginatus*.

The life history of *P. marginatus* is affected by host plant. However, it has the ability to develop, survive, and reproduce on a variety of host plant species. The information gathered from this study will be important in the management of *P. marginatus* by providing a better understanding of its life cycle and its ability to survive on different host plant species. This information is needed in development of IPM of this pest.

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