ECOLOGICAL AND BEHAVIORAL FACTORS ASSOCIATED WITH MONITORING AND MANAGING PINK HIBISCUS MEALYBUG (HEMIPTERA: PSEUDOCOCCIDAE) IN THE SOUTHERN US

Justin Matthew Vitullo

Dissertation submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

In

Entomology

J. Christopher Bergh
Aijun Zhang
Catharine Mannion
Richard Fell
Douglas G. Pfeiffer

June 25, 2009
Blacksburg, Virginia

Keywords: *Maconellicoccus hirsutus*, *Hibiscus rosa-sinensis*, monitoring, sex pheromone, mating disruption

Ecological and behavioral factors associated with monitoring and managing pink hibiscus mealybug (Hemiptera: Pseudococcidae) in the southern US

Justin Matthew Vitullo

ABSTRACT

The pink hibiscus mealybug (PHM), *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae) was investigated with regard to damage caused to hibiscus by feeding, dispersal of nymphs, evaluation of management tactics, and the use of sex pheromone based monitoring in southern Florida from 2005 to 2008. Understanding the ability of PHM to locate and colonize new hosts, and the response of hosts is essential to optimized monitoring and management strategies. Investigation of the onset and severity of PHM feeding symptoms by *Hibiscus rosa-sinensis* L. showed that severity of symptoms differed among cultivars and that PHM were found on plants that did not exhibit feeding symptoms. Aerially dispersing PHM were predominantly first instars. Dispersal occurred with a diel periodicity that peaked between 14:00 and 18:00 h and was significantly influenced by mean wind speed. Initial infestation with 5, 10, or 20 PHM adult females had no significant affect on the number of dispersing individuals captured from hibiscus plants and PHM were captured at 50 m from infested source plants.

The effects of mating disruption, the insecticide (dinotefuran), the parasitoid, *Anagyrus kamali* (Moursi), and the predator, *Cryptolaemus montrouzieri* (Mulsant) on PHM on hibiscus plants in screened field cages were evaluated. The total number of mealybugs captured in sticky band and pheromone traps during the study was reduced by dinotefuran and the predator. At the end of the study, the number of nymphs recovered from hibiscus terminals was reduced by the dinotefuran, predator and parasitoid treatments. Field experiments showed that the time of day during which male PHM were captured in pheromone traps in May and September was crepuscular, with most captures occurring from 18:00 to 21:00 h. Significantly more males were

captured in traps placed in non-host trees at an elevation of 2 m above the ground than 6 m, and more males were captured in traps placed within host plants than in those 3 m upwind. Pheromone traps placed in hibiscus treated with soil applied dinotefuran or left untreated captured equal numbers of males during the 3 wk prior to treatment and during the 12 wk after treatment. Release of parasitoids at residential sites did not have a significant effect on the total number of males captured in sex pheromone traps over 18 mo. The number of mealybugs found at both parasitoid release and untreated sites were highly variable and corresponded with males captured in sex pheromone traps, as high and low levels of mealybugs corresponded with high and low levels of males captured. The number of males captured in pheromone traps during a two week survey at residential sites in May were a strong predictor of subsequent captures in 2006 (r^2 = 0. 712), but not 2007 (r^2 = 0.019). The relationship between PHM populations and males captured in sex pheromone traps was influenced by a multitude of factors that can impact the ability of traps to accurately reflect populations at a given location. Pheromone traps have the potential to provide meaningful information towards monitoring and mitigating risk from PHM. The contributions of this dissertation towards optimizing PHM sex pheromone monitoring, as well as facets of PHM monitoring that have yet to be resolved are discussed.

For Nancy Tuman and Nick Vitullo

ACKNOWLEDGMENTS

The realization of this dissertation would not have been possible without the help of a group of great people. I'd like to thank my major advisor from Virginia Tech, Dr. Chris Bergh, whose thoughtful suggestions and mentoring have made me a better writer, presenter, thinker, and research scientist. I'd like to thank my major advisor from the USDA, Dr. Aijun Zhang, without him and his identification and synthesis of the pink hibiscus mealybug pheromone this dissertation would not have been possible. In addition to the great long distance support my advisors gave me as we were based in different states, their visits to south Florida were always memorable. I'd like to thank Dr. Catharine Mannion from University of Florida, while she may be listed as my committee member, her guidance in southern Florida entomological research, and the time I spent with her at TREC made her much more.

I am very grateful for the network of caring people that Virginia Tech provided me as their student by title, and for the two years that I physically spent in Blacksburg, VA. The expertise of Dr. Richard Fell and Dr. Doug Pfeiffer as committee members was integral in making sure my project was both fundamentally sound and feasible, and I was lucky to have them on my committee. Virginia Tech has a great entomology department with outstanding faculty, staff (Kathy Shelor, Sarah Kenley, and Robin Williams), and students. I would like to make special note of the support Daniel Frank and Derek Monthei provided me as we all faced a multitude of challenges during our simultaneous pursuit of PhD degrees. As a resident of the Donaldson-Brown graduate student life center for my time in Blacksburg I met exceptional

graduate students from varied disciplines that improved my VT experience, especially: Dan Noonan, John Leacock, Jim Bisha, and Dana Caracciolo.

I am also very grateful for the hospitality that the University of Florida showed me as I conducted my research in Dr. Mannion's lab at their TREC in Homestead, FL. My extensive experiments could not have been completed without the help of laboratory and field staff: Holly Glenn, Julio Almanza, Patricia Lopez, Robert Stubblefield, and Manny Soto. This research would not have been possible without the USDA-CSREES Southern Region IPM Program, Award 2007-34103-18140, Garden Depot of Miami, FL for donating plants and G. Azore from FDACS for help with all biocontrol related experiments. I also want to thank the members of the information technology unit at TREC, M.J. Frederick and Ian Maguire. Ian Maguire a Homestead native and all around nice guy, has mentored me on the culture of southern Florida and the sharing of mangoes.

I would like to thank the very diverse group of people of southern Florida that I have lived with, played sports with, and explored the area with. In an effort to keep this short, I will simply list them by name alphabetically: Luis Barquin, Daniel Carrillo, Tom Davenport, Andrew Derksen, Rita Duncan, Dalia Flores, Kaushi Lalalala, Omar Lugo, the Lugo family, the Maguire family, Pam Moon, Sikavas Nanalampang, Guillermo Padilla, Jorge Pena, Bruce Shaffer, and Tara Taranowski. All have added to my PhD experience in a very positive way and I hope to keep in touch in the future.

I also want to thank my family for their long distance support, my church family at South Baptist for their local support, and God for the help nobody else could give.

TABLE OF CONTENTS

Page

Page

Page

LIST OF TABLES

LIST OF FIGURES

Figure Page

CHAPTER 1: BIOLOGY AND MANAGEMENT OF PINK HIBISCUS MEALYBUG

INTRODUCTION

The pink hibiscus mealybug (PHM), *Maconellicoccus hirsutus* (Green) is an invasive pest of the US first reported in Hawaii in 1983 (Beardsley 1985), California in 1999 (Anonymous 1999), Florida in 2002 (Amalin et al. 2003), Louisiana in 2006 (LDAF 2006), Texas in 2007 (Bogran and Ludwig 2007), and Georgia in 2008 (Horton 2008). PHM has been recorded on over 300 host plants, including citrus, ornamentals, and vegetables (Hodges and Hodges 2005). Much of the southern US is climatically suitable for colonization by PHM, putting crops at economic risk (USDA 1998). Moffitt (1999) arrived at a conservative estimate of the cost of PHM invasion to agriculture in the continental US and the US Virgin Islands at $750 million per year in the absence of control, and suggested that the producers of ornamental and vegetable crops would bear the greatest burden. In Florida, scale insects, such as mealybugs are serious pests and received 12% of total ornamental pesticide applications in 1995 (Dunn et al. 1999). The Tropical Fruit Pest Management Strategic Plan developed in Homestead, FL in 2003 identified PHM as an emerging pest of avocado, mango and papaya, and suggested investigations on its biology as a key topic (Mossler and Nesheim 2003). Specific research needs identified by the IPM Roadmap applicable to PHM in the US include, clarification of pest biology and host/pest/climate interactions, enhanced capabilities to predict pest incidence, improved efficiency of suppression tactics and investigation of pest management alternatives. A

USDA (2003) internal document stated, “there is no question that PHM will become widely distributed throughout the Western Hemisphere in the next 10 years”.

Control of PHM using parasitoids has been successful in Hawaii, Grenada, the Bahamas, Belize, the US Virgin Islands, and Puerto Rico, but this tactic does not eradicate the pest (Ranjan 2004). In larger non-island settings with numerous hosts such as India, Egypt and Australia, PHM has long been a pest of agricultural and ornamental crops (Das and Singh 1986, Williams 1996). In the US, chemical controls are used as preventative broadcast sprays and as soil drenches in nurseries due to quarantine if PHM is detected (Osborne 2005). The large number of susceptible hosts, expense of producing biocontrol agents, low number of plant protection technicians relative to the size of potentially infested areas, and labor intensity of scouting, creates a need that pheromone-based monitoring can fill. Pheromone traps have the potential to effectively target infested areas for biocontrol releases, reduce the chance of shipping infested plants, provide thresholds that eliminate the need for preventative chemical treatment, and provide a sensitive method for monitoring PHM distribution and rate of spread across North America.

