LIFE HISTORY OF PAPAYA MEALYBUG (*Paracoccus marginatus*), AND THE EFFECTIVENESS OF THREE INTRODUCED PARASITOIDS (*Acerophagus papayae*, *Anagyrus loecki*, AND *Pseudleptomastix mexicana*)

By

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Affectionately dedicated to my late parents

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Native to Mexico and Central America, papaya mealybug (*Paracoccus marginatus*) is an adventive pest insect that can damage a large number of tropical and subtropical fruits, vegetables, and ornamental plants in the US, the Caribbean, and the Pacific islands. It is an important pest in Florida, and potentially poses a threat to other states such as California, Hawaii, and Texas. Currently, three introduced parasitoids are used as biological control agents. Information on papaya mealybug and its parasitoids is scarce. In this dissertation, the life history of papaya mealybug in relation to temperature and host plants, and the biology and the effectiveness of its parasitoids were investigated.

Temperature is one of the important abiotic factors that may decide the establishment and distribution of papaya mealybug into other areas in the US. Adult males and females required 303.0 and 294.1 degree-days, and 14.5 and 13.9°C, minimum temperature threshold, respectively. In addition, papaya mealybug was able to complete its life cycle on three ornamental plants, hibiscus, acalypha, plumeria, and the weed parthenium, which are commonly found plants in many US states.

In the field, *Acerophagus papayae* provided better control than the other parasitoids. *Pseudleptomastix mexicana* was not observed, while *Anagyrus loecki* had lower parasitism. In the laboratory, all parasitoids were able to develop and emerged successfully in all stages of *P. marginatus* except for first-instar nymphs. *Acerophagus papayae* and *P. mexicana* preferred second-instar *P. marginatus* while *A. loecki* preferred third instars. Developmental times of *A. papayae* and *A. loecki* were similar but *P. mexicana* had a longer developmental time. Overall, *A. papayae* provided better control of the host, when alone or with the other two parasitoids. *Pseudleptomastix mexicana* was less competitive when mixed with *A. papayae* and *A. loecki*.

Considering its low thermal requirement and high minimum temperature threshold, papaya mealybug has a smaller distribution range than anticipated. Southern parts of Texas and California, South Florida, and Hawaii are suitable areas for its development. Its final establishment and distribution may be influenced by other factors such as host plant range, and the rules and regulations governing plant movement from state to state.

CHAPTER 1
INTRODUCTION

Mealybugs

Mealybugs are soft-bodied insects, which belong to the family Pseudococcidae in the order Hemiptera (Borror et al. 1992). The name "mealybug" is derived from the mealy or waxy secretions that cover the bodies of these insects (Borror et al. 1992). This layer of fine mealy wax often extends laterally to form a series of short filaments. The mealy wax covering is frequently white and the color may vary among some species (Williams and Granara de Willink 1992). The body of the adult female is normally elongate to oval, and membranous (Williams and Granara de Willink 1992). Antennae normally have 6 to 9 segments. Legs are present; each with a single tarsal segment and a single claw (Williams and Granara de Willink 1992).

In common with other hemipterans, female mealybugs have piercing and sucking mouthparts and are generally active throughout their life (Ben-Dov 1994). In the tropics, their life cycle may be reduced to less than one month. They often attain high numbers, killing the host plant by depleting the sap and occasionally by injecting toxins, transmitting viruses, or by excreting honeydew, which is a suitable medium for the growth of sooty mold (Ben-Dov 1994). The mold often covers the plant to such an extent that normal photosynthesis is severely reduced (Williams and Granara de Willink 1992). Although some mealybugs are host plant specific, mealybugs such as *Maconellicoccus hirsutus* (Green), and *Phenacoccus madeirensis* Green are polyphagus mealybugs that can damage a large number of economically important plants (Sinacori 1995, Serrano and Lapointe 2002).

Reproduction of mealybugs under greenhouse conditions is year round, and in certain species is by the production of living nymphs or young often without fertilization. Some mealybug species reproduce parthenogenetically. The cassava mealybug, *Phenacoccus manihoti*

Matile-Ferrero reproduces by thelytokous parthenogenesis (Calatayud et al. 1998, Le Ru and Mitsipa 2000). Many species form an ovisac in which to lay the eggs. In sexually reproducing species, the adult males are normally minute without functional mouthparts. Male mealybugs are often winged but occasionally apterous. In contrast, females are always wingless (Williams and Granara de Willink 1992).

Many of the 2000 mealybug species already described are important insect pests of many agricultural crops (Williams and Granara de Willink 1992). Infestations may occur within vegetative shoots or apexes and can be extremely difficult to detect. This ability of mealybugs to form dense colonies, particularly within the shoot and apex, often makes chemical control of this pest quite difficult. With the introduction of many new systemic insecticides, control has improved; however, with insects that are polyphagus, and have numerous hosts, it becomes a challenge to manage them with just chemical control.

Many times mealybug populations in their countries of origin are not pest problems due to their parasitoids and predators. The most serious outbreaks occur when mealybugs are accidentally introduced to new countries without their natural enemies. The introduction of pests on infested plant material has unfortunately become fairly common. Florida is one of the important agricultural states in this country and it has weather and climatic patterns that are conducive for the establishment of many insects. In South Florida, the more subtropical climatic condition facilitates the growth of a variety of tropical and subtropical crops. This agricultural pattern, subtropical climatic condition, increase of world trade, and geographic location of the state, are the main reasons for the regular invasion of insect pests to Florida. Invasive insect species such as the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) (Mead 2007) and the pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) (Hemiptera:

Pseudococcidae) (Hoy et al. 2006), which were accidentally introduced to Florida in 1998 and 2002, respectively are good examples of the pest invasions to Florida. *Paracoccus marginatus* Williams and Granara de Willink is one of the mealybug species that has been accidentally introduced into the Caribbean, the US and the Pacific islands, from Central America.

Genus *Paracoccus*

The genus *Paracoccus* was first described by Ezzat and McConnell in 1956 by using the type species *Pseudococcus burnerae* Brain, by original designation (Ben-Dov 1994). Generic characters of *Paracoccus* were later described by Williams and Granara de Willink (Ben-Dov 1994). *Paracoccus* has a varied distribution from the "Austro-Oriental", Ethiopian, Madagasian, Nearctic, Neotropical, New Zealand, Pacific, Palaearctic, and Oriental regions (Ben-Dov 1994). There are about 79 species recorded from the genus *Paracoccus* (Ben-Dov 1994). Most of the *Paracoccus* species are not recognized as major economic pests except for two species. In South Africa, *Paracoccus burnerae* (Brain) is considered as a serious pest of citrus (Ben-Dov 1994). *Paracoccus marginatus* Williams and Granara de Willink (papaya mealybug) is a pest of papaya and other economically important fruits, vegetables and ornamentals in the Caribbean, the US, and several Pacific islands.

Paracoccus marginatus Williams and Granara de Willink

Specimens of papaya mealybug were first collected from Mexico in 1967, which were believed to be native to Mexico and/or Central America (Miller et al. 1999). Papaya mealybug is not a serious pest in Mexico, probably because of the availability of its natural enemies (Miller et al. 1999). This species was first described by Williams and Granara de Willink in 1992 from the specimens collected from neo-tropical regions in Belize, Costa Rica, Guatemala and Mexico (Williams and Granara de Willink 1992). In 2002, Miller and Miller re-described this mealybug species (Miller and Miller 2002).

In early 1990, papaya mealybug invaded the Caribbean region and became a pest of many tropical and subtropical fruits, vegetables, and ornamental plants (Miller and Miller 2002). Since 1994, it has been recorded in 14 Caribbean countries. In 2002, a heavy infestation of papaya mealybug was observed on papaya (*Carica papaya* L. (Caricaceae)) in Guam (Meyerdirk et al. 2004). Subsequently, papaya mealybug infestations were reported from the Republic of Palau in 2003 and in Hawaii in 2004 (Muniappan et al. 2006, Heu et al. 2007).

The papaya mealybug is an adventive pest insect species that has been found in the US. It was first recorded on hibiscus in Palm Beach County in Florida in 1998 (Miller et al. 1999) and subsequently spread into several other counties in the state. It has been collected from more than 25 different plant genera in many counties in Florida since then (Walker et al. 2003).

Paracoccus marginatus is yellow in color and has a series of short waxy filaments around the margins of the body, which are less than 1/4 the length of the body (Miller et al. 1999). The female papaya mealybug passes through three immature stages (first, second, and third instar) before emerging as an adult. The ovisac produced by the adult female is on the ventral side of the body and is generally two or more times the body length (Miller et al. 1999). Generally, first instars of mealybugs are called “crawlers". There is no distinguishable difference between male and female crawlers, and male and female early second instars. In the latter part of the second instar, the color of the male changes from yellow to pink. Later, it develops a cottony sack around itself. Male third instars are termed as “prepupa”. Unlike the female, the male has a fourth instar termed as "pupa", from which the adult male emerges (Miller et al. 1999).

Host Plant Species

Food is a component of the environment and may influence an animal's chance to survive and multiply by modifying its fecundity, longevity or speed of development (Andrewartha and Birch 1954). The economically important host range of the papaya mealybug includes papaya,

hibiscus, acalypha, plumeria, avocado, citrus, cotton, tomato, eggplant, pepper, beans and peas, sweet potato, mango, cherry and pomegranate (Miller and Miller 2002). In addition, weed species such as *Parthenium hysterophorus* L. are also recorded as host plants of papaya mealybug (Miller and Miller 2002). Infestations of papaya mealybug have been observed on papaya, plumeria, hibiscus and jatropha in Hawaii with the favored hosts appearing to be papaya, plumeria, and hibiscus (Heu et al. 2007). However, insects may settle, lay eggs, and severely damage plant species that are unsuitable for development of immatures (Harris 1990). There is no specific information about the life history of papaya mealybug on different host plant species. Although, papaya is the dominant host plant species of papaya mealybug, it is important to find out how it can develop on popular ornamental plants such hibiscus, acalypha, and plumeria as well as on a commonly found invasive annual weeds such as parthenium.

Hibiscus, which is believed to be native to China, is a popular ornamental and landscape shrub, and widely grown in the tropics and subtropics (Ingram and Rabinowitz 2004). Different hibiscus species are grown in many areas of the US (USDA 2007a). Hibiscus has been grown in Florida for many years (Ingram and Rabinowitz 2004), and its potential planting range in the US includes some areas of Texas and California (Gilman 1999b). Hibiscus is widely grown in Hawaii. Hibiscus is sold nationwide as potted flower plants, and maintained in greenhouses around the country. Pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae) is another important mealybug species that was introduced to Florida in 2002, and has been identified as one of the most important insect pests of hibiscus (Goolsby et al. 2002, Hoy et al. 2006).

Acalypha L. is a large, fast growing evergreen shrub, which can provide a continuous splash of color in the landscape with the bronze red to muted red and mottled combinations of

green, purple, yellow, orange, pink or white (Gilman 1999a). It is believed to be native to Fiji and nearby Pacific islands. *Acalypha* L. is grown in many parts of the United States (USDA 2007a). Aphids, mites, scales, and mealybugs are recorded as pests of acalypha (Gilman 1999a).

The genus *Plumeria* L. originates from Central America and its different species are popular ornamental plants that are widely distributed in the warmer regions of the world (Begum et al. 1994). Plumeria belongs to the family Apocynaceae (dogbane) (Criley 1998) and the sap of most of the plants belonging to this family is milky, and may contain toxic alkaloids or glycosides. In Southwestern Puerto Rico, a caterpillar of the sphinx moth, *Pseudosphinx tetrio* L. (Lepidoptera: Sphingidae), two mealybug species (*P. marginatus* and *Puto* sp.) and one unidentified Margaroididae are the frequently encountered herbivores of *Plumeria alba* (Sloan et al. 2007). The most common homopteran attacking *P. alba* in Puerto Rico is the papaya mealybug (Sloan et al. 2007). These homopterans attack the leaves, inflorescences, flowers, fruits and sometimes the stem of *P. alba* (Sloan et al. 2007). They feed on the sap of *P. alba* leaves when the standing crop of leaves is the greatest, causing the leaves to be frequently contorted, misshapen, and not fully expanded (Sloan, et al. 2007). Triterpenoids are chemicals commonly found in plants that belong to the family Apocynaceae, and in plumeria, these compounds can be feeding deterrents to most generalist insects. The aposematic coloration of *P. tetrio* suggests that it is able to detoxify and sequester secondary compounds in *P. alba*, but these compounds can make *P. alba* unpalatable to other generalist herbivores (Sloan et al. 2007).

Parthenium hysterophorus L. is an introduced, invasive weed species, which can be found in more than 17 states in the Eastern, Southern, and South Central US (USDA 2007a). Parthenium is considered a noxious annual weed because of its prolific seed production and fast spreading ability, allelopathic effect on other plants, strong competitiveness with crops and

health hazard to humans as well as animals (Tefera 2002, Raghubanshi et al. 2005). Parthenium contains sesquiterpene lactones and phenolic acids (Picman and Picman 1984, Mersie and Singh 1988). Terpinoids, from volatile monoterpenoids to involatile triterpenoids, are broadly defensive against herbivory on plants (Harbone 2001). Parthenin is a terpinoid found in parthenium weed, which is identified as a barrier to herbivore feeding (Harbone 2001). A leaf feeding beetle, *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae) and a stem-galling moth, *Epiblema strenuana* Walker (Lepidoptera: Tortricidae) are some of the natural enemies used in the biological control of parthenium in Australia (Dhileepan 2001, Dhileepan et al. 2005).

Temperature

Temperature is one of the important environmental factors that can affect the movement, establishment, and abundance of insects. Insect biology is influenced by various environmental factors and temperature is one of the most important and critical of the abiotic factors (Huffaker et al. 1999). The rate of insect development is affected by the temperature to which the insects are exposed (Campbell et al. 1974). Insect development occurs within a definite temperature range (Wagner et al. 1984). The temperature below which no measurable development occurs is its threshold of development. The amount of heat required over time for an insect to complete some aspect of development is considered a thermal constant (Campbell et al. 1974). The thresholds and the thermal constant are useful indicators of potential distribution and abundance of an insect (Huffaker et al. 1999). The importance of predicting the seasonal occurrence of insects has led to the formulation of many mathematical models that describe developmental rates as a function of temperature (Wagner et al. 1984). The thermal summation model (Campbell et al. 1974) and Logan 6 model (Logan et al. 1976) are widely used models to explain the relationship between developmental time and temperature of arthropods. Temperature had

pronounced effects on the development, survival, and reproduction of Madeira mealybug, *Phenacoccus madeirensis* Green (Chong et al. 2003). The female *P. madeirensis* was able to complete its development in temperatures ranging from 15 to 25°C within 66 to 30 days respectively (Chong et al. 2003). Between 15 to 25°C, survival rates of *P. madeirensis* were not affected by temperature but the temperature had a strong influence on fecundity, pre-oviposition time, and the duration of reproduction (Chong et al. 2003). Between 20 and 25°C, the cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero, and *Phenacoccus herreni* Cox and Williams, complete development within 46 to 36 days (Lema and Herren 1985) and 91 to 41 days respectively (Herrera et al. 1989). Comparison of whole-life developmental times of *P. herreni* to those of *P. manihoti* suggests that *P. herreni* develops slower than *P. manihoti* at cooler temperatures but faster than *P. manihoti* at warmer temperatures (Herrera et al. 1989). This is supported by the more tropical distribution of *P. herreni* (Columbia, The Guyana, and northern Brazil) compared to that of *P. manihoti*, which has subtropical distribution (Herrera et al. 1989).

Chemical Control of Papaya Mealybug

Organophosphate and carbamate insecticides such as dimethoate, malathion, carbaryl, chlorpyrifos, diazinone, and acephate (Walker et al. 2003) were commonly used insecticides to control mealybugs. Currently neonecotinoid insecticides such as acetamiprid, clothianidin, dinotefuran, imidacloprid, thiamethoxam, and insect growth regulators (IGR) such as pyriproxyfen are used to control scale insects and mealybugs (Buss and Turner 2006). However, there is no specific insecticide currently registered for control of papaya mealybug (Walker et al. 2003). Mealybugs are generally difficult to control chemically due to their thick waxy secretion covering the body, and their ability to hide in the damaged buds and leaves without being exposed to the insecticide. The adult mealybugs were more difficult to control than the young and repeated applications of chemicals targeting immatures were required in suppressing *P.*

madeirensis (Townsend et al. 2000). In addition, with polyphagous insects such as papaya mealybug, it would be difficult to manage it with just insecticides and to achieve long-term control with the wide variety of host plants. Development of insecticide resistance and non-target effects of insecticides on natural enemies make chemical control a less feasible option for the long-term control of papaya mealybug (Walker et al. 2003). Because of these reasons, biological control was identified as a preferred method to control the papaya mealybug.

Biological Control

Biological control is the use of parasitoid, predator, pathogen, antagonist, or competitor populations to suppress a pest population, making it less abundant and thus less damaging (Van Driesche and Bellows 1996). It is widely accepted that there are three general approaches to biological control: importation, augmentation, and conservation of natural enemies. Importation biological control is often referred to as "classical biological control" reflecting the historical predominance of this approach (Orr and Suh 1998). Classical biological control can be defined as importation and establishment of non-native natural enemy populations for suppression of non-native or native organisms (Orr and Suh 1998). Augmentation includes activities in which natural enemy populations are increased through mass culture, periodic release, and colonization. Conservation biological control can be defined as the study and modification of human influences that allow natural enemies to realize their potential to suppress pests (Orr and Suh 1998). Currently, the "classical" approach is probably the most recognized and heralded form of biological control among biological control practitioners.

Classical Biological Control of Papaya Mealybug

Many adventive insect species become pests because they are unaccompanied by natural enemies from their native home (Orr and Suh 1998). In the classical biological control of an adventive pest species, most often the natural enemies of the pest are searched for in its native

homeland by examining the pest population in its native environment (Van Driesche and Bellows 1996). These natural enemies are then collected and shipped to the country where the pest has invaded. After being subjected to appropriate quarantine and testing to ensure safety, these natural enemies are released and established. This type of introduction of natural enemies is self-maintaining and less expensive than chemical control over the long term (Van Driesche and Bellows 1996).