Geiger and Daane (2001) stated, “there are no simple and effective methods to monitor most mealybug species.” They may have been referring to the difficulty for research on pheromone based monitoring of mealybugs to go beyond the ability to determine presence or absence, and provide a basis for management decisions utilizing the number of males captured in sex pheromone traps. Information is needed on how PHM disperses in the environment to locate and colonize new hosts. With new information, effective monitoring and management strategies can be devised to reduce the risk associated with local PHM dispersal. Recent work on pheromone-based monitoring of mealybugs has addressed trap design and placement (Millar et

al. 2002, Vitullo et al. 2007). The next step is to give meaning to captures in traps, as has been attempted with the Comstock mealybug, *Pseudococcus comstocki* (Kuwana) (Meyerdirk et al. 1981), and vine mealybug *Planococcus ficus* (Signoret) (Millar et al. 2002, Walton et al. 2004). Any correlation between trap data and populations in the surrounding area will also need to take into account the effects that management tactics have on suppression of PHM and related changes in the capture of males. Tactics include the use of insecticides, the release of biocontrol agents, and potentially, mating disruption. While mating disruption has yet to be evaluated for PHM, it has shown some success for the closely related vine mealybug (Walton et al. 2006).

PEST STATUS AND HOST RANGE

World Distribution and Invasion of the Continental US. Pink hibiscus mealybug is the approved common name for *Maconellicoccus hirsutus* (Green) (synonym: *Phenacoccus hirsutus* Green), which has also been known as the grape mealybug (not to be confused with the grape mealybug, *Pseudococcus maritimus* (Erhorn)), the grapevine mealybug, the pink mealybug and the hibiscus mealybug (Gautam 2002, Anonymous 2005). PHM is one of nine species of *Maconellicoccus*. The genus is most likely of tropical Australian origin, since five of nine species are found there, or possibly Asia (Williams 1996). *Maconellicoccus hirsutus* is the only species with records on 6 continents in more than 50 countries (CABI/EPPO 2004) and has been an important economic pest of a broad range of agricultural and horticultural crops in Asia, parts of Africa and Australia (Williams 1996).

Its invasion of Florida in 2002 was preceded by introduction into Grenada in 1994 (Persad 1995) and spread in the Caribbean islands during the 1990s (Meyerdirk 1997), where it caused significant economic damage (Francois 1996, USDA 1997). The Caribbean Agricultural

Research and Development Institute put emergency response plans into action, suggesting field surveys and release of predators (Gautam 2002). During the same period, the first records of PHM were also reported from Mexico, Central and South America (Guyana) (Pollard 1998).

Between 2002 and 2006, PHM spread to 36 counties in Florida from the initial point of infestation in Broward County, including 25 counties with positive residential sites and 11 with non-residential sites where there are nurseries and nursery stock dealers (G. Azore, PHM Field Supervisor, Florida Department of Agriculture and Consumer Services (FDACS), Division of Plant Industry (DPI), Broward Co. FL, pers. comm.). As of 25 March 2009, 37 counties are positive for PHM in FL (G. Azore, pers. comm.). The spread of PHM is likely exacerbated by its use of several common native hosts, including Florida nettle (*Trema micranthum* L.) (Hodges and Hodges 2005). Between January and July 2004, potentially infested hibiscus plants were shipped from Homestead, FL to 36 states, including 11 states considered climatically suitable for establishment of PHM. The pest was confirmed on plants from this shipment in Kansas, Louisiana and North Carolina (Hodges and Hodges 2005). Chang and Miller (1996) suggested a strong possibility for the spread of PHM to Louisiana, Texas, Arizona and potentially to the milder, temperate zones of Mississippi, Alabama, Georgia, and South Carolina.

In addition to the environmental issues surrounding the introduction of an exotic pest, PHM has the potential to have a serious negative economic impact in Florida. Florida is the second largest producer of floriculture crops in the US with 22.9% of the nation's production, exceeding $900 million in 2007 (National Agricultural Statistics Service 2007). After the introduction of PHM into Florida, a zero tolerance policy was established. The FDACS, DPI provided PHM management guidelines for state inspectors, nursery producers of ornamental plants and ornamental plant stock dealers (FDACS 2005). Businesses confirmed with PHM

infested plant material are placed under quarantine and owners are given the following options: destroy all infested plants under the supervision of a DPI representative, followed by treatment of all remaining plants with a recommended pesticide, or treat all infested and non-infested plants with pesticides until all plants are confirmed free of PHM, a period usually lasting at least two weeks. PHM creates a loss of revenue during quarantine and additional expense due to consequent pesticide treatments. Based on information from the National Agricultural Statistics Service, PHM caused 1,008 days of quarantine among 22 nurseries in Florida in 2003 (Ranjan 2004). A more recent PHM Advisory from the Director of the FDACS (Gaskalla 2006) indicated that as of 2006, there had been over 100 quarantine actions taken in nurseries in Miami-Dade County due to nursery stock infested with PHM.

Host Range and Injury Symptoms. PHM is highly polyphagous (Hall 1921, Berg 1996) and its extensive list of hosts include numerous plants of economic significance to US agriculture. PHM has been found on 215 genera of plants (USDA 1997), attacking more than 300 species in 74 plant families (Bogran and Ludwig 2007) and as the common name suggests, hibiscus, *Hibiscus rosa-sinensis* L. (Malvaceae), has been considered a preferred host (Hall 1921, Mani 1989, Kairo 1998). Its wide host range favors rapid spread and complicates effective control. In spite of its wide distribution, PHM was a major pest only in India and Egypt prior to its arrival in the Caribbean. In India it reduced grape yields by 50 to 100%, and yield loss on other crops, such as sorrel, jute, mesta, and roselle, ranged up to 75% (Manjunath 1985). In Egypt, PHM is a pest of shade trees, such as lebbek, bauhinia, mulberry, pigeon pea, and guava (Hall 1926). Cotton is particularly susceptible, if planted near infested trees. In Grenada, PHM infests cocoa, many types of fruits and vegetables, and ornamental plants, such as hibiscus, oleander, and croton (Kairo et al. 2000). PHM has also affected Grenada's forests, killing

individual trees and even whole groves (USDA 1997). The USDA (2001) provided a comprehensive list of PHM's host range, including: citrus, coffee, sugar cane, annonas, plums, mango, okra, sorrel, teak, mora, peanut, maize, asparagus, chrysanthemum, beans, cotton, soybean, and cocoa, as well as native Florida hosts, *Calophyllum* sp., *Schefflera arboricola* (Hayata), *Viburnum odoratissimum* (Ker Gawler), and Florida nettle. PHM exploit hosts by locality as a reflection of changes in habitat, environment, and interactions with the local flora/fauna/predator/parasite complex (Meyerdirk 1999). Economic losses exceeded $3.5 million a year in Grenada and $125 million a year in Trinidad and Tobago (USDA 1997).

PHM forms colonies on host plants that if left unmanaged grow into large white, waxy masses covering branches, fruiting structures, leaves, and even whole plants, including large trees (Stibick 1997). PHM feeds on phloem sap, injecting saliva that results in malformed leaf and shoot growth, stunting, and occasionally death (Kairo et al. 2000). Leaves show a characteristic curling similar to damage caused by some viruses. Heavily infested plants have shortened internodes leading to rosetteing or "bunchy top". This symptom does not occur on all hosts (Stibick 1997), nor even on different varieties of the same host (Abdel-Moniem et al. 2005, Anonymous 2005). Some plant species have been recorded as hosts because crawlers were found on them and because they showed damage symptoms, but were not suitable for reproduction or development (Kairo et al. 2000). "Bunchy top" in mulberry caused by PHM is known as "Tukra", and was thought to be a viral disease transmitted by PHM (Babu et al. 2004). Viral involvement was ruled out and "bunchy top"/tukra is considered a manifestation of PHM attack, even though precise genesis of the symptoms are still not understood (Manjunath et al. 1996). In shoots exhibiting bunchy top, the ground meristem of leaf primordia did not differentiate into palisade and spongy mesophyll when leaves opened, leading to highly crumbled leaves with

uneven thickness, rough surfaces and a thick covering of epidermal appendages. Anomalous growth of bundle sheath extensions caused leaf curling (Babu et al. 2004). Morphological and structural alterations to the leaves and stem were enough to hinder normal functions (Babu et al. 2004). Typical of phloem feeding insects, PHM also produces honeydew which is colonized by sooty mold, reducing photosynthesis and plant growth and affecting the marketability of plants and/or their fruit (Chang and Miller 1996).

Biology and Life History. PHM adult males are slightly smaller than the 3 mm long female (Fig 1.1). The females have a pink to reddish brown body fluid, 1-2 pairs of lateral filaments, are wingless, ovoid in shape and covered by white, mealy wax (Stibick 1997), and reproduce sexually (Chong et al. 2008). Males have a pair of wings, two long waxy tails and are capable of flight (USDA 1999).

In cool climates, PHM overwinters in the soil or on the host plant, either in the egg stage or as an adult. In warm climates, the insects stay active and reproduce year round (USDA 1999). The mature female lays eggs in an egg sac of white wax, usually in clusters on the twigs, branches, or bark of the host plant, but sometimes on the plant's leaves and terminal ends. Mani (1989) summarized studies measuring the PHM life cycle and provided size and development time range: egg length (0.29-0.4 mm), egg width (0.15-0.21 mm), incubation (3-9 d), nymph (10-22 d), egg to adult (23-35 d), adult female length (2.5-3.0 mm), preoviposition (0.5-5.0 d), oviposition (4-8 d), and fecundity (84-654 eggs/female). Females only mate once and can live up to 28 d after eclosion at 20ºC (Chong et al. 2008). Initially, eggs are orange but turn pink as they age, then hatch into nymphs, called crawlers, that are very mobile and can survive without food for 1-2 days (Gautam 2002). In appearance, nymphs of both sexes resemble adult females. Female nymphs have three instars while male nymphs have four (Ghose 1971b, Gullan 2000).

Differences between sexes are detectable by the end of the 2nd instar (Mani 1989). Anatomical differences separate instars: first have 6-segmented antennae, second have 6-segmented antennae with tubular ducts on dorsal abdomen, third have 7-segmented antennae and adult females have 8-segmented antennae (Hodges and Hodges 2005). The last instar of the male is an inactive stage with wing buds within a cocoon of mealy wax. Adult males have nonfunctional mouthparts and their longevity decreases as temperature increases, living for approximately 3.5 days at 20ºC, 2.5 days at 25ºC, and 1.4 days at 30ºC (Chong et al. 2008). Fletcher (1919) reported 10 generations per year in India, although under optimum laboratory conditions, there can be as many as 15 generations per year (USDA 1999). Evaluation of the effects of host plants on the biology of PHM resulted in significant differences in days to complete life cycle, egg to adult survival, and total fecundity (Persad and Khan 2007). Ghose (1972) reported a 1:1 sex ratio for PHM reared on roselle (*Hibiscus sabdariffa*) and that 1 male was capable of mating with 3-4 females.

Seasonal climatic patterns influence PHM populations. Research reports from the Caribbean (Kairo 1998), South India (Mani and Thontadarya 1988) and Australia (Goolsby et al. 2002), showed that populations were low during the rainy season and winter, and peaked during the summer months. In cooler climates females remained inactive in sheltered parts of the plants or overwintered in the egg stage. Mani and Thontadarya (1988) showed that the maximum temperature tested had a positive correlation and relative humidity a negative correlation with mealybug populations. Higher temperatures shortened the incubation period, a 5˚C depression in temperature increased the life cycle duration twofold (Babu and Azam 1987c). It is believed that PHM can adapt to cooler weather by nymphs moving to sheltered locations, and adult females choosing protected places to position egg sacs (Mani and Thontadarya 1988).

New paragraph (may change pagination) The potential geographic distribution of PHM has been simulated using the Climex Simulation Model (USDA 1998). Locations infested with PHM were selected throughout the world and compared with matching locations in North America. Cold stress appears to be the limiting factor for geographical distribution and PHM could find favorable climates in: Mexico, California, Nevada, Arizona, New Mexico, Texas, Oklahoma, Arkansas, Louisiana, Mississippi, Alabama, Tennessee, Georgia, Florida, North and South Carolina, and Virginia. Central and South America, and the Caribbean, also have many suitable climates (USDA 1998).

Aerial dispersal. Knowledge of factors contributing to PHM dispersal is essential for following the course of infestation and planning control procedures (Rabkin and Lejeune 1954, Barrass et al. 1994). The USDA response guidelines suggest that egg, crawler, and male (adult) stages have the potential to be blown by wind currents in the upper atmosphere for hundreds of miles (Stibick 1997). Additionally, PHM is also capable of being spread through the movement of infested nursery stock.

Dispersal studies conducted on scale insect pests that lend their investigation methods to the PHM system are numerous. Soft scales (Hemiptera: Coccidae), *Saissetia oleae* (Olivier) dispersed aerially up to 137 m (Quayle 1916), pine tortoise scale, *Toumeyella numismaticum* (Pettit and McDaniel) dispersed aerially up to 5 km (Rabkin and Lejeune 1954) and brown soft scale, *Coccus hespiridum* L. dispersed aerially up to 55 m (Hoelscher 1967). Small gusts of wind resulted in more dispersal than consistent wind at higher velocities (Hoelscher 1967). Mealybugs (Hemiptera: Pseudococcidae) have also been shown to disperse aerially and via walking. Long-tailed mealybug adults, *Pseudococcus longispinus* (Targioni-Tozzetti) captured in sticky traps walking down tree trunks were a very good indicator of the total population on a given tree

(Debach 1949). Furness (1976) examined the distribution and movement of long-tailed mealybug on orange trees and observed different numbers of individuals on various parts of the plant throughout the year and similar patterns each year. Females would not disperse if already in a protected site, but would reproduce there (Furness 1976). Long-tailed mealybugs caught in sticky aerial dispersal traps resulted in 89% 1st and 11% 2nd instars, with dispersal positively related to wind speed and daily maximum temperature (Barrass et al. 1994). Barrass et al. (1994) suggested that males are only capable of weak flight and did not use wind to disperse to new locations.

Dispersing mealybug crawlers can be monitored using sticky traps placed on the plant or on stands at distances from infested plants. Numerous studies have used these methods to address questions about the spread of mealybug pests in different habitats via aerial dispersal (Quayle 1916, Strickland 1950, Brown 1958, Cornwell 1960, Hoelscher 1967, Greathead 1972, Agnello et al. 1992) or on plants via walking (Hill and Burts 1982, Geiger and Daane 2001).

Anecdotal observations report that first instars (crawlers) of PHM are the dispersive stage (Misra 1920, Hall 1921), which is typical for mealybugs. Newly hatched crawlers are quite active and may walk for considerable distances on a plant before settling to feed. In choice tests, crawlers were shown to prefer and walk toward plant odor sources compared with a blank control, and of 24 plant species tested, *H. rosa-sinensis* attracted the most crawlers (Persad and Khan 2006). Crawlers or egg sacs may be carried on the wind or by other animals, including insects, away from the plant on which they emerge. Given the broad host range of PHM, the probability of dispersing individuals arriving at a suitable host is greater than that of species with a narrower host range. Hall (1921) concluded that the rate and direction of spread of PHM around Cairo, Egypt was directly related to aerial dispersal from infested trees, spreading much further south than to the north on the prevailing northern winds. However, despite the

assumption that PHM is spread by walking and aerial dispersal (Chang and Miller 1996), there has been no research on either mode of dispersal in relation to biotic (e.g. host quality, population density) or abiotic (e.g. environmental) factors.

MANAGEMENT AND MONITORING

Chemical Control. PHM is considered difficult to control with foliar pesticides (Osborne et al. 2003) due to the establishment of colonies in sheltered locations and protection from hydrophobic wax filaments that often envelope colonies. This is especially true of the egg stage, which is protected by a white, waxy ovisac that is almost impossible to penetrate with many pesticides (McKenzie 1967). Quarantine of this pest has resulted in attempts at phytosanitation of agricultural commodities through the use of irradiation (Jacobson and Hara 2003), methyl bromide (Zettler et al. 2002), hot water immersion for surface disinfestations (Hara and Jacobsen 2005), and generic vapor heat treatment (Follet 2004). While many of these tactics are effective at killing PHM, such as methyl bromide, which produced 100% mortality of all life stages, they are not viable solutions for plant material shipments that would not withstand these tactics.

Chemical control recommendations by the University of Florida for PHM in nurseries or stock dealers, include soil drenches of imidacloprid, dinotefuran or thiamethoxam, and/or foliar applications of acephate, bifenthrin, chlorpyrifos, acetamiprid or buprofezin (Osborne 2005). Experience in the Caribbean showed that the use of pesticides in combination with cutting and burning infested host plants were not successful techniques for controlling PHM in the landscape (Sagarra and Peterkin 1999). The protective ability of PHM to conceal themselves within damaged plant parts and are somewhat protected from foliar applied chemicals, along with its wide host range and the large size of many infested hosts make it almost impossible to have a

spray program that will bear the cost and cope with the practicalities of treating the whole range of infested plants in an affected area (Sagarra and Peterkin 1999).

Biological Control. Biological control agents suppress PHM and are one of the most environmentally benign forms of long-term control (USDA 2001). At present, 21 parasites and 41 predators are known to attack PHM worldwide (USDA 1999). In the Caribbean, a biological control program was implemented involving the importation and/or mass-rearing and release of two parasitic wasps, *Anagyrus kamali* (Moursi) and *Gyranusoidea indica* (Shafee, Alam & Agarwal) (both Hymenoptera: Encyrtidae). Successful establishment of these parasitoids, in combination with the effects of predation by a coccinellid predator, *Cryptolaemus montrouzieri* (Mulsant) (Coleoptera: Coccinellidae), have significantly impacted PHM populations in the Caribbean (Kairo et al. 2000, Michaud and Evans 2000), Puerto Rico (Michaud and Evans 2000), and California (Roltsch 2006). In Egypt, parasitism of PHM by biological control agents in field samples ranged from 66-98% (Kamal 1951) and in India from 60-70% (Mani 1989).