The United States Department of Agriculture (USDA), Animal Plant Health Inspection Service (APHIS) initiated a classical biological control program for papaya mealybug using several natural enemies in 1999. The identified natural enemies of papaya mealybug are solitary endoparasitic wasps that belong to the family Encyrtidae in the Order Hymenoptera. These wasps were collected in Mexico as potential biological control agents. They were *Acerophagus papayae* Noyes and Schauff, *Anagyrus loecki* Noyes and Menezes, *Anagyrus californicus*, *Pseudophycus* sp. and *Pseudleptomastix mexicana* Noyes and Schauff (Meyerdirk et al. 2004). *Acerophagus papayae, A. loecki* and *P. mexicana* are three parasitoid species that are currently used in the biological control of papaya mealybug. They are mass reared in Puerto Rico and released in papaya mealybug infested areas in the Caribbean, the US, and the Pacific islands as needed (Meyerdirk et al. 2004).

Parasitoids

The term "parasitoid" embraces an exceedingly large number of insect species (Gauld 1986). Parasitoids are arthropods that kill their hosts and are able to complete their development on a single host (Vinson 1976). Parasitoids have been the most common type of natural enemy introduced for biological control of insects. They have been employed in the management of insect pests for centuries (Orr and Suh 1998). The last century, however, has seen a dramatic increase in their use as well as an understanding of how they can be manipulated for effective,

safe use in insect pest management systems (Orr and Suh 1998). Most parasitoids that have been used in biological control are in the orders Hymenoptera and, to a lesser degree, Diptera (Van Driesche and Bellows 1996). Of these, certain groups stand out as having more species employed in biological control projects than others. The most frequently used groups in the Hymenoptera are Braconidae and Ichneumonidae in the Ichneumonoidea, and the Eulophidae, Pteromalidae, Encyrtidae, and Aphelinidae in the Chalcidoidea. In the Diptera, Tachinidae is the most frequently employed group (Greathead 1986). Although parasitoids have been recorded in the orders Strepsiptera and Coleoptera, parasitism is not common in them (Van Driesche and Bellows 1996).

***Acerophagus papayae* Noyes and Schauff**

This species of parasitoid is named for the papaya plant (*C. papaya* L.) on which its host feeds. It is the smallest species out of the three introduced parasitoids of papaya mealybug. The female *A. papayae* is 0.58 to 0.77 mm long including its ovipositor, and males are generally 0.44 to 0.66 mm in length (Noyes and Schauff 2003). The male and female *A. papayae* are generally pale orange in color. Other than the un-segmented clava, and genitalia, males are very similar to their females (Noyes and Schauff 2003). *Acerophagus papayae* was originally recorded from *P. marginatus* in Mexico (Noyes and Schauff 2003).

***Pseudleptomastix mexicana* Noyes and Schauff**

This is the second parasitoid out of the three introduced parasitoids of *P. marginatus*; *P. mexicana* is named for its country of origin, Mexico (Meyerdirk 2003, Noyes and Schauff 2003). Larger than *A. papayae*, the length of the male and female *P. mexicana* is 0.56 to 0.84 and 0.76 to 1.03 mm, respectively. The head and thorax of the female are black in color and the gaster is dark brown with a coppery and purple or brassy sheen. *Pseudleptomastix mexicana* also was originally recorded from *P. marginatus* in Mexico (Noyes and Schauff 2003). In 2000, *P.*

mexicana was introduced into Puerto Rico with other exotic natural enemies from Mexico to control *P. marginatus* (Meyerdirk 2003). There are no other known introductions of exotic *Pseudleptomastix* species into various countries for the control of *P. marginatus* or any other mealybug species (Meyerdirk 2003).

***Anagyrus loecki* Noyes and Menezes**

The largest out of the three species, female *A. loecki* is 1.45 to 1.76 mm in length, and the male is 0.94 to 1.08 mm long respectively (Noyes 2000). In the female, the head and thorax are mostly orange in color and the gaster is light brown. The male is dark brown in color and varies from the female in its size and color (Noyes 2000). This species was recorded from several mealybug species. The holotype was reared from *Dysmicoccus hurdi* and some of the paratypic material was laboratory reared on *Phennacoccus madeirensis* and *P. marginatus* (Noyes 2000).

Developmental Time, Longevity, and Lifetime Fertility

Developmental time, longevity, and lifetime fertility are important fitness parameters when evaluating a parasitoid as a biological control agent (Hemerik et al. 1999). Developmental time of a parasitoid is the duration of time from oviposition to adult emergence. The time between adult emergence and death is termed as adult longevity. The lifetime fertility of an insect is the total number of progeny produced during its lifetime.

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 it is developmentally arrested through destructive feeding by the parasitoid larva (Hemerik et al. 1999). The development of *Venturia canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae), a solitary endoparasitoid of *Plodia interpunctella* (Hubner) (Lepidoptera: Pyralidae) depends on the ability of early stadia of its host to grow after parasitism and to reach their final stadium (Hemerik et al. 1999). The early emerging females of *Trichogramma evanescens* Westwood

(Hymenoptera: Trichogrammatidae), a gregarious egg parasitoid of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) were larger and produced more progeny and had higher fitness than late emerging females (Doyon and Boivin 2005). The adult size and the developmental time of the solitary endoparasitoid, *Aphidius ervi* Haliday were affected by the size of its host, *Acyrthosiphon pisum* (Harris) (Sequeira and Mackauer 1992).

The developmental time, longevity and the progeny production of parasitoids can be affected by the developmental temperature of the host (Hansen 2000). Between 15 to 30°C, the developmental time of the female *Trichogramma turkestanica* on the host *Ephestia kuehniella*, ranged from 32.9 to 7 days (Hansen 2000). The developmental time decreased with increasing temperature for the gregarious encyrtid endoparasitoid *Tachinaephagus zealandicus* reared on *Chrysomya putoria* (Ferreira de Almeida et al. 2002). *Amitus fuscipennis* MacGown and Nebeker, a potential biological control agent of *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae), had longer developmental time and adult longevity at lower temperatures (Manzano et al. 2000). The lifetime fecundity and the reproductive life were significantly affected by temperature for *Anagyrus kamali* Moursi, a parasitoid of *Maconellicoccus hirsutus* Green reared at 26 and 32°C (Sagarra et al. 2000a). Early emerged *Tachinaephagus zealandicus* lived longer than late emerged *T. zealandicus* (Ferreira de Almeida et al. 2002).

The host diet affected the developmental time, fecundity, sex ratio, and size of *Apanteles galleriae* Wilkinson (Hymenoptera: Braconidae), a parasitoid of *Achroia grisella* (F.) (Uckan and Ergin 2002). The mating status of a parasitoid can affect its fitness parameters. The mated solitary endoparasitoid female *Anagyrus kamali* Moursi had higher progeny production and had a female biased sex ratio in comparison with unmated females, which had lower progeny production and male only progeny (Sagarra et al. 2002). Unmated *A. kamali* lived longer than

the mated ones (Sagarra et al. 2002). Fecundity and survival of *Anagyrus kamali* was also affected by higher feeding and storage temperatures of 27°C than 20°C (Sagarra et al. 2000b).

Host Stage Susceptibility, Host Stage Suitability, and Sex Ratio

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 to those that develop in the late instars, in which they can produce more female progeny (Charnov et al. 1981, Sagarra and Vincent 1999). In no choice tests, *A*. *kamali* a parasitoid of the pink hibiscus mealybug, *M*. *hirsutus* Green, was able to parasitize all nymphal stages and adult females, while choice tests indicated that *A*. *kamali* prefers third instar and pre-oviposition adult females (Sagarra and Vincent 1999). Parasitoids emerged from hosts that were parasitized as second-instar *P*. *herreni* were strongly male-biased for *A*. *vexans* while apparently preferred later host stages yielded significantly more females than males (Bertschy et al. 2000).

Increased size of the host translates into both increased male and female fitness. For females, this measure is the lifetime production of eggs while for the male it is longevity (Charnov et al. 1981). The later the developmental stage of the host at oviposition, the faster the parasitoids develop and emerge (Bertschy et al. 2000). Within a particular host stage, the male had a shorter developmental time than the female for *Aenasius vexans* Kerrich, an encyrtid parasitoid of cassava mealybug, *Phenacoccus herreni* Cox and Williams (Bertschy et al. 2000). Depending on the instar they attack, the parasitoid progeny can be either male or female biased. The solitary endoparasitoid of cassava mealybug (*Phenacoccus herreni* Cox and Williams), *Aenasius vexans* Kerrich (Hymenoptera: Encyrtidae), shows male-biased sex ratio when it attacks second-instar *P. herreni*, and female-biased sex ratio when it parasitizes third instars (Bertschy et al. 2000).

The haplodiploid sex determination system of most parasitoid wasps provides females a means of controlling the offspring sex ratio, because they can adjust the proportion of fertilized eggs at oviposition (King 1987). Parasitoid wasps provision their young with food by ovipositing in or on a host. Upon hatching the wasp larva feeds on the host, usually killing it prior to the wasp's pupation. Because a few males can fertilize many females, female-biased broods facilitate the use of parasitoids wasps as biological control agents (King 1987). The factors that may influence the offspring sex ratio are parental characteristics, environmental characteristics, host characteristics, and factors influencing local mate competition. The parental characteristics are time delay between emergences and insemination, number of times a female has mated, maternal and paternal age, maternal size, maternal diet, and genetics (King 1987). Photoperiod, temperature, and relative humidity are the environmental characteristics that can affect sex ratio. Host characteristics such as host size, age, sex, and species can affect the progeny sex ratio of the parasitoids. Local mate consumption theory predicts that isolated females should produce primarily daughters with only enough sons to inseminate those daughters. Superparasitism, female density, number of offspring per host, and host density are factors affecting local mate consumption theory (King 1987). Sex ratio of the progeny can also be affected when a female hymenopteran lacks sperm and lays male eggs (Ridley 1988).

Interspecific Competition

According to Dent (1995), when two species compete with one another intensely enough over limited resources, then with time, one or the other can become extinct. When there is a dominant parasitoid, which can displace other parasitoid species, the releasing of several species might not provide the expected efficiency of a biological control program. In solitary insect parasitoids, generally only one offspring survives in a host (Vinson 1976). Females normally deposit one egg per host and this reduces the host availability to conspecific and heterospecific

parasitoids. The successful oviposition of a female, therefore, would be increased if she were the first to identify and oviposit only in hosts with no previously laid eggs (Lawrence 1981). Although, coexistence of several parasitoid species in the system can be more productive than a single parasitoid species, coexistence requires that some difference exist in niches among the species. When several parasitoid species attack the same host species, and one parasitoid prefers to attack early instars of the host and others prefer late instars or vice versa, there can be efficient control of the host species (Bokonon-Ganta et al. 1996). The pest instar they attack is the most important factor to decide the coexistence or competitive exclusion of biological control agents when several agents are released together. The competition of parasitoids can be affected by the temperature. Some parasitoids compete more for hosts at lower temperatures and some prefer to attack hosts when temperatures are higher (Van Strien-van Liempt 1983). The parasitoids of *Drosophila melanogaster* Meigen and *Drosophila subobscura* Collin, *Asobara tabida* Nees von Esenbeck, and *Leptopilina heterotoma* (Thomson) compete differently at different temperatures. *Asobara tabida* is a better competitor at lower temperatures and *Leptopilina heterotoma* performed better at higher temperatures (Van Strien-van Liempt 1983).

Research Objectives

Research studies on papaya mealybug and its parasitoids are lacking. There is no information on the life history of papaya mealybug, either in relation to its host plant species or to temperature. Understanding the life history of an insect is important in insect predictions, distribution, and its management. Determining thermal constants and temperature thresholds is also useful in predicting insect emergence, distribution, and its management. In addition, there is very little published research on papaya mealybug parasitoids. Information on the biology of *A. papayae*, *A. loecki*, and *P. mexicana*, and their interspecific competition, and the effectiveness in the field is scarce. It is important to find out whether populations of these parasitoid species are

established in the field, and if there is a need for inoculative releases. The goal of this study was to understand the life history of papaya mealybug and to identify the efficient parasitoids for successful utilization of currently used biological control agents to obtain an effective and sustainable biological control program for papaya mealybug infestation in the US. Therefore, research was conducted to determine the life history of papaya mealybug, and then to evaluate the effectiveness of three introduced parasitoids of papaya mealybug. There were five objectives for this study.

The first objective was to define the life history of papaya mealybug using four host plant species commonly found in Florida. The second objective was to understand the effect of constant temperature on development, reproduction and survival of papaya mealybug, and then to estimate its thermal constants and temperature thresholds for development. The third objective was to evaluate the effectiveness of currently released parasitoids of papaya mealybug, *A. papayae*, *A. loecki* and *P. mexicana* in the field. The fourth objective was to study the developmental time, longevity and the lifetime fertility of *A. papayae*, *A. loecki* and *P. mexicana*. The fifth and final objective was to investigate the host stage susceptibility and suitability, sex ratio, and interspecific competition of *A. papayae*, *A. loecki* and *P. mexicana*.

CHAPTER 2
LIFE HISTORY OF *Paracoccus marginatus* WILLIAMS AND GRANARA DE WILLINK (HEMIPTERA: PSEUDOCOCCIDAE) ON FOUR HOST PLANT SPECIES UNDER LABORATORY CONDITIONS

Introduction

Paracoccus marginatus Williams and Granara de Willink (Hemiptera: Pseudococcidae) is a polyphagus 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). *Paracoccus marginatus* was first described by Williams and Granara de Willink (1992) and re-described by Miller and Miller (2002). *Paracoccus 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. 2003). In 1998, *P. marginatus* was first reported in the US in Florida, in Palm Beach County on hibiscus (Miller et al. 1999). Thereafter, it was recorded in several other counties in Florida from more than 25 genera of plants (Walker et al. 2003). Heavy infestations of *P. marginatus* on *C. papaya* were recorded in Guam in 2002 (Walker et al. 2003, Meyerdirk et al. 2004) and in the Republic of Palau in 2003 (Walker et al. 2003, Muniappan et al. 2006). In 2004, *P. marginatus* was reported in Hawaii on papaya, plumeria, hibiscus, and *Jatroph*a sp. L. (Heu et al. 2007).

Since its introduction to the Caribbean, the US, and the Pacific islands, *P. marginatus* has established in most of the Caribbean islands, Florida, Guam, the Republic of Palau, and Hawaii. *Paracoccus marginatus* potentially poses a threat to numerous agricultural products in the US especially in Florida, and states such as California and Hawaii, which produce similar crops. In

southern parts of Texas, where the country's third largest citrus production exists (CNAS 2007) is also a susceptible area for *P. marginatus*. The potential planting range of hibiscus includes Southern Texas (Gilman 1999b).

Life history of *P. marginatus* has not been investigated. 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. Since there is a high possibility of spreading *P. marginatus* into other areas in the US, it is important to study its life history using host plant species that are either widely grown in the susceptible areas, or potted 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.) *cutivar 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 areas in the US.

Materials and Methods

Rearing Mealybugs. *Paracoccus marginatus* was initially collected from a papaya (*Carica papaya* L.) field in Homestead, FL. Red potatoes (*Solanum tuberosum* L.) (Ryan Potato Company, East Grand Forks, MN) were allowed to sprout and then used in rearing a colony of *P. marginatus*. Potatoes were soaked in 1% solution of bleach (Clorox ®, The Clorox Company, Oakland, CA; 6% sodium hypochlorite) for 15 minutes, and then 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 ± 1°C and 65% ± 2 R.H. Each week, 30 newly sprouted potatoes were infested with ovisacs of *P. marginatus* to maintain the colony. Each sprouted potato was infested with 3

to 5 ovisacs depending on the size of the potato and ovisacs. Infested potatoes were kept in 3.8-L plastic containers at the rate of 10 per container (Rubbermaid ®, Newell Rubbermaid Inc. Atlanta, GA). Prior to placing the infested potatoes, screens (Amber Lumite ®, Bio Quip, Gardena, CA) were glued to cut sections of lids in these containers to facilitate air circulation. The mealybug colony was held in an environmental growth chamber (Percivel I-36LL, Percival Scientific Inc. Perry, NC) at 25° ± 1°C, 65 ± 2% R.H., and a photoperiod of 12:12 (L:D).

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. All plant material was collected and prepared 24 hours before the experiment. Hibiscus cuttings were obtained from 1-yr old container-grown hibiscus and 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 approximately 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-diam Petri dish with a 0.6-cm-diam hole in the bottom for hibiscus, acalypha, and parthenium; 18-cm-diam 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 Inc., Englewood Cliffs, NJ). Eggs were collected within 24 h of oviposition. Dishes were checked daily for egg hatch and shed exuviae. The number of days to egg hatch, and emergence and survival of each instar, and number of emerging adult males and females were recorded. The developmental time and the survival of eggs and first instars were not separated by gender. The gender 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 counted separately. For each plant species, 35 Petri dishes (replicates) each with 10 eggs were used. This experiment was repeated twice at the end of the preceding experiment. All experiments were carried out inside an environmental growth chamber as above.

Reproduction. Newly emerged virgin females obtained from the developmental study of 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 the same plant species for sexual reproduction. Petri dishes were kept in an environmental growth chamber as above. The date oviposition began, the number of eggs laid, and adult mortality 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.

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. Data of the initial and repeated experiments were pooled together after a two-way analysis of variance (ANOVA) indicated no interaction among the experiments ($F = 0.69$, $df = 6, 408$, $P = <0.6539$). One-way ANOVA was performed using a general linear model (GLM) for all experiments (SAS Institute 1999). Means were compared at $P = 0.05$ significance level using the Tukey's HSD test. Data for proportions of females (sex ratio) and survival were square-root arcsine-transformed, when necessary prior to ANOVA (Zar 1984).

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

Results

Preliminary studies demonstrated that it takes approximately one month for eggs of *P. marginatus* to hatch and develop into adults. Use of tender leaves could avoid leaf senescence during this time. Hibiscus cuttings can root within two to three weeks time in water. Even after 30 days, acalypha cuttings were not rooted. Use of rooting hormones could have accelerated the process of rooting, however the impact of rooting hormones on the development of insects is not known. Therefore, the fresh cuttings were used. Cuttings obtained from parthenium, a soft herbaceous plant, were unable to survive 30 days 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 more than 30 days 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. Use of hard water in the containers to which the stems of the cuttings were submerged, could stain the bottom of the Perti dish and disturb the checking procedures for the mealybugs, which were dislodged from the leaf into the Petri dish. Use of distilled water did not significantly affect the development, reproduction, and survival of *P. marginatus* compared to hard water. Therefore, distilled water was used instead of hard water.

Development. There were differences in the developmental times of *P. marginatus* reared on four host species (Table 2-1). Males had longer developmental time than females. Adult females emerged earlier from the eggs on acalypha and parthenium than from the eggs on hibiscus and plumeria. Adult males had longer developmental time on acalypha and plumeria than on parthenium and hibiscus (Table 2-1).

Survival. Eggs survived similarly on all four plants (Table 2-2). 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 were not affected by the host species (Table 2-2).

Proportion of Females and Adult Longevity. Adults emerged on plumeria with a higher proportion of females than on the other three host species ($F = 8.15$, $df = 3, 416$, $P < 0.0001$). The mean proportion of adult females ranged from 53-59 % (acalypha: 53.9 ± 1.3, hibiscus: 53.7 ± 1.1, parthenium: 53.4 ± 1.0, and plumeria: 58.9 ± 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 ± 0.1 and 21.2 ± 0.1 d, respectively.

Reproduction. Virgin females did not lay any eggs on any of the four plant species. Mated females reared on plumeria laid a lower number of eggs (186.3 ± 1.8) than the number of eggs laid by 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) and oviposition periods (11.2 ± 0.1) were not affected by the plant species ($F = 0.23$, df = 3, 416, $P = 0.8739$, $F = 0.12$, df = 3, 416, $P = 0.9496$).

Discussion

Determining the life history of an insect is important to understand its development, distribution and abundance. In polyphagus 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 due to nutritive factors, allelochemical compounds, and physical differences in leaf structures, which may be involved in the variation in plant suitability, although these factors were not investigated for *P. marginatus* in this study. Use of different presentations may have confounded the results but preliminary studies found that these were the best ways to maintain these hosts in a condition suitable for the tests.

Different host plant species have affected the life history parameters of other mealybug species. Longer pre-reproductive period and a higher progeny production were observed for *Rastrococcus invadens* Williams reared on different varieties of *Mangifera indica* L. (Bovida 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 developed faster and had a higher fecundity when developed 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, *Maconellicoccus 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 to *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, there was less survival of the first and second instars on plumeria. Stickiness observed on plumeria leaves may have contributed to this low survival. This stickiness may have resulted from the experimental conditions such as the hydration method 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 *Plumeria alba* L. in Puerto Rico (Sloan et al. 2007) indicating its ability to develop well on this plant species. A loss of 17 to 18% of the first instars was also observed on hibiscus, acalypha, and parthenium. A low survival rate of first-instar mealybugs was also observed when *P. kraunhiae* were reared on *V. faba* seeds (Narai and Murai 2002). The loss of first instar *P.*

marginatus may be due to the movement of crawlers (first instars) away from the leaf tissues and they falling off the plants. This movement was observed on all plant species, although it was more evident on plumeria. 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 or the shoots. Preliminary studies demonstrated that the crawlers of *P. marginatus*, which were dislodged from the leaf, were not be able to survive, unless they moved back or were placed back on the leaf. Ultimately, the low percent survival of eggs and first instars was reflected in the low egg to adult survival of *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 due to restricted conditions and the experimental arena used. With a female developmental time of 24 to 25 d, even with the lowest fecundity observed from the females reared on plumeria, the number of eggs obtained was large enough to build up 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 the current study. The sex ratio was slightly female biased, thus there is no evidence for parthenogenetic reproduction in this species.

The ability of *P. marginatus* to develop on these plant species demonstrates the possibility of movement, distribution, and establishment of *P. marginatus* into new areas in the US. Hibiscus, acalypha, and plumeria are popular ornamental plants widely grown in Florida,

California, and Hawaii (Criley 1998, Gilman 1999a, USDA 2007a). Different hibiscus species are grown in many US states (Gilman 1999b, USDA 2007a), and potted hibiscus plants are transported to other parts of the US and Canada. Parthenium is a noxious annual weed commonly found among the ornamental plants in the landscape of urban areas, agricultural lands, and in disturbed soil in more than 17 states in the Eastern, Southern, and South central US (USDA 2007a). There is a 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* in the other areas in the US could be decided by the 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.

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 the development of integrated pest management of this pest.

Table 2-1 Mean number of days (± SEM) for each developmental stadium of *P. marginatus* reared on four host species (gender could not be determined before the second instar).

Host	Stadia							Cumulative	
	Egg	First	Second		Third		Fourth		
			Male	Female	Male	Female	Male	Male	Female
Acalypha	8.6 ± 0.1b	5.9 ± 0.1c	6.5 ± 0.1c	3.8 ± 0.1c	2.8 ± 0.1b	6.3 ± 0.1a	4.5 ± 0.1a	28.4 ± 0.1b	24.5 ± 0.1b
Hibiscus	8.4 ± 0.1c	6.2 ± 0.1b	6.8 ± 0.1bc	5.0 ± 0.1b	2.3 ± 0.1c	5.9 ± 0.1b	3.9 ± 0.1b	27.6 ± 0.1c	25.5 ± 0.1a
Parthenium	8.8 ± 0.1a	5.8 ± 0.1c	5.6 ± 0.1d	5.2 ± 0.1ab	3.4 ± 0.1a	4.7 ± 0.1d	4.1 ± 0.1b	27.7 ± 0.1c	24.4 ± 0.1b
Plumeria	8.5 ± 0.1b	6.6 ± 0.2a	9.6 ± 0.1a	5.3 ± 0.1a	2.7 ± 0.1b	5.1 ± 0.1c	2.6 ± 0.1c	30.0 ± 0.1a	25.5 ± 0.1a
F	25.44	63.78	358.88	122.56	32.37	109.51	128.68	239.96	74.78
df	3, 416	3, 416	3, 413	3, 416	3, 415	3, 416	3, 415	3, 415	3, 416
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

n = 105

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

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

Host	Egg	First	Second	Third		Fourth	Egg to Adult
				Male	Female	Male	
Acalypha	82.8 ± 0.7	83.2 ± 0.9a	89.4 ± 1.1a	89.6 ± 1.4	89.3 ± 1.4	89.7 ± 1.6	49.9 ± 0.8a
Hibiscus	83.3 ± 0.6	82.7 ± 0.9a	89.4 ± 1.0a	89.8 ± 1.5	89.0 ± 1.5	89.7 ± 1.6	50.4 ± 0.8a
Parthenium	83.5 ± 0.6	82.7 ± 0.9a	89.1 ± 0.9a	89.5 ± 1.4	89.7 ± 1.4	89.6 ± 1.5	50.1 ± 0.7a
Plumeria	82.2 ± 0.7	58.4 ± 1.4b	64.9 ± 1.7b	84.5 ± 2.3	81.8 ± 2.4	81.4 ± 3.5	20.0 ± 0.5b
F	0.89	73.44	58.67	0.42	1.55	0.48	379.44
df	3, 416	3, 416	3, 416	3, 416	3, 416	3, 416	3, 416
P	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 $\alpha = 0.05$ (Tukey's HSD test).

CHAPTER 3
EFFECT OF CONSTANT TEMPERATURE ON THE DEVELOPMENTAL BIOLOGY OF *Paracoccus marginatus* WILLIAMS AND GRANARA DE WILLINK (HEMIPTERA: PSEUDOCOCCIDAE)

Introduction

Understanding the developmental biology of an insect can provide useful information for pest management. Developmental biology, however, can be influenced by various environmental factors. Temperature is one of the most important and critical of the abiotic factors that can affect insect development. Insects require a certain amount of heat units to develop from one stage of their life cycle to another, which can be measured in degree-days (Gordan 1999). The ability of an insect to develop at different temperatures is an important adaptation to survive varying climatic conditions, and is important in insect population predictions and control strategies (Mizell et al. 1978). Temperature also influences the population dynamics of insect pests and their natural enemies (Huffaker et al. 1999). Temperature range and climatic condition of an area determine the ability of an adventive insect species to invade that area. There is no information on the effect of temperature on the development and survival of one such adventive pest species, *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae), which has been recently introduced in to the US. *Paracoccus marginatus* is a polyphagus insect that has been recognized as a significant pest of a large number of tropical and subtropical fruits, vegetables, and ornamental plants (Miller and Miller 2002).

First described by Williams and Granara de Willink in (1992) and re-described by Miller and Miller (2002), *P. marginatus* is believed to be native to Mexico and Central America (Miller et al. 1999). Its economically important hosts include papaya, hibiscus, avocado, citrus, cotton, tomato, egg plant, beans and peas, sweet potato, mango, cherry, and pomegranate (Walker et al.

2003). It is an important pest in the Caribbean, the US (Miller and Miller 2002) and some Pacific islands such as the Republic of Palau (Muniappan et al. 2006), Guam (Meyerdirk et al. 2004) and Hawaii (Heu et al. 2007). Since 1994, *P. marginatus* has been recorded in 14 Caribbean countries (Walker et al. 2003). In 1998, *P. marginatus* was first discovered in the US in Palm Beach County, Florida, on hibiscus plants, and since then has been recorded on more than 25 genera of hosts (Miller and Miller 2002). *Paracoccus marginatus* was subsequently found in several other counties in Florida, and potentially poses a threat to numerous agricultural products in Florida as well as to the other US states producing similar crops (Walker et al. 2003).

The ability of *P. marginatus* to spread into other states in the US may depend on its ability to develop and survive at different temperatures. Determining the effect of temperature on the life history of *P. marginatus* and estimating its thermal requirements will be useful in predicting where this pest can potentially spread in the US. This study focuses on the effect of constant temperature on the developmental biology and thermal requirements of *P. marginatus*.

Materials and Methods

Insect Rearing. *Paracoccus marginatus* was initially collected from a papaya (*Carica papaya* L.) field in Homestead, FL. Sprouted red potatoes (*Solanum tuberosum* L.) (Ryan Potato Company, East Grand Forks, MN) were used to rear a colony of *P. marginatus* at the University of Florida, Tropical Research and Education Center (TREC), Homestead, FL. Prior to sprouting, potatoes were soaked in a 1% solution of bleach (Clorox ®, The Clorox Company, Oakland CA; 6% sodium hypochlorite) for 15 minutes, and then rinsed with clean water and dried. Potatoes were placed in black cotton cloth bags, and kept inside a dark room at 27° ± 1°C to encourage sprouting. Each week, 30 sprouted potatoes were infested with *P. marginatus* ovisacs collected from the previously infested potatoes selected from the colony to maintain the mealybug population. The mealybug colony was held in an environmental growth chamber (Percivel I-

36LL, Percival Scientific Inc. Perry, NC) at 25° ± 1°C, 65 ± 2% R.H., and a photoperiod of 14:10 (L:D).

Development and Survival. Hibiscus (*Hibiscus rosa-sinensis* L.) leaves were used as the host tissues. These leaves were taken from hibiscus plants that were obtained from a local nursery and maintained outdoors. The experimental arena consisted of a 9-cm-diam Petri dish. A 0.6-cm-diam hole was made in the bottom of the Petri dish using a heated cork borer. A tender hibiscus leaf with a 4 cm long stem was placed in each Petri dish with the stem inserted through the hole at the bottom of the Petri dish and the lid was replaced. Each Petri dish with a hibiscus leaf was placed on a cup of water so that the stem below the petiole was immersed in water. The development and survival of *P. marginatus* was initially evaluated at five constant temperatures of 15, 20, 25, 30, and 35°C, all ± 1°C. Eggs maintained at 15 and 35°C hatched but these nymphs were unable to complete their first instar development. Three new temperatures of 18, 34, and 37°C, all ± 1°C were later included in the experiment to find out more about the egg and the first instar development. For each temperature, 35 gravid females were collected from the colony to obtain eggs. To acclimatize them for each temperature, gravid females were kept individually on hibiscus leaves prepared as above, and were transferred to environmental growth chambers (TCI model, Environmental Growth Chambers, Chagrin Falls, OH) at the experimental temperatures, 65 ± 2% R.H., and a photoperiod of 14:10 (L:D), 48 hours before the experiment. Eggs were collected within 24 hours of oviposition. Ten eggs collected from a single female were placed on each hibiscus leaf arranged in a Petri dish prepared as above. There were 35 replicates for each temperature. Immediately after placing the eggs, the Petri dishes were transferred to each environmental growth chamber with the specific temperature. Petri dishes were checked daily for egg hatch and molting. When eggs started to hatch, chambers were

maintained in complete darkness for 72 hours to encourage the first-instar nymphs (crawlers) to settle on the leaves. The gender of each individual was determined during the latter part of the second instar when the males change their color from yellow to pink. At this point, the developmental times of males and females were counted separately. The developmental time and the survival of eggs and first instars were not separated by gender. The number of surviving individuals at each stage was counted.

Reproduction. To determine the effect of constant temperatures on reproduction of *P. marginatus*, newly emerged males and females were separated as soon as they emerged as adults at 18, 20, 25, and 30°±1°C. Each adult female was placed on a hibiscus leaf, which was arranged in a Petri dish prepared as above. Each female was provided with 2-3 newly emerged adult males to ensure mating. Each female represented a replicate. There were 35 replicates for each temperature. The number of eggs laid by each female was counted daily. The number of days for the pre-oviposition period (number of days from adult emergence to oviposition) and the oviposition period (time between beginning and end of oviposition) were also counted.

Adult Longevity. The number of days from adult emergence to death was evaluated at four constant temperatures (18, 20, 25, and 30°±1°C) for both males and females. Each individual was placed in a Petri dish prepared as mentioned above. For each temperature, 35 individuals (replicates) each of both males and females were evaluated.

Developmental Thresholds and Thermal Constant. A linear regression analysis (PROC REG) (SAS Institute 1999) was carried out to calculate the thermal constant and lower developmental threshold (T_{min}) for *P. marginatus*, using rate of development (reciprocal of development) from egg to adult against the constant temperatures used. The linear degree-day model (thermal summation model) estimates the relationship between temperature and the rate of

development in a linear relationship (Campbell et al. 1974). This linear relationship is $Y = a + bT$, where Y is the rate of development (1/days), T, ambient temperature (°C), and the regression parameters intercept (a) and slope (b). The thermal constant K ($1/b$) is the number of degree-days above the threshold summed over the developmental period. Lower developmental threshold T_{min} ($-a/b$) is the minimum temperature at which the rate of development is zero or no measurable development occurs.

To describe the developmental rate over a wider temperature range, a nonlinear model (Logan 6 model) was used to calculate the upper developmental threshold (T_{max}) and the optimum temperature threshold (T_{opt}) (Logan et al. 1976). The upper developmental threshold T_{max} is the maximum temperature at which the rate of development becomes zero and life processes can no longer be maintained for a prolonged period. The optimum temperature T_{opt} is the temperature at which the maximum rate of development occurs (Walgama et al. 2006). The Logan model does not estimate the lower developmental threshold (T_{min}), because it is asymptotic to the left of the temperature axis. The relationship between developmental rate (1/D) and upper developmental threshold is described in the Logan 6 model as,

$$1/D = \psi\left[\exp(\rho T) - \exp(\rho T_{\max} - \frac{T_{\max} - T}{\Delta T})\right],$$

where ψ is a directly measurable rate of temperature dependent physiological process at some base temperature, ρ is the biochemical reaction rate and ΔT is the temperature range over which 'thermal breakdown' becomes the overriding influence (Logan et al. 1976). To determine the optimum temperature (T_{opt}) for development, the following equation (Logan et al. 1976) was used.

$$T_{opt} = T_{\max}\left[1 + \varepsilon(\frac{\ln(\varepsilon b_o)}{1 - \varepsilon b_o})\right]$$

Here, ε is $\Delta T / T_{max}$ and b_0 is ρT_{max}.

Statistical Analysis. The experimental design used for all experiments was completely random. Prior to the statistical analysis, the mean of the individuals in each Petri dish/replicate was calculated and used in the analyses. One-way analysis of variance (ANOVA) was performed using a general linear model (GLM) for all experiments (SAS Institute, Cary, NC). Means were compared at $P = 0.05$ significance level using the Tukey's HSD test. Proportions of females (sex ratio) and survival were square-root arcsine-transformed using

$$p' = \arcsin\sqrt{p}$$

where p = proportion of female/survival, to adjust the variances (Zar 1984) prior to ANOVA, but the untransformed data were presented in the tables.

A linear regression was performed to find the linear relationship between rate of development and temperature and to estimate the parameters a and b (PROC REG) (SAS Institute 1999). A non-linear regression (PROC NLIN) (SAS Institute 1999) was performed for the non-linear section of the relationship between rate of development and temperature to find the estimates for the parameters, ψ, ρ, T_{max} and ΔT of the Logan 6 model.

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 and Survival. Eggs hatched at all temperatures except 37°C (Table 3-1). The duration of development of all stages decreased with increasing temperatures. The egg developmental time was the same at 34 and 35°C. The egg developmental time at 15°C was approximately 5 times longer than the developmental time at 35°C. Eggs hatched at 15, 34, and 35°C were unable to complete their first-instar development (Table 3-1). The percent survival of

eggs increased with increasing temperature until 30°C, above which temperature the survival started to decrease (Table 3-2). First-instar developmental time at 18°C was more than four times longer than the developmental time at 30°C (Table 3-1). Developmental times for male and female nymphal stages, and cumulative adult male, were not different at 25 and 30°C (Table 3-1). The cumulative developmental time for the female was decreased over the temperature range from 18-30°C (Table 3-1).

A low percentage of eggs survived at 35°C (Table 3-2). Survival of first and second-instars was lowest at 18°C. The cumulative adult percent survival increased with increasing temperatures over the range from 18 to 30°C (Table 3-2). Since the gender of the eggs was difficult to differentiate, cumulative survival from egg to adult was not separately calculated for each gender.

Reproduction. Pre-oviposition and oviposition periods decreased with increasing temperatures with no difference at 25 and 30°C (Table 3-3). Fecundity increased from 18 to 25°C, and then drastically decreased at 30°C (Table 3-3). Females lived longer at lower temperatures than at higher temperatures, and with no difference at 25 and 30°C (Table 3-3). Adult male longevity was shorter at 25°C than that of at 18 and 20°C (Table 3-3). The proportion of females was lowest at 25°C (Table 3-3).