In anticipation of the arrival of PHM in Florida, State and Federal agencies developed contingency plans for responding with a biological control program involving release of the same two parasitoids. First detection of PHM in Florida occurred in Broward County on June 13, 2002, and initial releases of the parasitoids occurred within one week (Amalin et al. 2003). While PHM populations have been greatly reduced in Florida, in part due to the establishment and actions of biological control agents (Amalin et al. 2003), these natural enemies do not cause local extinction of populations (Ranjan 2004). In Florida, new infestation sites are expected to arise and re-infestation of previously infested sites is considered a possibility (Ranjan 2004). The FDACS established an insectary in Gainesville, FL to mass-rear the two parasitic wasps and one predatory beetle. Their projected production goal was 10,000 parasites and 5,000 predators per

week to be shipped to affected areas (FDACS 2004). The biological control efforts are estimated at a cost of $500,000 per year to implement, a fraction of the estimated $750 million per year in loses due to PHM in the absence of a control program (Moffitt 1999). However, the losses that occurred after the implementation of the biological control program are difficult to calculate.

The solitary Hymenopteran (Encyrtidae) endo-parasitoids *A. kamali* and *G. indica* are native to China and Egypt, respectively, and have been used due to their success in Egypt (Kamal 1951). The parasitoids *A. kamali, G. indica* and *Allotropa merida* (Walker) (Platygastridae) collected in southern Egypt were released in California in 1999 (Roltsch 2006). Upon release, PHM population densities averaged over 200 per terminal. In 4 years the number of PHM per infested shoot was reduced to fewer than 10. In California, *A. kamali* was the dominant parasitoid, while *G. indica* only represented about 10% of the individuals collected (Roltsch 2006). Due to the success of *A. kamali*, a considerable amount of research has been carried out to assess interactions between parasitoids and PHM.

Adult female *A. kamali* can parasitize all stages of PHM, excluding adult males. First and 2nd instar PHM nymphs parasitized with *A. kamali* resulted in progeny with a sex ratio close to one (all males), while 3rd instar and adult PHM resulted in progeny with a 0.4 sex ratio of *A. kamali* (Sagarra 1999). Sixty percent of parasitoid eggs laid in adult female PHM did not develop due to PHM's cellular defense of encapsulation and melanization of the parasitoid egg, resulting in more progeny emerging from nymphs (Sagarra 1999, Sagarra et al. 2000a, Sagarra et al. 2000c). Lifetime fecundity and reproductive life were significantly affected by temperature and photoperiod (Sagarra et al. 2000b). Large *A. kamali* lived longer than small, and while large females showed no preference for large males, large females produced more eggs and more females (Sagarra et al. 2001b, Sagarra et al. 2000c). Virgin *A. kamali* females are

parthenogenetically arrhenotokous (Sagarra et al. 2002). *A. kamali* has a very high level of host specificity making it a good candidate for biological control. In FL there are 3 hymenopteran hyperparasitoid species of *A. kamali*, but they have shown no significant impact on the overall regulatory ability of the parasitoids (Amalin et al. 2003).

The predator that has shown potential for success against PHM is *C. montrouzieri*, known as the mealybug destroyer, native to Australia, and has been used in Egypt and India (Mani and Thontadarya 1987). However, the mealybug destroyer has poor winter survival and low temperatures delay emergence of adults from pupae, as well as initiation of oviposition (Babu and Azam 1988). The mealybug destroyer is a high density feeder and does not maintain "low" pest populations (Kairo et al. 2000). Prior to pupation, larvae could eat an average of 881 eggs, 259 nymphs, or 27 adult PHM. The completion of the *C. montrouzieri* life cycle took more time when fed on eggs and nymphs, and less when fed adult PHM, and females lived 6 days longer than males (55d) (Mani and Thontadarya 1987). While trying to control PHM on grape in India, the use of certain fungicides showed instances of killing *C. montrouzieri*, causing pest resurgence (Babu and Azam 1987a,b).

Factors that reduce the effectiveness of biological control agents include the perimeters of nurseries/golf courses which are high-insecticide environments that can create PHM refuges, as well as sugar-loving ants that will protect PHM from parasites and predators (Ghose 1971a). Based on the principles outlined by Hanski (1998), the ability of biological control agents to successfully reduce PHM to acceptable levels on "island" ecosystems such as Hawaii, the Caribbean, Puerto Rico and the infestation point in California (surrounded by desert and ocean), does not necessarily translate to larger ecosystems like Egypt and India where the pest is still a problem and where there is much closer resemblance to the southern US ecosystem (Das and

Singh 1986, Williams 1996). The large area and fragmented patterning of the US landscape may decouple natural enemies and reduce the odds of parasitism (Roland and Taylor 1997). The varied habitats of the southern US coupled with other factors creates a challenge when attempting to control PHM.

Pheromone-Based Monitoring and Management. Sex pheromones have been identified for a variety of mealybugs, including the comstock mealybug, *P. comstocki* (Bierl-Leonhardt et al. 1982), the citrus mealybug, *Planoccocus. citri* (Risso) (Bierl-Leonhardt et al. 1981), the vine mealybug, *P. ficus* (Millar et al. 2002), the obscure mealybug, *Pseudococcus viburni* (Signoret) (Millar et al. 2005), the passionvine mealybug, *Planococcus minor* (Maskell) (Ho et al. 2007) and the grape mealybug, *P. maritimus* (Figadère et al. 2007).

Serrano et al. (2001) showed that PHM uses a female-produced sex pheromone to facilitate mate location. Virgin PHM females attracted males at a distance of up to 50 m from an infested source (Serrano et al. 2001). The PHM sex pheromone was identified as (*R*)-lavandulyl (*S*)-2-methylbutanoate and (*R*)-maconelliyl (*S*)-2-methylbutanoate blended at a ratio of 1:5 (Zhang et al. 2004a), has been synthesized (Zhang et al. 2004b, Zhang and Nie 2005), and evaluated in field trials (Zhang et al. 2006).

Extensive pheromone research has been conducted on armored scales (Hemiptera: Diaspididae) (Gieselmann and Rice 1990), demonstrating that funds spent identifying a pheromone, developing an effective trapping program and routine monitoring are justified. Identification of the sex pheromone of the California red scale, *Aonidiella auranti* (Maskell) cost $250,000 over 10 years but was estimated to save $9,000,000 per year in California alone (Gieselmann and Rice 1990). Cost savings result from spray reduction, reduction in labor, and

mapping of populations in the field by a more efficient means than visual inspection. The pheromone was over 2000 times more efficient than visual inspection.

The identification and synthesis of PHM sex pheromone has provided a highly sensitive and species-specific tool. Implementation of pheromones in an IPM system necessitates a large data base which includes longevity of the pheromone in the field, trap design, trap placement and density, economic threshold levels and a thorough knowledge of the pest ecology (Gieselmann and Rice 1990). Zhang and Amalin (2005) conducted a field test in Florida comparing the capture of male PHM in traps baited with a series of pheromone loadings ranging from 0 to 100 μg per lure. Significantly more males were captured in traps baited with 1 and 10 μg lures than with 0.1 μg lures. Interestingly, the number of male PHM captured in traps baited with 100 μg lures was significantly lower than in those with 1 or 10 μg and not significantly different from traps baited with 0.1 μg. These data strongly suggest that a pheromone source with a relatively high concentration is inhibitory to the response of male PHM and may indicate that this pest is a candidate for mating disruption. Another important feature of the data reported is that the number of PHM parasitoids captured in pheromone-baited traps was no different from the number captured in traps with no pheromone, suggesting the compatibility of pheromone-based monitoring and management with biological control programs (Zhang and Amalin 2005). It has been determined that the half-life of a 1μg septum lure of PHM sex pheromone is 3.5 months (Francis et al. 2007).

The comparison of five commercially available trap designs for the capture of PHM resulted in the Jackson trap (Trécé, Salinas, CA) capturing as many males per cm^2 of trapping surface as those with larger surfaces, and the time required to count males in Jackson traps was significantly lower (Vitullo et al. 2007) (Fig 1.2). Additionally, unlike the other traps evaluated,

which must be replaced entirely or inspected in the field and then redeployed, only the sticky liners of Jackson traps required replacement, enhancing the efficiency of trap servicing. Initial investigations on the phenology of PHM using pheromone traps in Port St. Lucie and Melbourne, FL, showed that males were relatively abundant from June through November in 2004, with peak activity during July (Hall and Lapointe 2005). Further trapping in Brevard, Miami-Dade, and Saint Lucie counties, FL determined that male populations were lowest from January through mid April and abundant during late summer and early fall, with peak populations in late August through early October (Hall et al. 2008).

Sex pheromone traps have the potential to be accurate and time saving indicators of PHM infestations. Visual sampling has been used to predict damage and make control decisions for *P. maritimus* (Geiger and Daane 2001), although visual sampling can be time consuming and costly. In California, sex pheromone traps were used to determine the number of generations per year of the Comstock mealybug (*P. comstocki*) in residential sites (Meyerdirk and Newell 1979), but did not predict damage. The correlation between the number of males captured in pheromone traps and the ability to make management decisions has not been resolved for most mealybugs, including PHM. Sex pheromone traps can capture males in areas where no visual indications of infestation are evident (Francis et al. 2007). A study on the Comstock mealybug found no correlation between the number of males attracted to 10 or more virgin females in pheromone traps and the relative population density on leaves of mulberry trees (Meyerdirk et al. 1981). In a study to monitor *P. ficus* in California vineyards, visual sampling methods and sampling of males using pheromone baited traps demonstrated a significant correlation with economic damage (Millar et al. 2002). These results were tested again in South African vineyards in an attempt to develop a model to estimate infestation levels using pheromone traps (Walton et al.