Thermal Requirements for Development. Between the temperatures of 18 to 25°C, there were excellent linear fits ($R^2 \geq 0.94$; P=<0.0001) for developmental rate versus temperature in the linear degree-day model for egg, male and female nymphal stages, and cumulative numbers of adult males and females (Table 3-4). Thermal constants (K) for development rates of egg, male and female nymphal stages were 100.0, 204.8, and 175.4 degree-days (DD), respectively. For cumulative development of adult male and female, the thermal constants were

303.0 and 294.1 DD, respectively. The estimated lower developmental thresholds (T_{min}) for egg, male and female nymphal stages were 13.3, 14.8, and 14.3°C respectively. For cumulative development of adult males and females, the estimated lower developmental threshold, T_{min} were 14.5 and 13.9°C respectively.

For the non-linear section of the developmental rate against temperature, the Logan 6 model also provided excellent fits (Pseudo- $R^2 \geq 0.97$; P=<0.0001) for each developmental stadium (Table 3-4). The estimated optimum temperatures (T_{opt}) for the developmental rates of egg and male and female nymphal stages were 34.8, 27.9 and 28.3°C, respectively. For adult male and female, estimated optimum temperatures were 28.7 and 28.4°C, respectively. The estimated maximum temperature thresholds (T_{max}) for egg, male and female nymphal stages were 41.6, 30.5 and 31.7° C respectively. For adult male and female papaya mealybug, the estimated T_{max} were 31.9 and 32.1°C, respectively.

Discussion

Insect systems function optimally within a limited range of temperatures. For a majority of insects, enzyme activity, tissue functioning, and the behavior of the whole insect is optimal at a relatively high temperature often in the range of 30-40°C (Chapman 1998). Temperature had a significant effect in the development of *P. marginatus*. Overall, the linear degree-day model and the nonlinear Logan 6 model, which were used in predicting temperature and developmental rate relationships in insects, estimated minimum, optimum, and maximum temperature thresholds for *P. marginatus* close to results obtained in this experiment.

The development of adult female *P. marginatus* was arrested at an estimated minimum temperature threshold of 14.5°C and a maximum temperature threshold of 31.9°C. It reached its optimal development at about 28.7°C. The cumulative developmental times of both male and female mealybugs at 18°C were three times longer than the developmental times at 30°C.

Although *P. marginatus* was unable to develop and complete its life cycle at 15°C, the Madeira mealybug, *Phenacoccus madeirensis* Green, a commonly-found mealybug species in greenhouses in Southeastern US with a worldwide distribution and with a wide host range (Ben-Dov 1994), was able to develop, reproduce and survive well at 15°C (Chong et al. 2003). At 15°C, the developmental time of female *P. madeirensis* was 66 days and was twice as long as the developmental time at 25°C (Chong et al. 2003). The minimum, optimum, and maximum temperature threshold for the female pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green), a polyphagus mealybug and a serious pest of many economically important crops, were 14.5, 29.0, and 35°C respectively. At 20°C, the developmental time of the female, *M. hirsutus* was 66 days, and was twice the developmental time at 30°C (Chong et al. manuscript in review).

High fecundity in insects is an important adaptation for a successful next generation. In nature, eggs of any insect can be exposed to natural enemies and other environmental factors such as wind, rain, sunlight, and radiation. Although *P. marginatus* females were able to develop in a shorter time and had a higher survival at 30°C than at the other tested temperatures, the fecundity at 30°C was considerably lower than at 20 and 25°C. The drastic drop in fecundity at 30°C suggests that even though the developmental time was shorter and survival was higher at 30°C than at 25°C, *P. marginatus* may have reached its optimal temperature for development and reproduction between the temperatures 25 and 30°C. The optimal temperature for development estimated using the Logan 6 model, for the female nymphal stage and the cumulative adult female was within this range, thus supporting the results obtained. Other mealybug species such as *P. madeirensis* and *M. hirsutus* showed differences in fecundity with increasing temperature and were able to reproduce successfully and increase their populations at temperatures such as 25°C. Compared to the fecundity at 20°C, the total number of eggs laid at

25°C was significantly lower for *P. madeirensis* (Chong et al. 2003). At 25°C, a female *P. madeirensis* can lay 288 eggs in 8 days and was able to emerge as an adult within 30 days (Chong et al. 2003). Similar to *P. marginatus*, the fecundity at 30°C was significantly lower for *M. hirsutus* compared to the fecundity at 25°C and adult females emerged within 31 days at 25°C and laid 300 eggs within 7 days (Chong et al. manuscript in review). At 25°C, female *P. marginatus* can emerge as an adult within about 26 days and can produce as many as 300 eggs in approximately 11 days. With its short life cycle, high survival and reproductive capacity, *P. marginatus* has a tremendous ability to increase its population to levels that can cause economic damage unless suitable management practices are implemented.

Although continuous constant temperatures were used in these experiments, in nature temperature can vary during the day and especially at night. Warmer day temperatures and colder night temperatures in the natural environment may allow *P. marginatus* to develop and survive at a higher temperature than 30°C. Developing *Oncopeltus* eggs reared at varying temperatures developed faster and used less metabolic energy than at the equivalent mean constant temperatures (Gordan 1999). The living system may be better adapted to normal environmental fluctuations than to an artificial constant state (Gordan 1999).

This information may also be helpful in monitoring the susceptible stages of *P. marginatus* for application of integrated management practices including releasing of biological control agents. The longer developmental time of eggs and immature stages, may increase the vulnerability by prolonged exposure to natural enemies and insecticides. On the other hand, at higher temperatures mealybugs grow quickly and become adults 2-3 times faster than at lower temperatures, allowing them an opportunity to reduce exposure time and presumably to increase survival and ultimately reproduce.

The estimated thermal constants for eggs and female *P. marginatus* were 100.0 and 294.1 DD, respectively, while those of *M. hirsutus* were 101.7 and 347.2 DD, respectively (Chong et al. manuscript in review). The estimated minimum temperature threshold for *P. citri* is 10.9°C and thermal constants for adult female at constant and fluctuating temperatures were 289 and 365 DD, respectively (Laflin and Parrella 2004). The thermal constant for females is considerably lower for *P. marginatus* compared to that of *M. hirsutus*. Although, *P. marginatus* and *P. citri* had similar thermal constants at constant temperature, the minimum temperature threshold for *P. citri* was much lower than that of *P. marginatus*. *Planococcus citri* has a wider distribution in the US compared to currently available information on the distribution of *M. hirsutus* (Ben-Dov 1994). Tropical insect species have higher values of minimum temperature thresholds than temperate insect species, and the thermal constants decrease with the increase of minimum temperature threshold (Trudgill et al. 2005). Considering its high minimum temperature thresholds and the low thermal constants, *P. marginatus* should have a smaller distribution range than anticipated earlier.

The developmental threshold and the thermal constant of an insect possibly are useful indicators of its potential distribution and abundance (Messenger 1959). Estimated developmental temperatures combined with degree-days were useful in predicting *Planococcus citri* (Risso) in greenhouse cut flower production in California, in temperatures maintained at 25 to 30°C (Laflin and Parrella 2004). The information on developmental temperatures combined with degree-days from this study, should be useful in predicting possible spread of *P. marginatus* in different areas in the US, the Caribbean, and the Pacific. According to the comparative climatic data from the National Climatic Data Center (NCDC 2005), some areas in Southern California, Southern Texas, Hawaii, and Florida have daily average temperatures that are

suitable for the development of *P. marginatus*. A large number of economically important fruits, vegetables and ornamental plants are grown in Southern California including citrus, avocado, beans, hibiscus and plumeria. Southern Texas has the third largest citrus production in the US (CNAS 2007). In Hawaii, where *P. marginatus* is already established on the big island and small islands of Maui, Oahu, and Kauai, a large number of fruits, vegetables, and ornamentals are grown including papaya, hibiscus, and plumeria. Papaya is the second most important fruit crop in Hawaii, after pineapple, and according to the National Agricultural Statistics Service (USDA 2007b), Hawaii currently grows 864 ha of papaya. In Florida, where approximately 100 ha of papaya are grown (Mossler and Nesheim 2002), *P. marginatus* has been found in most of the counties of Central and South Florida (Walker et al. 2003). Distribution and establishment of *P. marginatus* in the areas in California and Texas that are suitable with regard to temperature can be influenced by other factors such as type of crops grown and regulation of plant movement from state to state.

Paracoccus marginatus has the potential to spread to Southern California and Texas. These states produce economically important crops, which are also favored by *P. marginatus*. If *P. marginatus* spread to California and Texas through the movement of plants or commodity, we will expect the damage to be significant unless suitable control measures such as biological control and restrictions of movement of susceptible plants and commodities to uninfected areas are implemented in a timely manner to slow down the spread.

Table 3-1 Mean number of days (± SEM) for each developmental stadium of *P. marginatus* reared at different constant temperatures

T (°C)	Developmental Stadia							Cumulative	
	Egg	First	Second		Third		Fourth		
			Male	Female	Male	Female	Male	Male	Female
15	27.5 ± 0.2a	-	-	-	-	-	-	-	-
18	23.1 ± 0.2b	25.3 ± 0.5a	21.1 ± 1.6a	13.5 ± 1.3a	7.0 ± 1.8a	13.2 ± 0.9a	11.7 ± 1.8a	85.2 ± 1.8a	74.4 ± 1.4a
20	14.4 ± 0.2c	14.6 ± 0.5b	13.6 ± 0.8b	9.3 ± 0.7b	4.5 ± 0.7ab	8.9 ± 0.9b	8.9 ± 0.7a	53.4 ± 0.7b	45.9 ± 0.9b
25	8.7 ± 0.1d	6.5 ± 0.1c	6.6 ± 0.5c	5.5 ± 0.5c	2.4 ± 0.5b	5.2 ± 0.2c	4.1 ± 0.5b	28.5 ± 0.3c	25.9 ± 0.2c
30	7.3 ± 0.2e	6.1 ± 0.2c	6.3 ± 0.4c	5.7 ± 0.4c	2.6 ± 0.4b	4.4 ± 0.3c	3.6 ± 0.4b	24.9 ± 0.6c	23.2 ± 0.3d
34	5.9 ± 0.1f	-	-	-	-	-	-	-	-
35	5.5 ± 0.1f	-	-	-	-	-	-	-	-
F	1922.10	400.59	57.41	17.09	5.35	15.31	15.66	725.42	521.23
df	6, 212	3, 132	3, 97	3, 101	3, 87	3, 90	3, 91	3, 84	3, 90
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0020	<0.0001	<0.0001	<0.0001	<0.0001

n = 35

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

Table 3-2 Mean (± SEM) percent survival for each developmental stadium of *P. marginatus* reared at different constant temperatures.

Temp.	Developmental Stadia						
	Egg	First	Second	Third		Fourth	Egg to Adult
				Male	Female	Male	
15	60.9 ± 3.3cd	-	-	-	-	-	-
18	80.0 ± 2.9b	54.1 ± 4.7d	80.1 ± 4.8b	96.4 ± 1.9ab	73.2 ±7.3	98.4 ± 1.6a	30.5 ± 4.4c
20	90.1 ± 2.1a	77.2 ± 3.0c	94.1 ± 1.3a	98.1 ± 1.0a	83.5 ± 3.8	75.4 ± 5.7c	41.4 ± 3.5bc
25	83.3 ± 3.4ab	83.2 ± 4.3bc	97.5 ± 1.3a	89.2 ± 3.1b	79.9 ± 4.8	86.7 ± 2.9bc	51.4 ± 4.0b
30	85.9 ± 3.3ab	90.5 ± 2.5ab	91.0 ± 4.0ab	96.9 ± 1.5ab	92.6 ± 2.9	97.8 ± 1.6a	70.8 ± 4.9a
34	73.4 ± 5.1bc	-	-	-	-	-	-
35	33.4 ± 6.9d	-	-	-	-	-	-
37	0.0 ± 0.0						
F	16.70	16.52	4.89	3.46	1.82	10.36	15.43
df	6, 205	3, 131	3, 122	3, 91	3, 90	3, 89	3, 121
P	<0.0001	<0.0001	<0.0030	<0.0196	<0.1494	<0.0001	<0.0001

n = 35

Means within a column followed by the same letters are not significantly different at $\alpha = 0.05$ (Tukey's HSD Test)

Table 3-3 Mean (± SEM) proportion of females, adult longevity, fecundity, pre-oviposition and oviposition periods of *P. marginatus* reared at four constant temperatures

Temperature (°C)	Proportion of Females	Adult Longevity (Days)		Fecundity	Pre-oviposition Period (Days)	Oviposition Period (Days)
		Male	Female			
18	69.4 ± 8.2a	5.5 ± 0.5a	40.2 ± 1.1a	160.6 ± 13.8cd	16.7 ± 0.7a	19.6 ± 1.0a
20	81.7 ± 3.6a	4.8 ± 0.3a	35.7 ± 1.0b	231.6 ± 12.8bc	13.5 ± 0.5b	21.4 ± 1.1a
25	42.6 ± 5.3b	2.9 ± 0.2b	21.1 ± 0.7c	300.2 ± 40.4ab	6.8 ± 0.4c	11.4 ± 0.8b
30	71.1 ± 4.2a	-	19.2 ± 1.4c	82.0 ± 11.7d	7.6 ± 0.7c	11.6 ± 1.4b
F	10.38	14.91	93.25	15.13	70.33	24.99
df	3, 89	2, 73	3, 92	3, 92	3, 92	3, 92
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

n = 35

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

Table 3-4 Summary of statistics and the estimates (± SE) of the fitted parameters of the linear thermal summation model and the nonlinear Logan 6 model.

Statistics Parameters	Developmental Stadia				
	Egg	♀ Nymphal	♂ Nymphal	Total ♀	Total ♂
Thermal Summation Model: $Y = a + bT$					
F	1640.79	1286.51	2606.13	1653.01	3493.67
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
df	1, 113	1, 69	1, 63	1, 69	1, 67
R^2	0.9356	0.9491	0.9764	0.9599	0.9812
a ± SE	-0.13 ± 0.01	-0.08 ± 0.01	-0.07 ± 0.01	-0.05 ± 0.01	-0.05 ± 0.01
b ± SE	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
Logan 6 Model: $1/D = \psi\left[\exp(\rho T) - \exp(\rho T_{max} - \frac{T_{max} - T}{\Delta T})\right]$					
F	22354.5	6092.2	5620.06	10500.7	10252.5
P	< 0.0001	<0.0001	< 0.0001	< 0.0001	<0.0001
SS_R	0.00024	0.00003	0.00003	0.000009	0.000007
SS_{CT}	0.01177	0.00228	0.00221	0.00102	0.00099
Pseudo-R^2	0.9796	0.9868	0.9864	0.9912	0.9929
Ψ ± SE	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
ρ ± SE	0.11 ± 0.02	0.15 ± 0.04	0.21 ± 0.04	0.13 ± 0.03	0.15 ± 0.03
T_{max} ± SE	41.56 ± 0.99	31.71 ± 1.25	30.52 ± 0.31	32.09 ± 1.24	31.99 ± 1.52
ΔT ± SE	5.24 ± 0.06	1.93 ± 0.46	1.62 ± 0.42	2.05 ± 0.36	1.88 ± 0.47

Y = rate of development (1/days); T = ambient temperature (°C); a = intercept; b = slope; K ($1/b$) = thermal constant; T_{min} ($-a/b$) = lower developmental threshold; SS_R = residual sums of squares; SS_{CT} = corrected total sums of squares; pseudo-R^2 = $1-SS_R/SS_{CT}$ ψ = rate of temperature dependent physiological process at some base temperature; ρ = biochemical reaction rate; ΔT = temperature range over which 'thermal breakdown' becomes the overriding influence; T_{opt} = optimum temperature threshold; T_{max} = upper developmental threshold

CHAPTER 4
HOST STAGE SUSCEPTIBILITY AND SEX RATIO, HOST STAGE SUITABILITY, AND INTERSPECIFIC COMPETITION OF A*cerophagus papayae*, *Anagyrus loecki*, AND *Pseudleptomastix mexicana*: THREE INTRODUCED PARASITOIDS OF *Paracoccus marginatus* WILLIAMS AND GRANARA DE WILLINK

Introduction

Knowledge of host selection is indispensable for the efficient use of parasitoids, both for mass rearing and biological control of pests. A parasitoid's biology may be greatly influenced by the quality of the host. Host stage is an important ecological variable, which may have an influence on a parasitoid's rate of attack, survival of its immature stages, and sex ratio of its offspring (Waage 1986). Host selection behavior is most important in determining the sex ratio of arrhenotokous parasitoids, which show a haplodiploid sex determination mechanism (King 1987). A female parasitoid can manipulate the offspring sex ratio at oviposition by regulating fertilization. A particular host size may be more suitable for the development of one sex, so that, in general, a female-biased offspring sex ratio is produced from the larger hosts and a male biased one from the smaller hosts (King 1987).

Solitary parasitoids generally determine the host quality by the size of the host. Large hosts are supposed to be better quality, as they are believed to contain more resources than small ones. However, host size may not always be equated to host quality at the time of oviposition by a parasitoid. The influence of host size on parasitoid development may differ between idiobiont and koinobiont parasitoids (Waage 1986). Idiobiont parasitoids oviposit in host stages such as egg and pupa, or paralyze their hosts prior to oviposition (Waage 1986) and on the other hand, koinobiont parasitoids, which do not paralyze their hosts at the time of parasitism, and allow hosts such as larval stages to grow, for which host size is not directly a representative of larval resources.

Sympatric parasitoid species that share the same host species may be competitors (Van Strien-van Liempt 1983). The greater the part of the host population that is exploited by both species, the more they will affect each other's population density. Their competitive abilities then, among other factors, determine their relative abundance (Van Strien-van Liempt 1983). In solitary insect parasitoids, generally only one offspring survives in a host (Vinson 1976). Females normally deposit one egg per host and reduce the host availability to both conspecific and heterospecific parasitoids. Successful oviposition of a female depends on how efficient she is in finding and parasitizing un-parasitized hosts. This leads to interspecific competition among the parasitoids in classical biological control, where more than one parasitoid species is used (Lawrence 1981).