2004). Walton et al. (2004) stated, “the model’s use may be limited because of the lure’s high level of attractiveness, which often resulted in positive trap catches in vineyards where no females were located during visual searches.” A variety of factors influence the correlation of adult males captured in pheromone traps with infestation levels. Local dispersal and the influence of management tactics need to be investigated under natural and controlled conditions to determine their relationship to males captured in pheromone traps.

Gieselmann and Rice (1990) reported that the use of scale insect sex pheromones in IPM has aided: (1) discovering low-density populations that could easily escape visual detection; (2) identification of “hot spots” in an otherwise light infestation; (3) determination of economic threshold levels to decide if control measures are necessary; (4) timing of control measures against susceptible life stages; (5) reduction of population levels by male mass trapping; (6) disruption of mating communication by the confusion technique; (7) monitoring and manipulating predator and parasite populations; and (8) evaluating the effectiveness of dormant sprays or other pesticides used to control scale. The utility of sex pheromones suggested for scale insects may also be applicable to PHM.

Mating disruption has been attempted in California vineyards to reduce populations of the vine mealybug, *P. ficus* (Walton et al. 2006). Walton et al. (2006) used a sprayable microencapsulated formulation of the vine mealybug sex pheromone in combination with buprofezin to reduce season-long trap catches of adult males, mealybug densities, and crop damage in mating disruption plots, with some success.

In summary, PHM is a highly polyphagous pest with a distribution that spans most of the world's tropical and subtropical regions. Specific research needs applicable to PHM include clarification of pest biology and host/pest/climate interactions, enhanced capabilities to predict pest incidence, improved efficiency of suppression tactics and investigation of pest management alternatives. Specific objectives were to:

1. Compare the expression of PHM feeding symptoms among hibiscus cultivars and among PHM infestation densities.
2. Investigate short-range dispersal of PHM.
3. Evaluate the relationship between local PHM infestations, dispersal/colonization of new hosts, and males captured in sex pheromone traps.
4. Evaluate and compare conventional and experimental management tactics for PHM under semi-field and field conditions.
5. Evaluate factors that influence the capture of male PHM in sex pheromone traps.

Figure 1.1. A. Adult female pink hibiscus mealybug surrounded by nymphs and eggs, and B. adult male pink hibiscus mealybug.

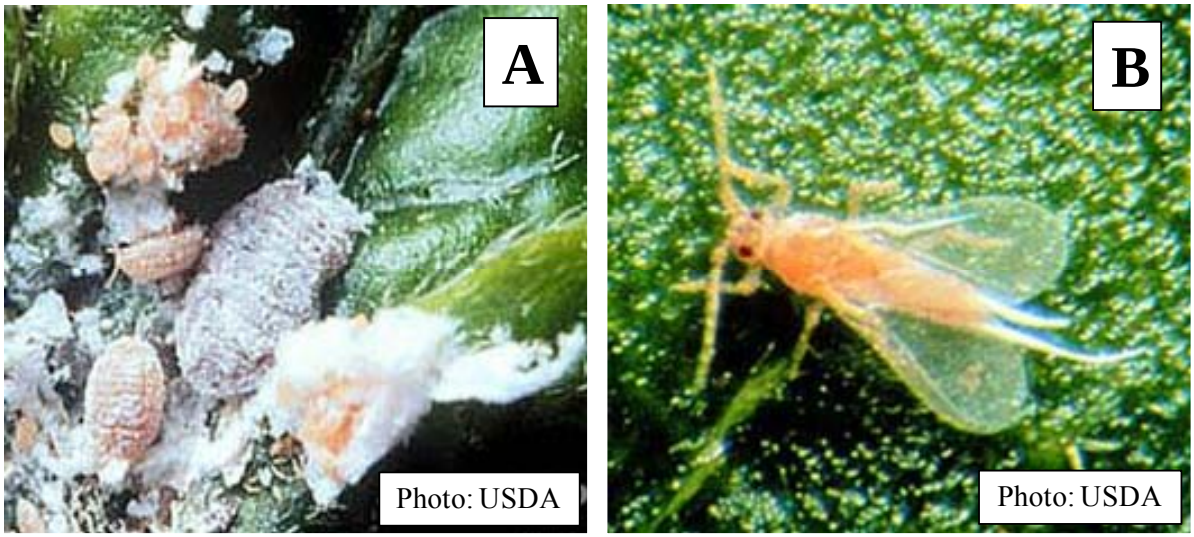


Figure 1.2. Jackson trap with removable liner.

CHAPTER 2: EXPRESSION OF FEEDING SYMPTOMS FROM PINK HIBISCUS MEALYBUG (HEMIPTERA: PSEUDOCOCCIDAE) BY COMMERCIALLY IMPORTANT CULTIVARS OF HIBISCUS

ABSTRACT

The pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green), is a highly polyphagous pest that invaded southern Florida in 2002 and is now widely established throughout most of the state. Although *Hibiscus rosa-sinensis* L. is a preferred and economically important host of *M. hirsutus*, the susceptibility and expression of feeding symptoms by different cultivars has not been evaluated. Cultivars of *H. rosa-sinensis* were infested with *M. hirsutus* and evaluated daily for 40 d for the onset and percentage of terminals expressing feeding symptoms. Under different initial densities of *M. hirsutus*, the cultivar 'President' showed no difference in the latency to expression of feeding symptoms, which occurred between 7 and 15 d after infestation, but did show significant differences between initial density and percentage of terminals expressing feeding symptoms from 10 d onward. When infested with 20 females, 80% of 'President' terminals exhibited symptoms 30 d after infestation. Four other cultivars initially infested with 10 female *M. hirsutus* showed significant differences in the onset and severity of feeding symptoms. All plants of the cultivars 'Florida Sunset' and 'Joanne' expressed damage symptoms at 12 ± 2 SE d and 10 ± 1 d, respectively, following infestation. Only a single plant of the cultivars 'Double Red' and 'Snow Queen' showed such symptoms, at 19 and 30 d after

infestation, respectively. Significant differences between cultivar and the percentage of terminals expressing feeding symptoms were observed from 20 d onward. Terminals sampled from all plants after 40 d revealed that egg, nymph, and adult female *M. hirsutus* were found on all plants, including those that did not exhibit feeding symptoms. These data have shown that hibiscus cultivars differ in their expression of *M. hirsutus* feeding symptoms, that *M. hirsutus* can reproduce on cultivars of hibiscus that do not express feeding symptoms and that feeding symptoms are not a reliable indicator of infestation by *M. hirsutus*, highlighting the need for further investigation of the mechanisms underlying differences among cultivars.

INTRODUCTION

The pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae), is a highly polyphagous pest that invaded southern Florida in 2002 (Amalin et al. 2003), Louisiana in 2006 (LDAF 2006), Texas in 2007 (Bogran and Ludwig 2007), and Georgia in 2008 (Horton 2008). *Maconellicoccus hirsutus* feeds in phloem tissue and injects saliva that can result in malformed leaf and shoot growth, stunting and plant death (USDA 2001). Many economically important agricultural and horticultural crops and common native plants that are potential hosts of *M. hirsutus* occur in Florida (Hodges and Hodges 2005) and throughout the southern US. Reported hosts include more than 300 plant species in 74 families (USDA 2001, Bogran and Ludwig 2007) and damaging populations have been recorded on numerous fruits, vegetables, and ornamentals (Hall 1926, Ghose 1972, Persad 1995, Chang and Miller 1996).

Hibiscus rosa-sinensis L. (Malvaceae), is considered a preferred host of pink hibiscus mealybug (Hall 1921, Mani 1989, Kairo 1998) and 15 *Hibiscus* species have been confirmed

with damaging populations (Stibick 1997). Based on the historical importance of hibiscus to ornamental production in southern Florida, *M. hirsutus* has had negative impacts on local nurseries. A zero tolerance policy has been in effect since 2004 (FDACS 2005) and numerous quarantine actions have occurred (Ranjan 2004, Gaskalla 2006). Retail distributors of ornamental plants in regions of the US not presently infested by pink hibiscus mealybug have reduced hibiscus orders to mitigate the risk of being sent infested stock (J. Cou, Garden Depot, Miami, FL, pers. comm.), especially since 2004, when infested hibiscus plants were shipped from a Homestead, FL nursery to 36 States (Hodges and Hodges 2005). Additionally, landscape design firms in southern Florida have reduced the planting of hibiscus since the invasion by *M. hirsutus*, and nurseries in southern Florida have significantly curtailed hibiscus production (J. Cou, pers. comm.). As with many other host plants, typical feeding symptoms from *M. hirsutus* on hibiscus include leaf curl and shortened internodes, leading to rosetteing or "bunchy top" (USDA 2001). However, during the course of our research in southern Florida, we noticed that certain cultivars of hibiscus growing in residential areas appeared to suffer more damage from *M. hirsutus* than others, which may be attributed to symptoms varying among hosts (Stibick 1997, Anonymous 2005), and/or *M. hirsutus* preferring certain hibiscus varieties (Abdel-Moniem et al. 2005).

Variations among *H. rosa-sinensis* cultivars in their susceptibility to *M. hirsutus* or their expression of feeding symptoms may have important ramifications for management programs. In response to the arrival of *M. hirsutus* in Florida, a biological control program involving the release of two encyrtid parasitoids, *Anagyrus kamali* (Moursi) and *Gyranusoidea indica* (Shafee, Alam and Agarwal), and the coccinellid predator *Cryptolaemus montrouzieri* (Mulsant) was implemented by the Florida Department of Agriculture and Consumer Services, Division of Plant Industry. New infestation sites for release of biological control agents are based on visual

scouting of preferred hosts in the landscape (USDA 2001). While *M. hirsutus* populations have been greatly reduced in Florida through the establishment and actions of biological control agents (Amalin et al. 2003), biological control does not cause local extinction of *M. hirsutus* populations (Ranjan 2004) and the pest has continued to spread in Florida. Sex pheromone traps for pink hibiscus mealybug deployed continuously between 18 May 2007 and 10 October 2008 have shown that males continue to be captured at locations in southern Florida where parasitoids were released in 2007 (Vitullo, Chapter 5). Given that management decisions for *M. hirsutus* are often based on the results of surveys recording the presence or absence of symptoms, understanding the variations in the expression or severity of these symptoms among cultivars is important. Furthermore, differences in susceptibility among cultivars may be helpful toward sustaining hibiscus production in Florida.

The following research addresses the response of several commercially important *H. rosa-sinensis* cultivars to feeding by pink hibiscus mealybug. Specific objectives were to determine the relationship between mealybug density and the expression of feeding symptoms in a susceptible cultivar and the expression of feeding symptoms in four cultivars infested with a single density of mealybugs.

MATERIALS AND METHODS

Insects and Test Plants. Experiments were conducted in a greenhouse set at 28°C at the University of Florida's Tropical Research and Education Center in Homestead, FL. Greenhouse temperature varied by 1°C with a relative humidity range of 40 to 98%. *Hibiscus rosa-sinensis* plants, approximately 1 m high and 0.66 m wide, were grown in 12 L plastic pots and maintained in accordance with standard horticultural recommendations (Ingram and Rabinowitz 1991).

Periodic releases of *Aphidius colemani* Viereck (IPM Laboratories, Inc., Locke, NY) kept aphid levels low in the greenhouse.

Pink hibiscus mealybug was reared on potatoes in a controlled environmental chamber at 26ºC and a 12:12 L:D regime (Serrano et al. 2001). Plants were infested by transferring adult female mealybugs with attached egg sac (Fig. 2.1) to the apical bud of terminals, using a micro-pin tool.

Expression of Feeding Damage in Relation to Mealybug Density. To evaluate the expression of feeding damage in a susceptible *H. rosa-sinensis* cultivar infested with different numbers of female mealybugs, 16 plants of the cultivar 'President' were placed in the greenhouse on 2 July 2007 (Fig. 2.1). Plants were infested with 1, 5, 10, or 20 female mealybugs per plant (n = 4 plants per infestation level). Due to constraints on the availability of mealybugs, the 4 replicates were infested on successive weeks, beginning 19 July.

The number of terminals on each plant was recorded (range = 14-22 terminals per plant) and plants were evaluated daily for 40 d for the number of terminals exhibiting "bunchy top" feeding symptoms. This experimental duration allowed for the completion of the first infesting generation of mealybugs and the dispersal of the second generation crawlers on each plant (Mani 1989).

Expression of Feeding Damage by *Hibiscus* Cultivars. To evaluate feeding damage expressed by different *H. rosa-sinensis* cultivars, 3 plants of 'Double Red', 'Snow Queen', 'Florida Sunset' and 'Joanne' were placed in the greenhouse on 18 January 2008 (Fig. 2.3). These cultivars were selected following consultation with Mr. J. Cou (Garden Depot, Miami, FL) and based on horticultural differences among them and their commercial popularity. 'Double Red' has cordate leaves densely packed along the shoot and red full double blooms. 'Snow

Queen' has variegated cordate leaves with a sprawling habit and fringed single red blooms. 'Florida Sunset' has cordate leaves and yellow regular single blooms with an orange eye zone. 'Joanne' has ovate leaves and orange cartwheel overlapped single blooms with a dark orange eye zone. Plants were pruned to have between 17 and 20 terminals, then infested with 10 female mealybugs on 12 March and evaluated daily for 40 d for the expression of feeding symptoms.

After 40 d, 6 terminals (15 cm long) were pruned from each plant. Since terminals could now be grouped into strata by presence or absence of feeding symptoms, a stratified random sample was used (Ott and Longnecker 2001). When possible 3 terminals exhibiting "bunchy top" were removed from each plant, the remaining terminals exhibiting no feeding symptoms were selected to complete the sample size of 6 terminals. Terminals were examined under a dissecting stereomicroscope at 250X magnification and the number of adult females, nymphs and egg sacs per terminal was recorded.

Data Analysis and Statistics. The latency to the first expression of feeding symptoms (days) for 'President' plants with different initial infestation levels was analyzed using a randomized complete block design ANOVA with four replicated blocks (SAS Institute 2001). The latency to the first expression of feeding symptoms was compared among hibiscus cultivars initially infested with an equal number of mealybugs using descriptive statistics, since plants that did not become infested could not be included in the analysis.

Repeated measures ANOVA (PROC MIXED, SAS Institute 2001) was carried out to determine effects of initial infestation density, days after infestation and their interaction on severity of feeding symptoms using compound symmetry (cs) and first-order autoregressive (ar(1)) models (Ott & Longnecker 2001). Severity of feeding symptoms was then compared among 'President' plants with different initial infestation levels on days 10, 20, 30, and 40 after

infestation using a randomized complete block design ANOVA (SAS Institute 2001), and evaluated for block effect. Assessment of the severity of feeding symptoms was based on the percentage of terminals exhibiting feeding symptoms on each plant analyzed as arcsine square-root transformed percentages (Zar 1999). Severity of feeding symptoms was compared among cultivars infested with the same number of females using the same tests as above. The number of unhatched egg sacs, nymphs and adult female mealybugs recovered from terminals sampled after 40 d, regardless of feeding symptom, was compared among cultivars using ANOVA (SAS Institute 2001). The number of mealybugs at different developmental stages found on cultivars with varied feeding symptoms was compared using descriptive statistics. Analyses of data from all experiments were considered significant at $\alpha = 0.05$. Prior to ANOVA, tests for normality using PROC UNIVARIATE (SAS Institute 2001) were performed. Means of significant effects using ANOVA were separated using a Tukey Honestly Significant Difference (HSD) test.

RESULTS AND DISCUSSION

Infestation of the cultivar 'President' with pink hibiscus mealybug resulted in the expression of "bunchy top" feeding symptoms in all plants within 15 d. Initial infestation levels (mean latency in days ± SE) of 1 (11.0 ± 1.8), 5 (8.5 ± 1.2), 10 (7.3 ± 0.8), or 20 (7.0 ± 0.6) adult females and their egg sac did not significantly affect the latency to the first terminal exhibiting feeding symptoms ($F = 2.23$; df = 3,12; $P = 0.137$). Low levels of variance are evidence of an insignificant block effect.

There were negligible differences between compound symmetry and first-order autoregressive analysis for all repeated measures comparisons for severity of feeding symptoms and initial infestation density of the 'President' cultivar, days after infestation and their interaction. Compound symmetry analysis on severity of feeding symptoms demonstrated a

significant difference for initial infestation density ($F = 50.24$; df = 3,48; $P < 0.001$), day after infestation ($F = 65.65$; df = 3,48; $P < 0.001$) and their interaction ($F = 2.17$; df = 9,48; $P = 0.042$). As such, these data provide a good indication of how the severity of feeding symptoms changes over time under differing initial infestation densities (Fig. 2.4). On day 10, plants infested with 1 and 20 females differed significantly ($F = 4.98$; df = 3,12; $P = 0.018$). On day 20, plants infested with 10 and 20 females differed significantly from those with 1 female ($F = 9.37$; df = 3,12; $P = 0.002$). Plants infested with 20 females differed significantly from all other plants at day 30 ($F = 12.65$; df = 3,12; $P < 0.001$) and 40 ($F = 11.79$; df = 3,12; $P < 0.001$) (Fig. 2.4). When day was excluded, severity of feeding symptoms was significantly different for infestation density ($F = 9.98$; df = 3,48; $P = 0.001$), but not block ($F = 1.14$; df = 3,48; $P = 0.3436$), or their interaction ($F = 0.48$; df = 9,48; $P = 0.881$).

Infestation of four cultivars with 10 pink hibiscus mealybug females and their egg sacs did not result in the expression of bunchy top feeding symptoms on all plants. One of three 'Double Red' plants exhibited symptoms, first occurring 19 d after infestation and totaled two shoots during the trial. One of three 'Snow Queen' plants exhibited feeding symptoms, with one terminal exhibiting bunchy top at 30 d after infestation. All 'Florida Sunset' and 'Joanne' plants exhibited bunchy top, with a mean (± SE) number of days to first expression of feeding symptoms of 12 ± 2 and 10 ± 1, respectively.