Classical biological control was identified as an important pest management practice for *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae), a polyphagus mealybug species that was first identified in the US, in Florida in 1998 (Miller and Miler 2002). *Paracoccus marginatus* is a pest of a large number of topical and subtropical fruits, vegetables, and ornamentals (Miller and Miler 2002). Currently there are three parasitoid species (Hymenoptera: Encyrtidae) used in the classical biological control of *P. marginatus* in the US, the Caribbean, and the Pacific islands (Meyerdirk et al. 2004). *Acerophagus papayae* Noyes and Schauff, *Anagyrus loecki* Noyes and Menezes, and *Pseudleptomastix mexicana* Noyes and Schauff are currently mass reared in Puerto Rico and released by the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) in areas infested with *P. marginatus* (Meyerdirk et al. 2004). These parasitoids have been released in Guam and the Republic of Palau, and were successful in controlling *P. marginatus* in these countries (Meyerdirk et al. 2004, Muniappan et al. 2006).

There is very little information available on the biology of *A. papayae*, *A. loecki*, and *P. mexicana*, or on how efficient they are in the control of *P. marginatus*. Knowledge of host selection and interspecific competition of parasitoids should lead to better understanding of the population dynamics of the host and the parasitoids. Hence, they are important in evaluating and understanding the success of biological control and integrated pest management programs. The present study focused on the host stage susceptibility and sex ratio, host stage suitability, and interspecific competition of *A. papayae*, *A. loecki*, and *P. mexicana*, three introduced koinobiont parasitoids of *P. marginatus.*

Materials and Methods

Rearing Mealybugs. *Paracoccus marginatus* was initially collected from a papaya (*Carica papaya* L.) field in Homestead, FL. Sprouted red potatoes (*Solanum tuberosum* L.) (Ryan Potato Company, East Grand Forks, MN) were used to rear a colony of *P. marginatus* at the University of Florida, Tropical Research and Education Center (TREC), Homestead, FL. Prior to sprouting, potatoes were soaked in a 1% solution of bleach (Clorox ®, The Clorox Company, Oakland CA; 6% sodium hypochlorite) for 15 minutes, then rinsed with water, and dried. Potatoes were placed in black cotton cloth bags to encourage sprouting. These bags were kept inside a dark room at 27° ± 1°C and 65 ± 2% R.H. Each week, 36 sprouted potatoes were infested with ovisacs to maintain the colony. An environmental growth chamber (Percivel I-36LL, Percival Scientific Inc. Perry, NC) was used at 25° ± 1°C, 65 ± 2% R.H., and a photoperiod of 12:12 (L:D) to rear the mealybug colony.

To obtain a particular stage of mealybugs, newly laid ovisacs were selected from the mealybug colony. A tender leaf with a 5 cm long stem was obtained from hibiscus (*Hibiscus rosa-sinensis* L.) plants that were purchased from a local nursery and maintained in a shadehouse at TREC. A 9-cm-diam Petri dish with a 0.6-cm-diam hole at the bottom was used to place the

hibiscus leaf. The stem of each hibiscus leaf was inserted through the hole in the Petri dish and each dish was kept on a 162 ml translucent plastic soufflé cup (Georgia Pacific Dixie, Atlanta, GA) filled with water, which allowed the stem below the petiole to be in water. On each leaf, a single ovisac was placed and the eggs were allowed to hatch and then develop into the desired stage. 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 in this study to reduce the variation in host quality.

Rearing Parasitoids. The potatoes with second and third instar *P. marginatus* were used for parasitoid rearing. These potatoes were initially infested with ovisacs of *P. marginatus*, obtained from the mealybug colony. Colonies of *A. papayae*, *A. loecki*, and *P. mexicana* were maintained in an insectary at TREC at 25° ± 2°C temperature, a 12:12 (L:D) photoperiod, and 65 ± 2% R.H. Initial colonies of parasitoids were obtained from the Biological Control Laboratory, Department of Agriculture, Puerto Rico, through USDA-APHIS. Parasitoid colonies were established in plexiglass cages (30x30x30 cm) using sprouted red potatoes infested with *P. marginatus*. In order to obtain a continuous supply of newly emerged parasitoids, mealybug-infested potatoes were provided to each parasitoid species weekly, and potatoes with parasitized mealybugs were moved to a new cage. A solution of honey and water (1:1) was streaked on 4 pieces (5x5 cm) of Benchkote surface protector paper (Fisherbrand ®, Fisher Scientific, Pittsburgh, PA) attached to the cage using labeling tape (Fisherbrand ®, Fisher Scientific, Pittsburgh, PA). Water was provided in two clear plastic 73.9-ml containers (Tristate Molded Plastic Inc., North Dixon, KY) per cage. In each container, 1-cm-diam 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.

To obtain mated-female parasitoids, newly emerged females of each species were placed singly in glass disposable culture tubes (1.2x7.5 cm) (Fisherbrand ®, Fisher Scientific, Pittsburgh, PA) and closed with two-ply tissue (Kimwipes ® EX-L, Kimberly-Clerk Global Sales Inc. Roswell, GA) secured with a piece of rubber tubing (0.95x 2.5 cm) (Fisherbrand ®, Fisher Scientific, Pittsburgh, PA). Five newly emerged males were placed in each tube with a female and were allowed to mate for 24 hours. A streak of honey and water (1:1) was provided for each tube. After 24 hours, males were removed from each tube and the female was used in the experiment. All experiments were carried out at 25° ± 2°C temperature, a 12:12 (L:D) photoperiod, and 65 ± 2% R.H.

Host Stage Susceptibility and Sex Ratio. For host stage susceptibility, a no-choice test was carried out for first and second instars, third-instar nymphs (female), and for newly emerged adult females. A hibiscus leaf with a 5 cm long stem was selected as the experimental unit. Petri dishes were prepared as mentioned previously. The leaf was placed inside the Petri dish with the stem inserted through the hole and then the lid was replaced. The Petri dish with the leaf was placed on a cup filled with water with the stem inserted in the water as mentioned above. For each mealybug stage, ten individuals were selected 24 hours before the experiment and placed on each hibiscus leaf using a paintbrush (No.000) (American Painter 4000, Loew-Cornell Inc., Englewood Cliffs, NJ). These 10 individuals of each mealybug stage were considered as a single experimental unit and a replicate. The dishes were covered with a piece of black cotton cloth, encouraging mealybugs to settle on leaves. A streak of honey and water (1:1) was placed on the inside of the lid. After 24 hours of placing the mealybugs in the Petri dish, a single mated female

parasitoid obtained as mentioned above was placed on each leaf and the lid was replaced. The Petri dish was covered with a piece (15x15 cm) of chiffon cloth material (Jo-Ann Fabrics and Crafts, Miami, FL), and secured with a rubber band to avoid parasitoid escape. Each parasitoid was allowed to oviposit for 24 hours, and then it was removed from the Petri dish. Mummified mealybugs were individually placed in glass culture tubes and were secured as mentioned previously. Parasitoids emerged from these tubes were sexed and the proportion of parasitism was estimated. There were 25 replicates for each mealybug stage and parasitoid combination.

Host Stage Suitability. To test host stage suitability, choice tests were conducted with two host stage combinations. For mealybugs, five individuals from each stage were used in the combinations of the second instar and third-instar female, the second instar and adult female, and the third instar and adult female. A mated female parasitoid was introduced to each Petri dish, and was allowed to oviposit for 24 hours and then removed. Immediately after removal of the parasitoid, five individuals of one stage of the host in each Petri dish were moved to a new hibiscus leaf prepared as above. The five individuals of the other host stage remained on the hibiscus leaf. These leaves were prepared at the same time as the other leaves. This was done for easier identification of mummified hosts for a particular stage. The choice tests were carried out in a similar manner as the host-stage susceptibility tests. The number and the gender of parasitoids emerging from the mummified mealybugs were recorded for each parasitoid species. Each mealybug combination for each parasitoid species had 25 replicates.

Interspecific Competition. Interspecific competition of parasitoids was studied using 10 individuals from the second instar and third-instar females. Each host stage was separately placed on a hibiscus leaf as prepared above. The parasitoid combinations used were *A. papayae* and *A. loecki*, *A. papayae* and *P. mexicana*, *A. loecki* and *P. mexicana*, and *A. papayae*, *A. loecki*,

and *P. mexicana*. A mated female of each parasitoid species was used in all combinations. They were allowed to parasitize for 24 hours, and were then removed. The mealybugs were allowed to mummify on the hibiscus leaves and mummified mealybugs were treated similar to those mentioned above. The number and the species of parasitoids, which emerged from each combination in each mealybug instar were counted. The mean percent parasitism was calculated from the 10 mealybugs used for each host stage in each parasitoid combination. Each parasitoid combination for each host stage had 25 replicates.

Statistical Analysis. The experimental design was completely random for all experiments. A two-way analysis of variance (ANOVA) was performed using a general linear model (PROC GLM) of SAS (SAS Institute 1999) to find the interaction between parasitoids and host stages of *P. marginatus* in host stage susceptibility and sex ratio experiments. Means were compared at $P = 0.05$ significance level using least square means (LSMEANS) of SAS (SAS Institute 1999). For host stage suitability tests, means between two stages of *P. marginatus* for each parasitoid species were compared at $P = 0.05$ significance level using a t-test (PROC TTEST) of SAS (SAS Institute 1999). In interspecific competition studies, PROC GLM was used for significance among the parasitoids and means were compared at $P = 0.05$ significance level using least square means (LSMEANS). Proportions of females (sex ratio) and proportions of parasitism were arcsine transformed using

$$p' = \arcsin\sqrt{p}$$

where p = proportion of female/parasitism, to adjust the variances (Zar 1984) prior to ANOVA, but untransformed data are presented in tables.

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 the Tropical Research and Education Center, University of Florida.

Results

Host Stage Susceptibility and Sex Ratio. All three parasitoids were able to develop and emerge successfully in second instar, third-instar females, and adult females of *P. marginatus* (Table 4-1). No parasitoids emerged from first-instar nymphs. The mean percent parasitism decreased with increasing host size for both *A. papayae* and *P. mexicana*.

The proportion of emerged female parasitoids increased with increasing host size (Table 4-2). Although *A. papayae* had a similar number of males and females that emerged from second-instar hosts, *A. loecki* and *P. mexicana* had a lower number of females than males emerging from second instars. In the third instar and the adult females, all three parasitoid species had higher female than male emergence.

Host Stage Suitability. *Acerophagus papayae* and *P. mexicana* preferred the second instar to the third-instar female and the adult female, while *A. loecki* preferred the third-instar female and the adult female to the second instar (Table 4-3). Between the third-instar female and the adult female, *A. papayae* and *A. loecki* preferred the third-instar mealybugs while *P. mexicana* had no preference.

Interspecific Competition. *Acerophagus papayae* had a higher mean percent parasitism when present with either *A. loecki* or *P. mexicana* or both in second-instar hosts (Table 4-4). In third-instar females, *A. loecki* had a higher parasitism when present with either *A. papayae* or *P. mexicana* or both. Overall, *P. mexicana* had a lower mean percent parasitism when present with either *A. papayae* or *A. loecki* or both except for the presence with *A. loecki* in the second-instar hosts.

Discussion

Size of the host is one of the factors that solitary endoparasitoids consider when they select a host stage for oviposition (Vinson and Iwantsch 1980). *Acerophagus papayae*, *A. loecki*, and *P. mexicana* did not prefer first-instar nymphs as a suitable host stage for parasitoid development. This makes the first-instar nymph of *P. marginatus*, which is approximately 0.4 mm in size (Miller and Miler 2002), less vulnerable to these parasitoids.

In parasitoid behavioral studies, first-instar *Rastrococcus invadens* Williams were preferred for host feeding by the parasitoid, *Anagyrus mangicola* Noyes (Bokonon-Ganta et al. 1995). Parasitoids such as *Anagyrus kamali* Moursi can oviposit in first-instar nymphs of *Maconellicoccus hirsutus* Green but the percent parasitism was less than 20% (Sagarra and Vincent 1999). In most situations, the ovipositor of *A. kamali* remained stuck within the first-instar host, precluding further foraging of the parasitoid (Sagarra and Vincent 1999). The second-instar nymphs of *Planococcus citri* (Risso) were often impaled on the ovipositor of *Anagyrus pseudococci* (Girault), thus preventing the female from further egg deposition (Islam and Copland 1997).

By choosing a larger host, the parasitoid accessed a larger food supply and increased the fitness of its progeny. In larger hosts, a female biased progeny was recorded for many parasitoids (King 1987). Further, increased host size translates into both increased male and female fitness (Charnov et al. 1981). For females this measure is the lifetime production of eggs, and for males, it is the length of life (Charnov et al. 1981). Although, all three parasitoid species of *P. marginatus* were able to develop and complete their life cycle in second-instar hosts, only *A. papayae* produced a higher proportion of female progeny. Having more males than females in its progeny is not a desirable characteristic for a parasitoid to have as an efficient biological control agent. The solitary endoparasitoid, *Aenasius vexans* Kerrich, which were able to oviposit

in second-instar nymphs of *Phenacoccus herreni* Cox and Williams also recorded a considerably higher proportion of males in the second instar than in the larger instars of *P. herreni* (Bertschy et al. 2000).

Except for the parasitism of *A. loecki* in second-instar *P. marginatus*, the percent parasitism of all three parasitoid species decreased with increasing host size. Mealybugs show strong physical defense and escape behavior, which could be increased with the body size. The third-instar *P. citri*, which was often encountered by the parasitoid *A. pseudococci*, showed strong physical defense and escape behavior (Islam and Copland 1997). The higher success of oviposition in the second-instar *P. marginatus* may be due to less or absence of these defense and escape behavior in early instar mealybugs.

Interspecific competition was evident by *A. papayae*, *A. loecki*, and *P. mexicana*, when competing for same host instar. Out of the three parasitoid species, *A. papayae* had the highest parasitism level indicating its superior ability to compete. Intensive studies of parasitic complexes in connection with biological control programs have shown that interspecific competition can be extremely important (Schroder 1974). This may be one reason, why *A. papayae* was well established and recovered from the field in the Republic of Palau (Muniappan et al. 2006). In field tests conducted in Florida in 2005 and 2006, both *A. papayae* and *A. loecki* were recovered, but *P. mexicana* was not (Chapter 6). *Pseudleptomastix mexicana* was also not recovered in the field studies conducted in the Republic of Palau (Muniappan et al. 2006). This information suggests that *P. mexicana* may be less competitive than the other two parasitoids.

Since the preference for a host stage is similar for *A. papayae* and *P. mexicana* but different for *A. loecki*, it reduces the competitiveness between *A. papayae* and *A. loecki*. Different host stage preference of *A. papayae* and *A. loecki* also greatly reduces competition for

the same host stage. However, the developmental time of *A*. *loecki*, which is similar to the developmental time of *A*. *papayae*, is shorter than the developmental time of its preferred host stage of *P*. *marginatus* (Chapter 5). Developmental times of *A*. *papayae* and *A*. *loecki* coincide with the developmental time of the second instar *P*. *marginatus* (Chapter 5). At the beginning of the season with the absence of overlapping generations of *P*. *marginatus*, *A*. *loecki* and *A*. *papayae* can compete for second instars due to unavailability of preferred third instar host stages for *A*. *loecki*. In addition to being a parasitoid of *P*. *marginatus*, *A*. *loecki* can develop in *Dysmicoccus hurdi* and *Phenacoccus madeirensis* Green (Hemiptera: Pseudococcidae) (Noyes 2000). *Phenacoccus madeirensis* is one of the commonly found mealybug species in South Florida. Since *A*. *loecki* is not host-specific, it has the advantage of searching for other suitable hosts such as *P*. *madeirensis* in the absence of suitable stages of *P*. *marginatus*. This may be one reason for the lower parasitism of *A*. *loecki* observed in the field studies and less competitiveness of *A*. *loecki* in the control of *P*. *marginatus* (Chapter 6). On the other hand, *P*. *mexicana*, which also prefers the second instar *P*. *marginatus* (as does *A*. *papayae*) has longer developmental time than the other two parasitoids (Chapter 5). Developmental time of *P*. *mexicana* does not coincide with the developmental time of the second instar *P*. *marginatus*. This allows *A*. *papayae* females for which developmental time of the host stage overlaps with its developmental time, to parasitize preferred host stages when it emerges as an adult. In mass rearing of parasitoids, second-instar *P*. *marginatus* is a suitable stage for *A*. *papayae* and *P*. *mexicana* while third-instar females are suitable for *A*. *loecki*.

Females of *A*. *papayae* and *P*. *mexicana* had a similar host stage preference for parasitism while it was a different host stage preference for female *A*. l*oecki*. *Acerophagus papayae* shows superior adaptability by being able to oviposit in second instar to adult-female *P*. *marginatus* as

well as causing a higher percent parasitism when present with either *A. loecki* or *P. mexicana* or both. The information gathered from this study, will be helpful in explaining the adaptability of these three parasitoids of *P. marginatus* in the field.

Table 4-1 Mean percent parasitism (± SEM) of *A. papayae*, *A. loecki*, and *P. mexicana* reared in different developmental stages of *P. marginatus* to evaluate host stage susceptibility using no-choice tests.

	Mean Percent Parasitism (%) for Developmental Stages of *P. marginatus*		
Parasitoid	Second	Third female	Adult female
A. papayae	82.8 ± 2.1aA	71.2 ± 2.6bB	60.8 ± 2.9bC
A. loecki	41.2 ± 2.8cB	82.4 ± 1.9aA	74.8 ± 3.2aA
P. mexicana	70.8 ± 1.9bA	50.8 ± 2.5cB	40.8 ± 3.6cB
	ANOVA Results		
Source	F	df	*P*
Model	36.32	8, 216	<0.0001
Parasitoid	34.06	2, 216	<0.0001
Stage	8.67	2, 216	0.0002
Parasitoid*Stage	51.27	4, 216	<0.0001

n = 25

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 (LSMEANS) Test).

Table 4-2 Mean proportion of females (sex ratio) (± SEM) of *A. papayae*, *A. loecki*, and *P. mexicana* reared in different developmental stages of *P. marginatus* to evaluate host stage susceptibility using no-choice tests.

	Mean Proportion of Females (Sex Ratio) (± SEM) for Developmental Stages of *P. marginatus*		
Parasitoid	Second	Third-female	Adult-female
A. papayae	0.50 ± 0.01aB	0.56 ± 0.01aA	0.57 ± 0.01abA
A. loecki	0.40 ± 0.01cC	0.51 ± 0.01bB	0.54 ± 0.01bA
P. mexicana	0.48 ± 0.01bC	0.55 ± 0.01aB	0.56 ± 0.01aA
	ANOVA Results		
Source	F	df	*P*
Model	72.34	8, 216	<0.0001
Parasitoid	73.68	2, 216	<0.0001
Stage	196.98	2, 216	<0.0001
Parasitoid*Stage	9.35	4, 216	<0.0001

n = 25

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 (LSMEANS) Test).

Table 4-3 Mean percent parasitism (± SEM) of *A. papayae*, *A. loecki*, and *P. mexicana* reared in different stage combinations of *P. marginatus* to evaluate host stage suitability using choice tests.

Host stage combination of *P. marginatus*		Parasitoid	Mean (± SEM) Percent Parasitism		T Statistics		
Stage 1	Stage 2		Stage 1	Stage 2	t	df.	P
Second	Third- Female	*A. papayae*	77.6 ± 1.8	58.4 ± 2.6	6.54	48	<0.0001
		A. loecki	30.4 ± 2.9	76.0 ± 2.0	-13.60	48	<0.0001
		P. mexicana	69.6 ± 2.6	40.8 ± 3.6	6.73	48	<0.0001
Second	Adult-Female	*A. papayae*	76.8 ± 1.9	50.4 ± 4.2	-5.98	48	<0.0001
		A. loecki	32.0 ± 3.1	68.8 ± 2.6	9.11	48	<0.0001
		P. mexicana	68.8 ± 2.6	32.0 ± 3.3	-8.78	48	<0.0001
Third	Adult-Female	*A. papayae*	60.0 ± 3.1	48.0 ± 5.0	-1.81	48	0.0471
		A. loecki	79.2 ± 0.8	64.8 ± 3.1	-4.58	48	<0.0001
		P. mexicana	41.6 ± 3.4	32.8 ± 3.2	-1.85	48	0.0691

n = 25

Host stage combinations: second instar (Stage 1) and third-instar female (Stage 2), second instar (Stage 1) and adult female (Stage 2), and third-instar female (Stage 1) and adult female (Stage 2).

Table 4-4 Mean percent parasitism (± SEM) of combinations of *A. papayae*, *A. loecki*, and *P. mexicana* reared in second and third-instar *P. marginatus* to evaluate interspecific competitions of parasitoids.

Stage of *P. marginatus*	Combination of Parasitoids			Mean (± SEM) Percent Parasitism		
Second	Parasitoid 1	Parasitoid 2	Parasitoid 3	*A. papayae*	*A. loecki*	*P. mexicana*
	A. papayae	*A. loecki*	-	69.6 ± 2.3A	19.6 ± 1.7B	-
	A. papayae	-	*P. mexicana*	78.4 ± 1.2A	-	20.0 ± 1.3B
	-	*A. loecki*	*P. mexicana*	-	40.4 ± 3.5B	50.4 ± 3.3A
	A. papayae	*A. loecki*	*P. mexicana*	59.6 ± 2.9A	14.8 ± 1.8C	23.6 ± 2.1B
Third	*A. papayae*	*A. loecki*	-	42.4 ± 3.3B	54.8 ± 3.5A	-
	A. papayae	-	*P. mexicana*	59.2 ± 4.3A	-	35.6 ± 3.5B
	-	*A. loecki*	*P. mexicana*	-	75.2 ± 2.7A	20.4 ± 1.4B
	A. papayae	*A. loecki*	*P. mexicana*	38.8 ± 4.4B	47.6 ± 4.5A	11.2 ± 0.6C
	ANOVA Results					
Source	F	df	*P*			
Model	49.8	17, 432	<0.0001			
Stage	0.71	1, 432	0.4887			
Combination	61.67	8, 432	<0.0001			
Stage*Combination	44.09	8, 432	<0.0001			

N = 25

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

CHAPTER 5
DEVELOPMENTAL TIME, LONGEVITY, AND LIFETIME FERTILITY OF *Acerophagus papayae*, *Anagyrus loecki*, AND *Pseudleptomastix mexicana*; THREE INTRODUCED PARASITOIDS OF *Paracoccus marginatus* WILLIAMS AND GRANARA DE WILLINK

Introduction

Developmental time, longevity, and lifetime fertility are important fitness parameters when evaluating a biological control agent. Determining developmental time of a parasitoid is necessary to determine its efficiency in controlling the host. Generally, the developmental time of a biological control agent should be shorter than the developmental time of the host (Greathead 1986). According to Greathead (1986), high fecundity and short generation time are some of the desirable characters of a parasitoid. Courtship and mating are energy and time consuming activities in insects, which can affect the outcome of the longevity, lifetime fecundity, and progeny production of hymenopteran parasitoids (Ridley 1988). Mating is required to achieve their full reproductive potential in some 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). Some parasitoid species are arrhenotokous, e.g. fertilized eggs lead to female progeny and unfertilized eggs give rise to males. Lifetime fertility or progeny production of a parasitoid is important in its long-term establishment as a biological control agent. A parasitoid with higher lifetime fertility with female-biased progeny can parasitize a higher number of hosts. Female-biased progeny are desirable in classical biological control (King 1987).

Classical biological control was identified as an important pest management practice for *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae), a polyphagus mealybug species that was first identified in the US in Florida in 1998 (Miller and Miler 2002). *Paracoccus marginatus* is a pest of a large number of tropical and subtropical

fruits, vegetables, and ornamentals (Miller and Miler 2002). Prior to invading the US, *P. marginatus* had been established in the Caribbean since 1994 (Miller et al. 1999). After the establishment in Florida, *P. marginatus* was identified in the Pacific islands of Guam (Meyerdirk et al. 2004), the Republic of Palau (Muniappan et al. 2006), and several Hawaiian islands (Heu et al. 2007). With the joint efforts of the Dominican Republic, Puerto Rico, and the US (Walker et al. 2003), currently there are three parasitoids (Hymenoptera: Encyrtidae) used in the classical biological control of *P. marginatus* in the US, the Caribbean, and the Pacific islands (Meyerdirk et al. 2004). The three solitary endoparasitoid species, *Acerophagus papayae* Noyes and Schauff, *Anagyrus loecki* Noyes and Menezes, and *Pseudleptomastix mexicana* Noyes and Schauff are currently mass reared in Puerto Rico and released in *P. marginatus* affected areas by the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) (Meyerdirk et al. 2004). They have been released in Guam, and the Republic of Palau, and are successfully controlling *P. marginatus* (Meyerdirk et al. 2004, Muniappan et al. 2006).

No information is available on the fitness parameters of these parasitoids of *P. marginatus*. Fitness parameters of parasitoids are specifically important when evaluating their efficiency and understanding long-term effects in a system where more than one parasitoid species have been released as classical biological control agents. This study focuses on the developmental time, longevity, and the lifetime fertility of *A. papayae*, *A. loecki*, and *P. mexicana*, three introduced parasitoids of *P. marginatus*.

Materials and Methods

Rearing Mealybugs. Red potatoes (*Solanum tuberosum* L.) (Ryan Potato Company, East Grand Forks, MN) were allowed to sprout and then used in rearing a colony of *P. marginatus* at the University of Florida, Tropical Research and Education Center (TREC), Homestead, FL.

Initially, *P. marginatus* was collected from a papaya (*Carica papaya* L.) field in Homestead, FL. Prior to sprouting, potatoes were soaked in a 1% solution of bleach (Clorox ®, The Clorox Company, Oakland CA; 6% sodium hypochlorite) for 15 minutes, and then rinsed with water, air-dried and placed in black cotton cloth bags to encourage sprouting. Bags were kept inside a dark room at 27° ± 1°C and 65 ± 2% R.H. Each week, 36 sprouted potatoes were infested with *P. marginatus* ovisacs to maintain the colony. An environmental growth chamber (Percivel I-36LL, Percival Scientific Inc. Perry, NC) was used at 25° ± 1°C, 65 ± 2% R.H., and a photoperiod of 12:12 (L:D) to rear the mealybug colony.

To obtain a particular stage of mealybugs, the newly laid ovisacs were selected and each was reared on a hibiscus leaf inside a 9-cm-diam Petri dish with a 0.6-cm-diam hole made in the bottom. Leaves were obtained from hibiscus (*Hibiscus rosa-sinensis* L.) plants maintained in a shadehouse at TREC. A tender hibiscus leaf with a 5-cm-long stem was placed in each Petri dish with the stem inserted through the hole at the bottom of the Petri dish. Each Petri dish was kept on a 162 ml translucent plastic soufflé cup (Georgia Pacific Dixie, Atlanta, GA) filled with water, which allowed the stem below the petiole to be in water. A single ovisac was placed on each leaf and the eggs were allowed to hatch and then to develop to the desired stage. It was not possible to differentiate the sex of the mealybugs for the first and second instar while the third-instar nymphs and the adults used were females. To reduce the variation in each mealybug instar used, newly molted individuals recognized by their size and the presence of shed exuviae were selected for each experiment.

Rearing Parasitoids. Colonies of *A. papayae*, *A. loecki*, and *P. Mexicana* were maintained in an insectary at TREC at 25° ± 2°C temperature, a 12:12 (L:D) photoperiod, and 65 ± 2% R.H. Initial colonies of parasitoids were obtained from the Biological Control Laboratory,

Department of Agriculture, Puerto Rico, through USDA-APHIS. Parasitoid colonies were established in plexiglass cages (30x30x30 cm) using sprouted red potatoes with second and third-instar *P. marginatus*. In order to obtain a continuous supply of newly emerged parasitoids weekly, potatoes with second and third-instar mealybugs were provided to each parasitoid species every week. After 7 days, potatoes were moved to a new cage for parasitoid emergence and new mealybug-infested potatoes were provided for oviposition. A solution of honey and water (1:1) was streaked on 4 pieces (5x5 cm) of Benchkote surface protector paper (Fisherbrand ®, Fisher Scientific, Pittsburgh, PA) and attached to the cage using labeling tape, for emerging parasitoids (Fisherbrand ®, Fisher Scientific, Pittsburgh, PA). Water was provided in two clear plastic 73.9 ml containers (Tristate Molded Plastic Inc., North Dixon, KY) per cage. In each of the containers, a 1-cm-diam 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.

To obtain mated female parasitoids for the experiments, newly emerged female parasitoids of each species were selected, and placed singly in glass disposable culture tubes, (1.2x7.5 cm) (Fisherbrand ®, Fisher Scientific, Pittsburgh, PA) closed with two-ply tissue, (Kimwipes ® EX-L, Kimberly-Clerk Global Sales Inc. Roswell, GA) and secured with a piece of rubber tubing (0.95x2.5 cm) (Fisherbrand ®, Fisher Scientific, Pittsburgh, PA). For each tube with a female, five newly emerged males were added and allowed to mate for 24 hours. A streak of honey and water (1:1) was provided for each tube. After 24 hours, males were removed from each tube, and the mated female was used in the experiment. All experiments were carried out at 25° ± 2°C temperature, a 12:12 (L:D) photoperiod, and 65 ± 2% R.H.

Specimens of *P. marginatus*, *A. papayae*, *A. loecki*, and *P. mexicana* were sent to Systematic Entomology Laboratory (SEL), USDA, Beltsville, MD, for species verification.

Developmental Time. To evaluate the developmental time of each parasitoid species in different mealybug instars, 10 individuals each from second instar, third-instar females, and adult female *P. marginatus* were selected and placed separately on new hibiscus leaves. The Petri dishes with leaves were prepared 48 hours before the experiment. Mealybugs were placed on the leaves 24 hours before the experiment to allow them to settle on the leaves. The 10 individuals of each mealybug stage on a hibiscus leaf were considered a replicate and were used as an experimental unit. A mated female parasitoid was placed in each Petri dish and the lid was replaced. Each Petri dish with the lid was covered with a piece (15x15 cm) of chiffon cloth material (Jo-Ann Fabrics and Crafts, Miami, FL) and secured with a rubber band to avoid parasitoid escape, before it was placed on a cup of water with the stem submerged. Parasitoids were allowed to oviposit for 24 hours and were then removed. Parasitized mealybugs became mummified on the hibiscus leaves. Mummies were individually placed in disposable, glass culture tubes and closed with two-ply tissue and secured with a piece of clear polyvinyl chloride (PVC) tubing. These tubes with the parasitized mealybugs were held in the insectary until the emergence of adults. The time for adult emergence and the sex of the adults were noted and the mean of the 10 individuals on each hibiscus leaf was used in analyses for developmental time and sex ratio of each parasitoid. This procedure was followed for all three parasitoid species with 50 replicates for each species.

Longevity. Longevity was studied for three mating conditions for each female parasitoid (unmated, mated-without oviposition, and mated-with oviposition), and two mating conditions for each male parasitoid (unmated and mated). To collect both males and females for each

species, third-instar mealybugs on sprouted potatoes were placed in plexiglass cages and mated females were released to each cage. After 24 hours, females were removed and mealybugs were allowed to mummify. Mummified mealybugs were collected, and were individually placed 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 individually transferred 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 before placing the lid. A circular area of 8.5-cm-diam was removed from the 12-cm-diam 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 hours, and then the females were removed. The mated males were retained in the culture tube. For mated females without-oviposition, one female was placed in a glass culture tube with a streak of honey, and five males were provided for 24 hours and then the males were removed and the females were retained. The same procedure was followed for the mated females with oviposition, except they were individually transferred to clear plastic 500 ml deli cups and provided with mealybugs on potatoes to oviposit as for unmated females with oviposition, as mentioned above. The number of days each parasitoid lived was counted for both males and females in all the above mating conditions. These procedures were repeated for all three parasitoid species. For each mating condition in each sex, 50 replicates were used.

Lifetime Fertility. Lifetime fertility of mated and unmated females of each parasitoid species was studied. A newly emerged virgin female was either held alone or allowed to mate with five newly emerged males for 24 hours in a glass culture tube provided with a streak of honey and water. After removing the males, the females were individually transferred to clear plastic 3.8 liter round jars (Rubbermaid ®, Newell Rubbermaid Inc. Atlanta, GA). Before placing the lid, each jar was covered with a piece of chiffon cloth material. A 9-cm-diam 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 approximately 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 Analysis. The experimental design was completely random for all experiments. A two-way analysis of variance (ANOVA) was performed using a general linear model (GLM) (SAS Institute 1999) to find interaction between parasitoids and mealybug instar for developmental time, parasitoids and mating conditions in the longevity experiment, and for reproductive period, and male and cumulative progeny in the lifetime fertility study. Means were compared at $P = 0.05$ significance level using least square means (LSMEANS) (SAS Institute 1999). For the developmental time and the longevity studies, means were compared within the mealybug instar and mating condition for each parasitoid, and among the parasitoids for each mealybug instar and mating condition. One-way ANOVA was performed using a general linear model (GLM) for number of female progeny and sex ratio. Means were compared

at $P = 0.05$ significance level using the Tukey's HSD test. The proportion of female (sex ratio) was square-root arcsine-transformed by using

$$p' = \arcsin\sqrt{p}$$

where, p = proportion of female, to adjust the variances (Zar 1984) prior to ANOVA, but untransformed data were presented in tables.

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 the Tropical Research and Education Center, University of Florida, Homestead, FL 33031.

Results

Developmental times were shorter with increasing host age for male *A. papayae* and *P. mexicana*, and female *A. loecki* (Table 5-1). *Acerophagus papayae* and *A. loecki* had shorter developmental times for both males and females, compared to male and female developmental times of *P. mexicana*.

Longevity was highest for *P. mexicana* and the lowest was for *A. papayae* in all mating conditions with increasing host size for both males and females (Table 5-2). There was no difference in the longevity between unmated and mated males in all three species. The longevity was similar for females that were unmated and mated, both without oviposition. The females that were unmated and mated both with oviposition had similar longevity in each species but lived a shorter time than the ones that did not oviposit.

Unmated females of all three species produced male progeny, and *A. loecki* and *P. mexicana* produced more progeny than *A. papayae* (Table 5-3). In mated females, there were more male and female progeny for *A. loecki* and *P. mexicana* than for *A. papayae*. The progeny of all three species had similar sex ratios with approximately 1:1 for male:female. The

reproductive period was longest for *P. mexicana*, and *A. papayae* had the shortest reproductive period.

Discussion

Differences in fitness parameters including developmental time, longevity, and the lifetime fertility of *A. papayae*, *A. loecki*, and *P. mexicana* are useful in evaluating them as efficient biological control agents of *P. marginatus*. There were differences in developmental time, longevity, and the lifetime fertility of *A. papayae*, *A. loecki*, and *P. mexicana*.

Although increasing host size had a significant effect on developmental time of male *A. papayae*, *A. loecki*, and *P. mexicana*, developmental times of female *A. papayae*, and *P. mexicana* were not influenced by host size. Host stage had affected developmental time of other mealybug parasitoids as well. Developmental time of *Anagyrus dactylopii* (Howard) was not different among the various stages of *Maconellicoccus hirsutus* (Green) (Mani and Thontadarya 1989). However, *Anagyrus kamali* Moursi, a parasitoid of *M. hirsutus*, had shorter developmental times when reared in the third instar and adult female *M. hirsutus* than when reared in the first and second-instar *M. hirsutus* (Sagarra and Vincent 1999). *Anagyrus kamali* had similar developmental times in the first and second instars, and in third and adult females of *M. hirsutus* (Sagarra and Vincent 1999). *Aenasius vexans* Kerrich, an encyrtid parasitoid of cassava mealybug, *Phenacoccus herreni* Cox and Williams, had a shorter developmental time in older hosts than in early instar *P. herreni* (Bertschy et al. 2000). Developmental time of female *Anagyrus pseudococci* (Girault), a koinobiont endoparasitoid of citrus mealybug, *Planococcus citri* (Risso) was similar on second and third instar, and adult *P citri*, but the developmental time of male *A. pseudococci* was longer on second instars than on third instar and adult *P. citri* (Chandler et al. 1980, Islam and Copland 1997). *Gyranusoidea tebygi* Noyes, a parasitoid of mango mealybug, *Rastrococcus invadens* Williams, when developed on second and third instar

had similar developmental times compared to the longer developmental time in first instar *R. invadens* (Bovida et al. 1995a). However, *Anagyrus mangicola* Noyes, the primary parasitoid of *R. invadens*, had a similar developmental time on different host stages of *R. invadens* with no differences in the size of emerging parasitoids (Cross and Moore 1992).