Repeated measures comparisons utilizing compound symmetry analysis on severity of feeding symptoms demonstrated a significant difference for cultivar ($F = 82.73$; df = 3,32; $P < 0.001$), day after infestation ($F = 19.29$; df = 3,32; $P < 0.001$) and their interaction ($F = 3.72$; df = 9,32; $P = 0.003$). As such, these data provide a good indication of the change in severity of feeding symptoms over time for different cultivars (Fig. 2.5). The severity of feeding symptoms

and cultivar at 10 d intervals showed significant differences from 20 d after infestation onward; 20 d ($F = 28.55$; df = 3,8; $P < 0.001$), 30 d ($F = 25.59$; df = 3,8; $P < 0.001$) and 40 d ($F = 15.51$; df = 3,8; $P < 0.001$). 'Florida Sunset' and 'Joanne' were not significantly different, but were different from 'Snow Queen' and 'Double Red', which did not differ (Fig. 2.5).

When terminals were removed from cultivars on day 40, *M. hirsutus* egg sacs, nymphs, and adults were found on all cultivars with or without feeding symptoms (Table 2.1). The number of *M. hirsutus* found on cultivars regardless of feeding symptom resulted in 'Florida Sunset' having significantly more unhatched egg sacs present than 'Double Red' and 'Snow Queen', but not 'Joanne' ($F = 5.94$; df = 3,68; $P < 0.001$). 'Snow Queen' had significantly fewer nymphs than all other cultivars ($F = 10.50$; df = 3,68; $P < 0.001$). 'Florida Sunset' had significantly more adults than 'Snow Queen', but not 'Double Red' or 'Joanne' ($F = 5.67$; df = 3,68; $P = 0.002$). The mean (±SE) number of pink hibiscus mealybugs found on terminals without feeding symptoms (n = 51) was 8 ± 2 egg sacs, 379 ± 56 nymphs, and 10 ± 2 adults, and terminals with symptoms (n = 21), had 44 ± 6 egg sacs, 1279 ± 140 nymphs, and 49 ± 7 adults. The presence of adults and egg sacs at day 40 indicate that *M. hirsutus* was able to reproduce on the hosts, based on life cycle duration (Mani 1989).

During our research using pheromone traps for assessing populations of *M. hirsutus* in residential areas in southern Florida, we noticed that not all hibiscus plants exhibited the typical feeding symptoms associated with pink hibiscus mealybug, despite capturing many males in traps suspended from these hosts (Vitullo, Chapter 5). It is important for growers and landscape managers to know that the number of pink hibiscus mealybugs it takes to elicit feeding symptoms and the latency from first infestation to the expression of symptoms is not the same for all cultivars. The latency to the first terminal exhibiting feeding symptoms for the 'President'

cultivar was not density dependent. A single female with her egg sack produced "bunchy top" feeding symptoms in 15 d or less.

Timing of management action can be greatly aided with information on differences in the latency to the expression of the first feeding symptoms among different hibiscus cultivars. For 'President', 'Florida Sunset', and 'Joanne', symptoms occurred in 7 to 15 d following infestation, while 'Double Red' and 'Snow Queen' symptom expression occurred much later or did not occur at all. There may be different characteristics that confer degrees of susceptibility. Abdel-Moniem et al. (2005) evaluated the vertical distribution of *M. hirsutus* on three *Hibiscus sabdariffa* L. varieties in an Egyptian nursery and found that only one in three varieties tested was attacked. Although feeding symptoms were not evaluated, they suggested that morphological and physiological characteristics of *H. sabdariffa* may increase its susceptibility to *M. hirsutus*. While much additional research would be necessary to reveal the mechanisms of tolerance or resistance of hibiscus cultivars to pink hibiscus mealybug, traditional breeding for reduced susceptibility to feeding injury may be possible.

While infestation density had no effect on the latency to first expression of feeding symptoms, cumulative plant injury over time was density dependent (Fig. 2.4). When the cultivar 'President' was infested with a single female and her egg sack, 33% of the terminals exhibited "bunchy top" after 40 d, whereas feeding by twenty females deformed 90% of the terminals. Of the cultivars tested, only 'Joanne' infested with 10 females expressed similar levels of damage as 'President' at day 20. There was little difference in the severity of symptoms from day 20 to 40 for 'Double Red', 'Snow Queen', 'Florida Sunset', and 'Joanne' (Fig. 2.5). Anecdotal observations suggest that newly-hatched crawlers of *M. hirsutus* may walk for considerable distances on a plant before settling to feed (Misra 1920). Feeding symptoms before

day 20 were likely caused by the infesting generation. Based on the duration of the *M. hirsutus* lifecycle (Mani 1989), symptoms from day 20 to 40 were likely caused by the second generation. The colonization and resulting feeding symptoms from the second generation would likely depend on the size and structure of the plant being evaluated.

Expression of feeding symptoms varies between cultivars, but may also be dependent on the growth of the plant. The feeding symptoms of *M. hirsutus* are an inability of the ground meristem of leaf primordia to differentiate into palisade and spongy mesophyll when leaves open (Babu et al. 2004), suggesting that feeding symptoms will only occur during this developmental time. The limiting factor for hibiscus growth is temperature (Ingram and Rabinowitz 1991), suggesting that the temperature in the region and time of year may also have an effect on the appearance of feeding symptoms during hibiscus growth periods.

Many plants have been evaluated and shown to be suitable for the development and reproduction of *M. hirsutus* (Serrano and Lapointe 2002), others expressed damage symptoms but were not suitable for reproduction or development (Kairo et al. 2000). *Maconellicoccus hirsutus* can reproduce on cultivars of hibiscus without producing symptoms, including 'Double Red' and 'Snow Queen' (Table 2.1). Given that feeding by an initial density of one pink hibiscus mealybug per plant elicited feeding symptoms on 'President' and that feeding by 10 females caused minimal or no damage on 'Snow Queen', it appears that feeding symptoms are not a reliable indicator of the presence or level of infestation of all hibiscus cultivars by *M. hirsutus*.

When making landscape design selections, knowledge of how pink hibiscus mealybug infestations affect different hosts will aid in creating aesthetic injury thresholds. Selection of plants that are tolerant to *M. hirsutus* feeding symptoms may make it possible to create low management input landscapes for infested areas when in conjunction with biological control.

Table 2.1. Mean ± SE number of pink hibiscus mealybugs and their developmental stage found on terminals of hibiscus cultivars on day 40.

Cultivar	Presence of feeding symptoms	No. of terminals	Nymph	Egg sac	Adult female
'Double Red'	No	16	620 ± 93	15 ± 4	18 ± 5
'Double Red'	Yes	2	907 ± 262	23 ± 7	28 ± 1
'Snow Queen'	No	17	86 ± 23	4 ± 1	5 ± 1
'Snow Queen'	Yes	1	101	17	17
'Florida Sunset'	No	9	690 ± 169	8 ± 4	11 ± 6
'Florida Sunset'	Yes	9	1421 ± 160	63 ± 10	68 ± 10
'Joanne'	No	9	194 ± 39	2 ± 1	2 ± 1
'Joanne'	Yes	9	1352 ± 245	34 ± 7	38 ± 8

Figure 2.1 Adult female pink hibiscus mealybug with attached egg sac.

Figure 2.2. ‘President’ hibiscus on day 1 prior to infestation and on day 20 after infestation, exhibiting “bunchy top” feeding symptoms.

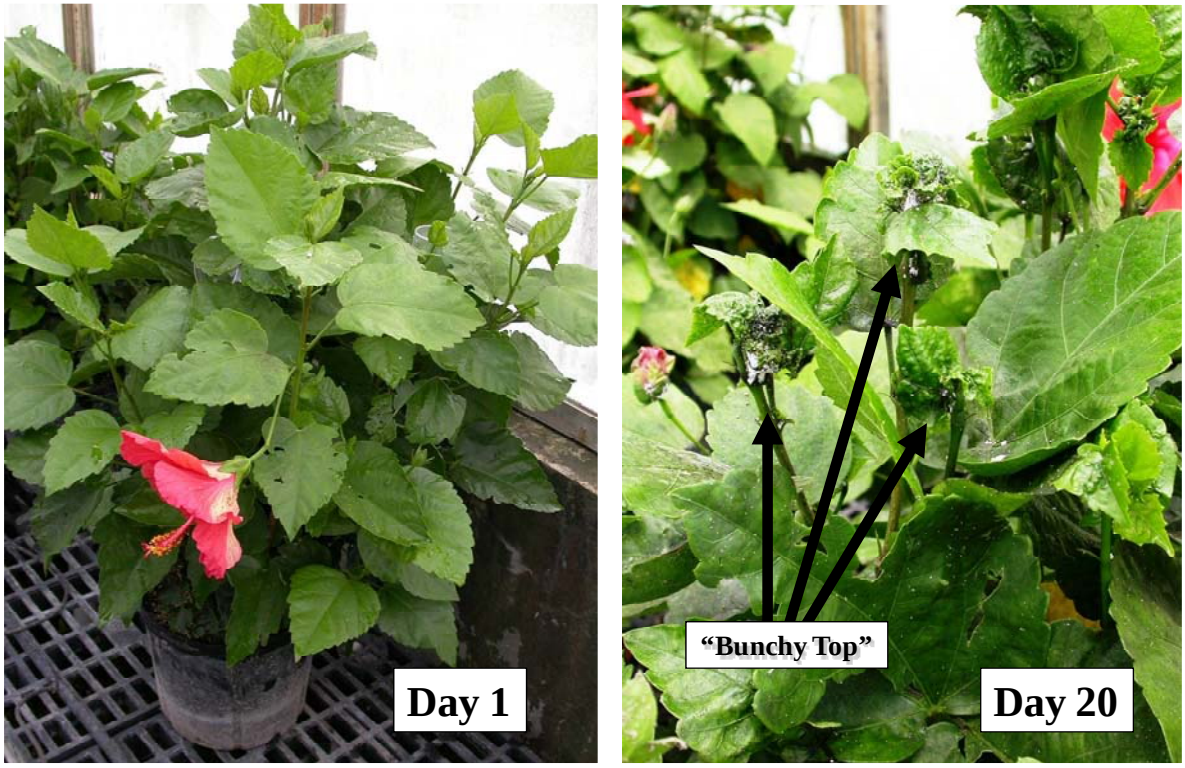


Figure 2.3. Hibiscus cultivars: ‘Double Red’, ‘Joanne’, ‘Florida Sunset, and ‘Snow Queen’.