When the developmental time of a parasitoid is shorter than the developmental time of the host, there is an advantage for the parasitoid. Later in the season with overlapping host generations, it can produce its progeny at a faster rate than the host and can parasitize the host populations in a shorter time. The adult female *P. marginatus* can develop on hibiscus in 25.9 days, and the second instar and third-instar females can emerge within 15.2 and 20.8 days respectively (Chapter 2). *Acerophagus papayae* and *P. mexicana* prefer second instars compared to third-instar *P. marginatus* (Chapter 4). Developmental time of female *A. papayae* overlaps the developmental time of the second-instar *P. marginatus*, providing an advantage for *A. papayae* over female *P. mexicana,* which needs a longer time to emerge as adults in second-instar *P. marginatus*. Although *A. loecki* prefers third instars compared to second-instar *P. marginatus* (Chapter 4), its developmental time is shorter than the developmental time of the third-instar *P. marginatus*. Early in the season with the absence of overlapping generations of mealybugs, the longer developmental time of the third-instar *P. marginatus*, which does not overlap the emergence of *A. loecki*, allows *A. loecki* able to parasitize the available second instar *P. marginatus*. When developed in second-instar *P. marginatus*, *A. loecki* produces male-biased progeny compared to *A. papayae*, which produces female-biased progeny (Chapter 4). Male-biased progeny would be less desirable than female-biased progeny in biological control (King 1987). However, because it is not a host-specific parasitoid of *P. marginatus*, female *A. loecki*

has the ability to select suitable stages of its other hosts such as Madeira mealybug, *Phenacoccus madeirensis* Green (Noyes 2000), in the absence of preferred host stages of *P. marginatus*.

In parasitic Hymenoptera, female eggs are preferentially laid in larger hosts compared to male eggs, which are laid in smaller hosts (King 1987). Hosts that were parasitized at different stages may represent resources of different quality during parasitoid development and the wasp may have adapted its sex allocation accordingly (Bertschy et al. 2000). Although both male and female *P. mexicana* are larger than male and female *A. papayae* (Noyes and Schauff 2003), *P. mexicana* females prefer to lay their eggs in the second instar rather than in third-instar *P. marginatus* (Chapter 4). Since the developmental time of *P. mexicana* is longer than the developmental times of *A. papayae* and *A. loecki*, a second-instar host may be more desirable than a third instar for the development of *P. mexicana*.

A fitness parameter such as the lifetime fertility of a parasitoid is important in long-term establishment of the parasitoid as a biological control agent. A parasitoid species with more female progeny has the ability to parasitize a higher number of hosts than one with fewer female progeny. Body size of a parasitoid is frequently related to fecundity, longevity, and host finding ability (Hemerik and Harvey 1999). A significant relationship between size and both longevity and lifetime fecundity was found in fitness parameter studies in *Trichogramma evanescens*, a gregarious, polyphagous egg parasitoid (Doyon and Boivin 2005). The smallest of the three species (Noyes 2000, Noyes and Schauff 2003), *A. papayae* produced the least progeny compared to *A. loecki* and *P. mexicana*, both of which produced twice the progeny of *A. papayae*. In addition, *A. papayae* had the shortest lifespan compared to *A. loecki* and *P. mexicana* for both males and females with different mating status. Females of all three parasitoid species outlived males. This has been recorded in other parasitoids species as well. In

the longevity studies of *Anagyrus kamali* Moursi, females lived longer than the males (Sagarra et al. 2000b).

There are 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 in evaluating their efficiency as parasitoids of *P. marginatus*. 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*, and *P. mexicana*.

Table 5-1 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*.

Mean Developmental Time of Parasitoids (Days)				
		Stage of *P. marginatus*		
Sex	Parasitoid	Second-instar	Third-instar female	Adult-female
Male	*A. papayae*	13.8 ± 0.2bA	13.5 ± 0.2bAB	13.1 ± 0.2bB
	A. loecki	13.7 ± 0.2bA	13.4 ± 0.2bAB	13.1 ± 0.3bB
	P. mexicana	21.8 ± 0.2aA	21.5 ± 0.2aAB	21.0 ± 0.2aB
	ANOVA Results			
	Source	F	df	*P*
	Model	339.74	8, 4414	<0.0001
	Parasitoid	1350.27	2, 441	<0.0001
	Stage	8.61	2, 441	0.0002
	Parasitoid*Stage	0.04	4, 441	0.9970
Female	*A. papayae*	14.8 ± 0.2bA	14.5 ± 0.2bAB	14.1 ± 0.2bB
	A. loecki	14.7 ± 0.2bA	14.4 ± 0.2bAB	14.0 ± 0.2bB
	P. mexicana	22.9 ± 0.2aA	22.7 ± 0.2aAB	22.1 ± 0.3aB
	ANOVA Results			
	Source	F	df	*P*
	Model	328.75	8, 441	<0.0001
	Parasitoid	1306.44	2, 441	<0.0001
	Stage	8.42	2, 441	0.0003
	Parasitoid*Stage	0.06	4, 441	0.9927

n = 50

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 (LSMEANS) Test) for males and females

Table 5-2 Mean longevity in days (± SEM) for male (unmated and mated), and female (unmated, mated-without oviposition, and mated-with oviposition) *A. papayae*, *A. loecki*, and *P. mexicana*.

Sex	Mating Condition	Longevity (Days) Parasitoid		
		A. papayae	*A. loecki*	*P. mexicana*
Male	Unmated	23.3 ± 0.4C	37.3 ± 0.7B	47.5 ± 1.8A
	Mated	22.0 ± 0.4C	36.6 ± 0.5B	45.9 ± 0.9A
	ANOVA Results			
	Source	F	df	*P*
	Model	141.97	5, 294	<0.0001
	Parasitoid	353.58	2, 294	<0.0001
	Mating Status	2.41	1, 294	0.1216
	Parasitoid* Mating Status	0.14	2, 294	0.8722
Female	Mating Status	*A. papayae*	*A. loecki*	*P. mexicana*
	Unmated-without oviposition	33.1 ± 0.6aC	48.9 ± 1.0aB	63.1 ± 1.8aA
	Unmated-with oviposition	13.8 ± 0.2bC	23.9 ± 0.5bB	41.1 ± 0.7cA
	Mated-without oviposition	32.3 ± 1.0aC	47.6 ± 1.2aB	58.4 ± 1.2bA
	Mated-with oviposition	13.9 ± 0.3bC	23.0 ± 0.4bB	40.1 ± 0.7cA
	ANOVA Results			
	Source	F	df	*P*
	Model	322.95	11, 588	<0.0001
	Parasitoid	922.75	2, 588	<0.0001
	Mating Status	558.98	3, 588	<0.0001
	Parasitoid* Mating Status	5.01	6, 588	<0.0001

n = 50

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 (LSMEANS) Test) for males and females.

Table 5-3 Mean (± SEM) number 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 ± 1.0c	48.3 ± 1.2b	92.8 ± 1.9c	0.52 ± 0.006	13.9 ±0.7c
	A. loecki	97.8 ± 1.3b	99.8 ± 1.6a	197.6 ± 2.5a	0.51 ± 0.004	20.1 ± 0.7b
	P. mexicana	103.0 ± 3.4b	105.9 ± 3.3a	208.9 ± 6.6a	0.50 ± 0.013	30.8 ± 0.9a
Unmated	*A. papayae*	88.0 ± 2.9b	-	88.0 ± 2.9c	-	11.9 ± 0.6c
	A. loecki	173.2 ± 10.2a	-	173.2 ± 10.2b	-	18.3 ± 0.7b
	P. mexicana	159.5 ± 7.7a	-	159.5 ± 7.7b	-	31.6 ± 0.9a
	ANOVA Results					
Model	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

Means within a column followed by the same lowercase letters are not significantly different at α = 0.05 (Least Square Means (LSMEANS) Test) for number of male progeny, cumulative progeny, and reproductive period.

Means within a column followed by the same lowercase letters are not significantly different at α = 0.05 (Tukey's HSD test) for number of female progeny and sex ratio.

CHAPTER 6
FIELD ASSESSMENT OF THREE INTRODUCED PARASITOIDS OF *Paracoccus marginatus* WILLIAMS AND GRANARA DE WILLINK (HEMIPTERA: PSEUDOCOCCIDAE)

Introduction

Paracoccus marginatus Williams and Granara de Willink is a polyphagous pest insect that can damage fruits, vegetables and ornamentals, including *Carica papaya* L. (papaya), *Hibiscus* spp. L. (hibiscus), *Citrus* spp.(citrus), *Persea americana* Mill. (avocado), and *Solanum melongena* L. (eggplant) (Miller and Miller 2002). This mealybug species was first described in 1992 (Williams and Granara de Willink 1992) and was re-described in 2002 (Miller and Miller 2002). Believed to be native to Mexico or Central America, *P. marginatus* has been established in the Caribbean since 1994 (Miller et al. 1999). In 1998, *P. marginatus* was first detected in the US, in Palm Beach County, Florida on hibiscus. Since then, it has been found on more than 25 genera of plants in the US. In recent years, *P. marginatus* has invaded the Pacific islands, and it is now established in Guam (Meyerdirk et al. 2004), the Republic of Palau (Muniappan et al. 2006), and in several Hawaiian islands (Heu et al. 2007).

Paracoccus marginatus potentially poses a threat to numerous agricultural products in the US especially in Florida and states such as California, Hawaii, and Texas, which produce similar crops. Classical biological control was identified as an important component in the management of *P. marginatus* and a program was initiated as a joint effort among the United States Department of Agriculture, Puerto Rico Department of Agriculture, and Ministry of Agriculture in the Dominican Republic in 1999 (Walker et al. 2003). Currently, there are three solitary endoparasitoid hymenopterans mass reared in Puerto Rico, and released in *P. marginatus* infested areas in the US, the Caribbean, and some Pacific islands (Meyerdirk et al. 2004). They are *Acerophagus papayae* Noyes and Schauff, *Anagyrus loecki* Noyes and Menezes, and

Pseudleptomastix mexicana Noyes and Schauff (Hymenoptera: Encyrtidae) (Noyes and Schauff 2003). In July 2003, *A. papayae*, *A. loecki*, and *P. mexicana* were obtained from the Biological Control Laboratory, Department of Agriculture, Puerto Rico, and released in 21 locations in South Florida, in Miami-Dade and Broward counties (11 locations in Miami, 5 locations in Homestead, and 5 locations in Pembroke Pines and Miramar) (D. M. Amalin, personal communication). A total of 6,000 parasitoids (1,400 *A. papayae*, 1,200 *A. loecki*, and 3,400 *P. mexicana*) were released in South Florida in a single release attempt in July 2003 (D. M. Amalin, personal communication). No subsequent releases have been recorded.

Information on parasitoids of *P. marginatus* and the field evaluation of their effectiveness is limited in the US. Assessing the effect of a natural enemy or natural enemy complex on its/their host populations in the field is important to evaluate the success of a biological control project (Neuenschwander et al. 1986). This could be done by comparison of two separate pest populations, one population with the natural enemy and the other without (Hodek et al. 1972). Pest populations without natural enemies can be found either in pre-release situations or can be created artificially, by using physical or chemical means to exclude the natural enemy from the plot (Smith and DeBach 1942). Experimental exclusion methods are the fastest and most direct way to demonstrate the effect of a natural enemy on a pest population (Smith and DeBach 1942). In this field study, a physical exclusion method using sleeve cages was used to find the ability of *A. papayae*, *A. loecki*, and *P. mexicana* to control *P. marginatus* in Homestead, FL.

Materials and Methods

Insect Rearing. A colony of *P. marginatus* was reared on sprouted red potatoes (*Solanum tuberosum* L.) at the University of Florida, Tropical Research and Education Center (TREC), Homestead, FL. Initially, *P. marginatus* was collected from a papaya (*Carica papaya* L.) field in Homestead, FL. Prior to sprouting, the potatoes (Ryan Potato Company, East Grand Forks, MN)

were soaked in a 1% solution of bleach (Clorox ®, The Clorox Company, Oakland CA; 6% sodium hypochlorite), for 15 minutes, and then rinsed with clean water and dried. Potatoes were placed in black cotton cloth bags to encourage sprouting. The bags were kept inside a dark room at 27° ± 1°C. Each week, 36 sprouted potatoes were infested with *P. marginatus* ovisacs to maintain the colony. Depending on the size, each potato was infested with 3 to 5 ovisacs. The infested potatoes were kept in 3.8-L plastic containers (Rubbermaid ®, Newell Rubbermaid Inc. Atlanta, GA) with 12 potatoes per container. To facilitate the air circulation to developing eggs and mealybugs, screens (Amber Lumite ®, Bio Quip, Gardena, CA) were glued to the cut sections of lids of these plastic containers. The mealybug colony was maintained in an environmental growth chamber (Percivel I-36LL, Percival Scientific Inc. Perry, NC) at 25° ± 1°C, 65 ± 2% R.H., and a photoperiod of 14:10 (L:D).

Field Experiments. The research plots were selected at three homeowner locations in Homestead, FL. The field experiments were carried out in July to August 2005 and 2006, using the same experimental locations in both years. *Paracoccus marginatus* was observed in all three locations at the time of selection. In each location, 10 hibiscus (*Hibiscus rosa-sinensis* L.) plants, approximately 2.5 to 3.0 m tall, were selected. Each selected plant was considered a replicate. The three treatments used in this experiment were closed sleeve cage, open sleeve cage, and no cage. The sleeve cages were made of white chiffon cloth material (Jo-Ann Fabrics and Crafts, Miami, FL), 72 cm in length and 50 cm in width. Along the length of the material, a groove was sewn at 15 cm from each end. The piece of cloth with the groove was then folded in half along the width, and the two ends along the width were placed together and sewn at the edge to make a cylinder of 15 cm diameter. A piece of stainless steel (20 gauge) wire (Tower Manufacturing Company, Madison, IN), 72 cm in length was inserted through each groove and

tied at the ends to make a ring to shape the cage into a cylindrical cage. Three branches 1-1.5 m above ground were selected from each hibiscus plant. The branches selected were evenly distributed among the hibiscus plants, and each branch had 7-10 leaves. All the selected branches were cleaned with moist tissues (Kimwipes ® EX-L, Kimberly-Clerk Global Sales Inc. Roswell, GA) to make them free from any insects and eggs. Each clean branch was enclosed in a closed sleeve cage mentioned above for 7 days to observe for any insect presence or development. To avoid the cloth material of the cage getting in contact with the leaves, a stainless steel wire (22 gauge and 25 cm in length) was tied to the branch at the middle at each end of the cage, and the ends were fixed to the cage along the diameter. Sleeves of the cage were secured with a stainless steel wire tied around the enclosed branch. During this time, all enclosed branches were checked daily for the presence of any insects by opening the sleeve at the terminal end of the branch of each cage. If any insects were observed in a cage, the branch was cleaned again using the above procedure.

After 7 days, five gravid females of *P. marginatus* collected from the mealybug colony, were carefully placed on the terminal leaves of the branch within each sleeve cage using a paint brush (No.000) (American Painter 4000, Loew-Cornell Inc., Englewood Cliffs, NJ). Immediately after placing the females, the open sleeve was tied back on to the branch, closing the cage. Approximately 21 days was allowed for the gravid females to lay eggs and the eggs to develop into second and third-instar mealybugs. When the number of second and third instars was at a 1:1 ratio by visual inspection, all the sleeve cages were removed, and were replaced according to the three treatments mentioned above. Each of the three treatments was randomly assigned among the three branches on each plant, using cages similar to those described above and placing them over the branches infested with mealybugs. In the closed sleeve cage

treatment, cages were kept closed. The purpose of this treatment was to evaluate the development of mealybugs in the microclimate created inside a closed cage. In the open sleeve cage treatment, the cages were left open and the sleeves were folded back along the cylindrical part of the cage and were fixed to the cage with four safety pins. The purpose of this treatment was to provide the parasitoids access to the mealybugs and to provide microclimate conditions similar to the closed sleeve cage treatment. In the no cage treatment, branches with mealybug colonies were left un-caged. This treatment was used to assess the effect of the sleeve cages themselves on the mealybug population growth and parasitism level. The treatments were checked for mealybug destroyer adults and larvae (*Cryptolaemus montrouzieri* Mulsant), ants, and spiders at 24, 48, and 72-hour intervals without disturbing the treatments.

At 72 hours, all treatments were covered with closed sleeve cages, and the branches were removed from the plant. Cages were brought to the laboratory, and the number of mealybugs was noted. The number of adults and larvae of the mealybug destroyer (coccinellid predator), ants, and spiders was also recorded. From each replicate, 100 second and third-instar mealybugs were randomly collected and placed on a sprouting potato for further development. These potatoes were kept singly in 500 ml deli cups (Georgia Pacific Dixie, Atlanta, GA). Each cup was covered with a piece of chiffon cloth held in a place with the cup lid with a circular area of 8.5 cm diam removed to facilitate air circulation. The cups were held in an insectary, maintained at 25° ± 1°C, 12:12 (L:D) photoperiod, and 65 ± 2 % R.H. Mealybugs were allowed to mummify on potatoes. Collection of mummified mealybugs was started 10 days after placing them on potatoes. Mummified mealybugs were placed individually in disposable, glass culture tubes of 1.2 cm diameter and 7.5 cm length (Fisherbrand ®, Fisher Scientific, Pittsburgh, PA). Each tube was covered with two-ply tissue (Kimwipes ® EX-L, Kimberly-Clerk Global Sales

Inc. Roswell, GA), secured with 2.5-cm- long piece of clear polyvinyl chloride (PVC) tubing (Fisherbrand ®, Fisher Scientific, Pittsburgh, PA) until the emergence of parasitoids. The emerging parasitoids from the culture tubes were sexed and were identified as to their species. Samples of parasitoids, mealybug destroyers, and ants were sent to the Systematic Entomology laboratory, USDA, Beltsville, MD for verification of identification. Samples of spiders were sent to Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, FL for species identification.

Statistical Analysis. The experimental design was completely random with 10 replicates at each location. A three-way analysis of variance (ANOVA) was performed using the general linear model (PROC GLM) of SAS (SAS Institute 1999) to find the interaction among year, location, and treatment for mealybugs, mealybug destroyers, ants, and spiders. A one-way ANOVA was performed using the general linear model (PROC GLM) for mean number of mealybugs collected from the three treatments. Means were compared at $P = 0.05$ significance level using the Tukey's HSD test. A repeated measure ANOVA using the general linear model (PROC GLM) was performed for mean number of adults and larvae of mealybug destroyers, spiders, and ants collected at 24, 48, and 72-hour intervals to check the interaction between the interval and the treatment. Means were compared between treatments using a t-test (PROC TTEST) of SAS (SAS Institute 1999) at $P = 0.05$. The closed sleeve cage treatment was excluded from the analysis since there were no natural enemies present in this treatment.

Proportions of parasitism of *A. papayae* and *A. loecki* for both open sleeve cage and no cage treatments were arcsine-square-root transformed using,

$$p' = \arcsin \sqrt{p}$$

where, p = proportion of parasitism, to adjust the variances (Zar 1984) prior to ANOVA, but untransformed data were presented in tables. A three-way ANOVA (PROC GLM) was performed to find the interaction among year, location, and treatment for proportion of parasitism for each parasitoid species. A two-way ANOVA was performed for proportions of individual and cumulative parasitism of *A*. *papayae* and *A*. *loecki* between treatments, and means were compared at $P = 0.05$ significance level using least square means (LSMEANS) of SAS (SAS Institute 1999).

Voucher Specimens. Voucher specimens of mealybugs, mealybug destroyer adults and larvae, ants, spiders, and parasitoids were deposited in the Entomology and Nematology Department insect collection, at the Tropical Research and Education Center, University of Florida.

Results

There was no interaction in the mean number of *P*. *marginatus* collected from each treatment by location and year ($F = 0.12$, df = 4, 162, $P = 0.9737$). Therefore, the data for *P*. *marginatus* were pooled by location, year, and treatment, and pooled data were used in the analyses. The mean number of *P*. *marginatus* collected from the closed sleeve cage (410.9 ± 1.6), was higher than the numbers collected from the open sleeve cage (171.6 ± 1.3) and the no cage treatment (109.1 ± 0.7) by 58.2% and 73.4% respectively ($F=16800.4$, df= 2, 177, $P <0.0001$). There were 36.4% more mealybugs in the open sleeve cage, compared to the no cage treatment.

Natural enemies such as mealybug destroyer adults and larvae, and spiders were observed at all three locations used in this experiment. However, no natural enemies were present in the closed sleeve cage treatment. There was no interaction in the mean number of individuals collected by location, year, and treatment for mealybug destroyer adults ($F = 0.01$, df = 2, 346, P

= 0.9998) and larvae (F= 0.04, df = 2, 346, *P* = 0.9599), ants (F = 0.04, df = 2, 346, *P* = 0.9653), and spiders (F= 0.14, df = 2, 346, *P* = 0.8733). Therefore, the pooled data for each of these insects were used in the analyses. The repeated measures ANOVA for within subject effects indicated that there was no interaction between the interval and the treatment (F= 0.01, df = 2, 944, *P* = 0.9931). There were higher mean numbers of mealybug destroyer adults and larvae (Table 6-1), ants, and spiders (Table 6-2) in the no cage than in the open sleeve cage treatment at 24, 48, and 72-hour intervals.

The spiders collected from the treatments were comprised of *Gasteracantha cancriformis* (Linnaeus), *Cyclosa walckenaeri* (O. P. Cambridge) (Araneae: Araneidae), *Lyssomanes viridis* (Walckenaer) (Araneae: Salticidae), *Misumenops* sp. (Araneae: Thomisidae), *Hibana* sp. (Araneae: Anyphaenidae), *Theridion melanostictum* O. P.Cambridge (Araneae: Theridiidae), and *Leucauge* sp. (Araneae: Tetragnathidae). None of the species of spiders collected was dominant in any of the treatments. The ants collected form the treatments were comprised of *Tapinoma sessile* Say, *Pheidole* sp., and *Technomyrmex* sp. (Hymenoptera: Formicidae). *Tapinoma sessile* was the predominant ant species collected from the three locations in both 2005 and 2006, and is a common and widely distributed North American ant species (Smith 1928).

There was no interaction in the mean proportion of parasitoids emerged from the mealybug samples collected by treatment, location, and year for *A*. *papayae* (F = 0.86, df = 2, 108, *P* = 0.4260), and *A*. *loecki* (F = 0.23, df = 2, 108, *P* = 0.7919). Therefore, the data for parasitoids were pooled by location and year, and pooled data were used in the analyses. *Acerophagus papayae* had higher percent parasitism in the open sleeve cage than in the no cage treatment by 30.9% (Table 6-3). Within a treatment, *A*. *papayae* had a higher parasitism than *A*. *loecki* by 92.6% in the open sleeve-cage and by 92.5 % in the no-cage treatment respectively.

Percent parasitism of *A. loecki* in the open sleeve cage was 30.8% higher than in the no cage treatment (Table 4-3). The open sleeve cage had 30.9% higher cumulative percent parasitism than the no cage treatment. There was no activity of *P. mexicana* in any of the treatments.

Discussion

In recent years, classical biological control has been used to control several invasive mealybugs. Use of *Apoanagyrus lopezi* to control the cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero in Africa (Neuenschwander 2001), *Gyranusoidea tebygi* Noyes for mango mealybug, *Rastrococcus invadens* (Williams) control in West Africa (Bokonon-Ganta and Neuenschwander 1995), and the use of *Anagyrus kamali* Moursi to control pink hibiscus mealybug, *Maconellicoccus hirsutus* Green in the Caribbean (Kairo et al. 2000) are some of the examples. Use of *A. papayae*, *A. loecki*, and *P. mexicana* to control *P. marginatus* in the Caribbean, the US, and the Pacific, is another example of utilizing classical biological control to manage an invasive mealybug species.

To determine the ecological and the economic impact of a biological control program, it is necessary to evaluate the efficacy of the biological control agents. In order to understand this, it is important to evaluate the pest insect population in an environment where it is not exposed to the natural enemies (Boavida et al. 1995b). One of the principal obstacles of the host evaluation has been the difficulty of excluding the natural enemies from the host population (Smith and DeBach 1942). In this study, sleeve cages were used as the exclusion method to investigate the host population without its natural enemy. A physical exclusion method using sleeve cages can be an effective way to evaluate the effect of presence and absence of natural enemies on the survival of their host populations (Smith and DeBach 1942). Limitations and applicability of physical exclusion methods on different natural enemies have been evaluated (Kiritani and Dempster 1973, Van Lenteren 1980). One limitation of this method is that it may cause

conditions within the sleeve cage to depart too far from the normal conditions outside the sleeve cage (Smith and DeBach 1942). To overcome these limitations, open sleeve cage and no cage treatments were included in this study. The closed sleeve cage protected the mealybugs from natural enemies as well as from environmental factors such as the rain and the wind, while the open sleeve cage likely provided some protection from adverse environmental conditions, and no protection provided by the no cage treatment. The greater host population in the closed environment indicates that when there was no outside interference from natural enemies, or no direct impact of the wind and rain, insects survive better than in the open environment where they are more exposed to direct environmental factors as well as their natural enemies. Similar results have been reported for *Rastrococcus invadens* Williams in field assessment studies conducted to find the impact of the introduced parasitoid, *Gyranusoidea tebygi* Noyes in West Africa (Boavida et al. 1995b).

The presence of predators such as *C. montrouzieri* adults and larvae, and spiders may have a negative impact on percent parasitism. *Cryptolaemus montrouzieri* was also collected in relatively low numbers in field assessment studies of the parasitoids of *P. marginatus*, conducted in the Republic of Palau (Muniappan et al. 2006) and in Guam in 2002 (Meyerdirk 2004). The presence of *C. montrouzieri* could have had an effect on parasitism, but due to the presence of a large number of mealybugs and the high percent parasitism observed in these areas, the effect of *C. montrouzieri* may not be significant. There is a possibility that parasitized mealybugs were preyed on by *C. montrouzieri*. Most coccinellid predators feed on more than one prey species; thus, disruption of existing biological control by introduced coccinellids and the potential for indigenous coccinellid species to disrupt introductions can happen (Rosenheim et al. 1995). Common forms of intraguild predation include predators that attack herbivores that harbor a

developing parasitoid (Rosenheim et al. 1995). This may be one reason that higher parasitism was observed in the open sleeve cage treatment than in the no cage treatment, because there were more predators in the no cage treatment, and *P. marginatus* was directly exposed to the environment.

Anagyrus loecki is not a host specific classical biological control agent (Noyes 2000). The low parasitism by *A. loecki* in both open sleeve cage and no cage treatments was possibly due to its multiple host preference. In addition to being a parasitoid of *P. marginatus*, *A. loecki* can develop in *Dysmicoccus hurdi* and *Phenacoccus madeirensis* Green (Hemiptera: Pseudococcidae) (Noyes 2000) and *P. maderiensis* is one of the commonly found mealybug species in South Florida (Williams and Granara de Willink 1992, Ben- Dov 1994). Other than *P. marginatus*, no other hosts have been recorded for *Acerophagus papayae* and *P. mexicana* (Noyes and Schauff 2003).

Not recovering a single *P. marginatus* that was parasitized by *P. mexicana* or the emergence of any hyper-parasitoids from the collected *P. marginatus* raises an interesting question of whether *P. mexicana* successfully established in the experimental area. In July 2003, 3,400 *P. mexicana* were released in Florida, as a one-time release in 21 locations in Miami-Dade, and Broward Counties including five locations in Homestead where these field studies were conducted (D. M. Amalin, personal communication, Meyerdirk 2003). On the other hand, only 1,400 *A. papayae* and 1,200 *A. loecki* were released at the same time and in the same locations as *P. mexicana*, but they both were recovered from the field. Even after several releases, *P. mexicana* has not been recovered in field assessment studies conducted in the Republic of Palau (Muniappan et al. 2006). A similar study has been conducted in Guam in 2002, although the results were reported without the recovery data of parasitoids (Meyerdirk et al. 2004). There is

very little information on *P. mexicana*, and there is no information on why it was not recovered from the field in previous studies. Further field experiments focusing on *P. mexicana* may be needed to clarify why it was not recovered from the field.

In laboratory studies, *P. mexicana* and *A. papayae* showed a preference for second-instar *P. marginatus* while *A. loecki* preferred the third instars. At 25°C, the developmental time of female *P. mexicana* was 22 days, and was longer than the 14-day developmental time of female *A. papayae* and *A. loecki* (Chapter 5). The second-instar *P. marginatus* can emerge within 14.6 days at 25°C (Chapter 5). The developmental time of second-instar *P. marginatus* coincides with the developmental time of female *A. papayae* and *A. loecki* (Chapter 5). The preference for the third-instar *P. marginatus* by *A. loecki* makes *A. papayae*, the dominant species in the competition for the second-instar *P. marginatus*. The longer developmental time can be an important reason for less effectiveness of *P. mexicana* in the field. *Pseudleptomastix mexicana* also was less efficient when competing with *A. papayae* and *A. loecki* in laboratory studies (Chapter 4).

The shorter developmental time and lack of competitors for preferred second-instar hosts may have placed *A. papayae* as the dominant species over *A. loecki* and *P. mexicana* (Chapter 5). In laboratory studies, *A. papayae* had better control of the host, when present singly or with *A. loecki* and *P. mexicana* (Chapter 4). *Acerophagus papayae* is also the predominant parasitoid species recovered from field studies in both Guam (Meyerdirk et al. 2004) and the Republic of Palau (Muniappan et al. 2006). Out of the three parasitoid species, *A. papayae* is the smallest parasitoid species (Noyes and Schauff 2003). Because of its smaller size, *A. papayae* has the advantage of parasitizing *P. marginatus* that were concealed in crevices of the host plant species.

Because of this concealed nature, there is a possibility of less predation of these mealybugs by *C. montrouzieri* larvae and adults, and spiders.

Higher numbers of ants present in the no cage treatment may have affected the foraging behavior of parasitoids. This may be one of the reasons for lower cumulative parasitism in the no cage treatment compared to the open sleeve cage treatment. Generally, mealybugs and ants have mutualistic relationships. Mealybugs benefit from ant association when ants promote sanitation in mealybug populations and/or protect mealybugs from natural enemies (Gonzalez-Hernandez 1999). It has been repeatedly observed that some pests have higher population densities on plants where ants are active than on plants free of ants (Hodek et al. 1972). There is considerable direct evidence of aggressive behavior toward predators or parasites in honeydew seeking ants. *Pheidole megacephala* (F.) significantly decreased *Dysmicoccus brevipes* (Cockerell) mortality, by *Anagyrus ananatis* Gahan and *Nephus bilucernarius* Mulsant (Coleoptera: Coccinellidae) adults via interference with natural enemy searching behavior (Gonzalez-Hernandez 1999). Presence of ants in both open sleeve cage and no cage treatments may have some influence on the parasitism by *A. papayae* and *A. loecki*, although the effect of ants on mealybugs and parasitoids was not investigated in this study.

Out of the three currently used parasitoids of *P. marginatus*, *A. papayae* is well established in the field, and is the main contributor to the mortality of this mealybug species. Multiple host preference may have caused the low effectiveness of *A. loecki* compared to *A. papayae*. Further research is needed to address the ability of *P. mexicana* to control *P. marginatus* as well as its ability to establish after release in the field.

Table 6-1 Mean (± SEM) number of mealybug destroyer (*Cryptolaemus montrouzieri*) adults and larvae collected per cage from open sleeve cage and no cage treatments using pooled data of 2005 and 2006 in three experimental locations

Treatment	Mealybug-destroyer (adult)/per cage			Mealybug-destroyer (larva)/per cage		
	Interval (Hours)			Interval (Hours)		
	24	48	72	24	48	72
Open sleeve	2.0 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.3 ± 0.1
No cage	3.0 ± 0.1	3.0 ± 0.1	3.1 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	2.1 ± 0.1
t	-7.33	-6.79	-6.86	-8.42	-8.21	-8.42
df	118	118	118	118	118	118
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

n = 60

Table 6-2 Mean (± SEM) number of ants and spiders collected from open sleeve cage and no cage treatments using pooled data of 2005 and 2006 in three experimental locations

Treatment	Ants			Spiders		
	Interval (Hours)			Interval (Hours)		
	24	48	72	24	48	72
Open sleeve	21.4 ± 0.2	21.3 ± 0.2	21.5 ± 0.2	2.0 ± 0.1	2.1 ± 0.1	2.1 ± 0.1
No cage	30.9 ± 0.3	30.9 ± 0.2	31.0 ± 0.2	2.9 ± 0.1	3.1 ± 0.1	3.0 ± 0.1
t	-27.32	-30.52	-29.93	-6.88	-7.77	-7.06
df	118	118	118	118	118	118
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

n = 60

Table 6-3 Individual and cumulative mean percent parasitism (± SEM) of *P. marginatus* by *A. papayae*, *A. loecki*, and *P. mexicana* in open sleeve cage, and no cage treatments using pooled data of 2005 and 2006 in three experimental locations

	Percent Parasitism		
Treatment	*A. papayae*	*A. loecki*	Cumulative
Open sleeve cage	31.0 ± 0.3aB	2.3 ± 0.1aC	33.3 ± 0.3aA
No cage	21.4 ± 0.3bB	1.6 ± 0.1bC	23.0 ± 0.3bA
Source	F	df	*P*
Model	3606.07	5, 354	<0.0001
Parasitoid	8038.28	2, 354	<0.0001
Treatment	1387.10	1, 354	<0.0001
Parasitoid* Treatment	283.36	2, 354	<0.0001

n = 60

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 (LSMEANS) Test).

CHAPTER 7
SUMMARY AND CONCLUSIONS

Paracoccus marginatus Williams and Granara de Willink (papaya mealybug) is a polyphagous pest insect that can damage a large number of tropical and subtropical fruits, vegetables, and ornamental plants. A native to Mexico and/or Central America, *P. marginatus* is currently established in the Caribbean, the US and several Pacific islands. Classical biological control was identified as a suitable method for the control of *P. marginatus*. Currently there are three solitary hymenopteran endoparasitoid species mass reared in Puerto Rico and released in *P. marginatus* infested areas in the Caribbean, the US and Pacific islands. They are *Acerophagus papayae*, *Anagyrus loecki*, and *Pseudleptomastix mexicana*. However, there is a lack of information about *P. marginatus* and its parasitoids. This dissertation focused on the life history of *P. marginatus* in relation to host plant species and temperature, and evaluated the effectiveness of the three introduced parasitoid species.

Life history studies of *P. marginatus* indicated that it could successfully develop, reproduce, and survive on a wide variety of economically important ornamental plants as well as on an aggressive weed species *Parthenium hysterophorus*. Egg to adult emergence occurred in 30 days or less in all plant species indicating its fast development on different host plants.

Paracoccus marginatus showed its tropical characteristics by completing its life cycle in the temperatures ranging from 18 to 30°C. Its high minimum temperature threshold of 14°C and the low thermal constant further clarified this characteristic. The optimum development of *P. marginatus* can be expected around 28°C, while the maximum temperature threshold can go up as high as 32°C. These characteristics may limit establishment of *P. marginatus* into many areas in the US, while some areas in California, Texas, Florida and Hawaii are more vulnerable. The ultimate movement of *P. marginatus* to new areas in the US that are suitable in regards to

temperature will also be influenced by other environmental factors, availability of host plant species, plant movement from state to state, and the rules, regulations, and restrictions of plant movement.

Of the three parasitoid species currently used in the biological control of *P. marginatus*, *A. papayae* had higher parasitism in the field. Natural enemies including *Cryptolaemus montrouzieri*, ants, and spiders were observed in the treatments exposed to the environment, and overall their activity may have contributed to the low parasitism. Parasitism of *P. mexicana* was not observed while *A. loecki* had a lower parasitism compared to *A. papayae*.

The smallest parasitoid species out of the three species, *A. papayae* had lower lifetime fertility than the other two species. In addition, *A. papayae* and *P. mexicana* compete for the second-instar *P. marginatus* while *A. loecki* prefers the third-instar hosts. At the beginning of the season, in the absence of overlapping generations, the longer developmental time of *P. mexicana* makes it unavailable at the second-instar mealybug emergence, providing an advantage to *A. papayae* in the competition for the hosts. *Acerophagus papayae* had high parasitism when present with both *A. loecki* and *P. mexicana*. The efficiency of *A. loecki* may have been affected by not being host specific, and being a parasitoid of commonly found *P. madeirensis*. Longer development time of *P. mexicana* reduces its competitiveness with *A. papayae* and *A. loecki*.

Information gathered from these studies will provide the insight needed to understand the life history of *P. marginatus* in relation to its host plants and temperature, and to explain the effectiveness of its three introduced parasitoids, *A. papayae*, *A. loecki* and *P. mexicana* in the classical biological control of *P. marginatus* in the field.

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BIOGRAPHICAL SKETCH

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