Figure 2.4. Mean cumulative percent of terminals of ‘President’ hibiscus plants exhibiting feeding symptoms in response to different initial infestation densities of pink hibiscus mealybugs.

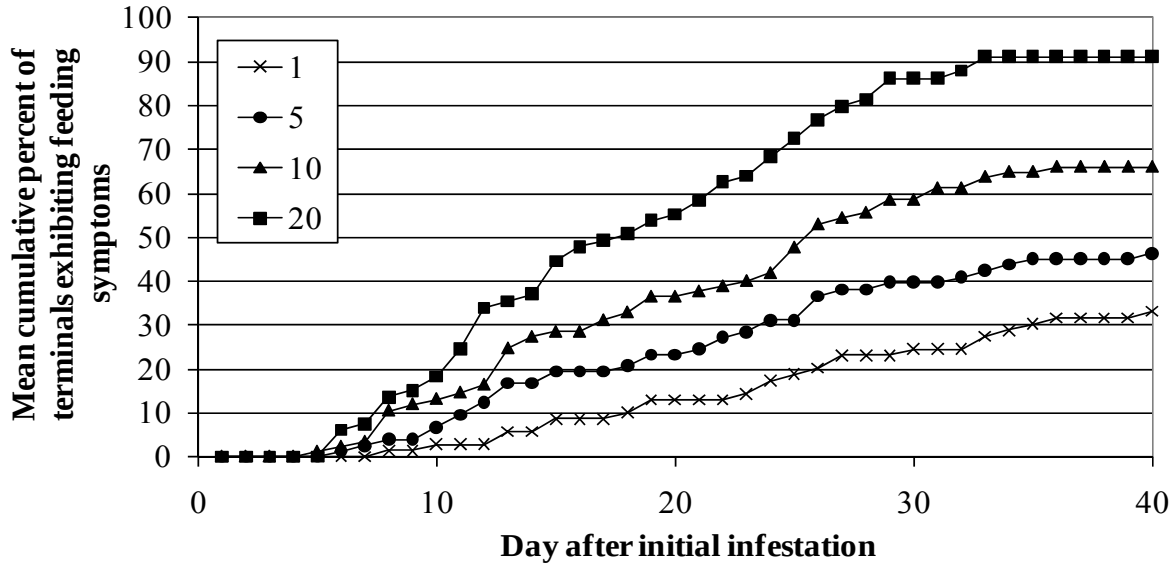
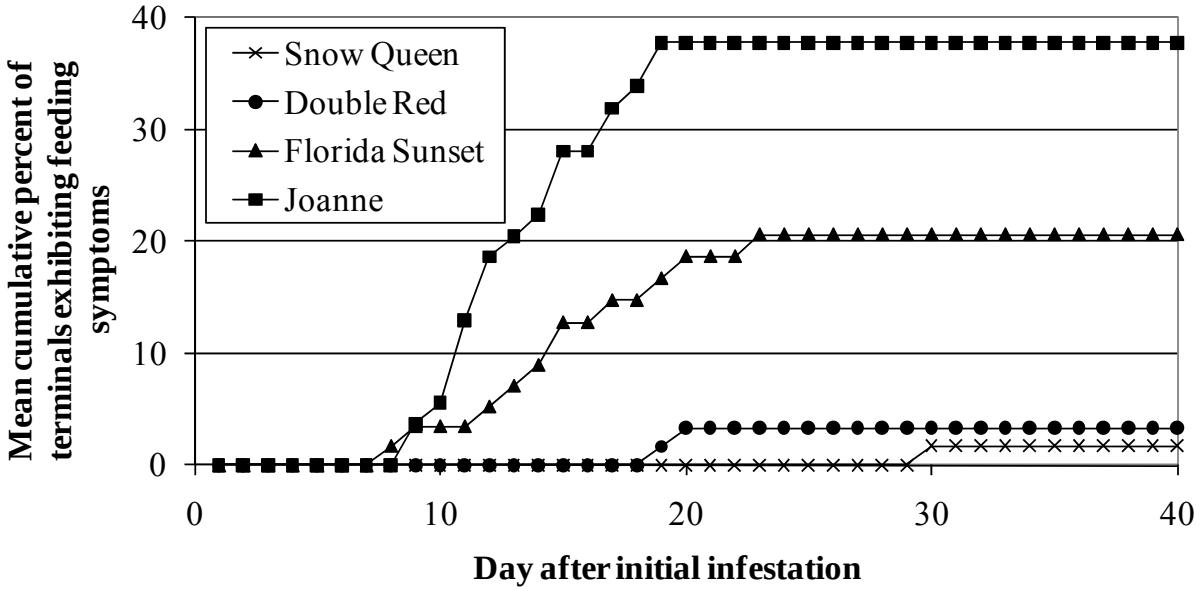


Figure 2.5. Mean cumulative percent of terminals of four hibiscus cultivars exhibiting feeding symptoms in response to an initial infestation density of 10 female pink hibiscus mealybugs.



CHAPTER 3: FACTORS ASSOCIATED WITH SHORT-RANGE DISPERSAL OF PINK HIBISCUS MEALYBUG (HEMIPTERA: PSEUDOCOCCIDAE) IN SOUTHERN FLORIDA

ABSTRACT

Aerially dispersing pink hibiscus mealybugs, *Maconellicoccus hirsutus* (Green) in southern Florida were captured in traps and were predominantly (97%) first instar crawlers. Dispersal showed a diel periodicity that peaked between 14:00 and 18:00 h and was significantly influenced by mean wind speed. Maximum wind speed, relative humidity, temperature, solar radiation, and rain fall had minimal effect. Dispersing crawlers were carried passively on air currents, indicated by the correspondence between the directionality of captures and the prevailing wind direction. Initial infestation with 5, 10, or 20 adult females had no significant effect on the number of dispersing individuals captured from plants. The number of crawlers captured at distances of 5, 10, 25, or 50 m from infested plants were not significantly different. Male captures in pheromone traps during a 2-wk survey in May were an accurate predictor of future captures in 2006 (r^2 = 0. 712), but not 2007 (r^2 = 0.019). The number of potted sentinel hibiscus plants that became infested by mealybugs and the latency to their expression of feeding symptoms did not differ among residential sites ranked according to the relative number of males captured (low, moderate, or high) during the 2 wk survey. The on-going development of guidelines for pheromone based monitoring of pink hibiscus mealybug is discussed in relation to mitigating the risk from the spread of populations to commercial nursery operations.

INTRODUCTION

The pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae), is a highly polyphagous pest that invaded southern Florida in 2002 (Amalin et al. 2003) and subsequently spread to 37 Florida counties where many of its economically important agricultural, horticultural, and common native host plants occur (Hodges and Hodges 2005). The spread of *M. hirsutus* is a threat to numerous southern states with climates considered suitable for its establishment (Ranjan 2004). First reports of the pest occurred in Louisiana in 2006 (LDAF 2006), Texas in 2007 (Borgan and Ludwig 2007), and Georgia in 2008 (Horton 2008).

Biological control programs against *M. hirsutus* have successfully suppressed its populations in California (Roltsch 2006), Puerto Rico (Michaud and Evans 2000), and the Caribbean (Kairo et al. 2000), although Ranjan (2004) stated that it is not eradicated by such programs. The spread of *M. hirsutus* in Florida has occurred simultaneously with the widespread release of two encyrtid parasitic wasps, *Anagyrus kamali* (Moursi) and *Gyranusoidea indica* (Shafee, Alam and Agarwal) since June 2002 (Amalin et al. 2003) and the effects of a coccinellid predator, *Cryptolaemus montrouzieri* (Mulsant). Extensive damage to hibiscus plants has continued in residential areas and other managed urban landscapes throughout Miami-Dade County (Vitullo, personal observation) and data from sex pheromone traps for monitoring pink hibiscus mealybug indicate widespread prevalence of the pest in southern Florida. Consequently, ornamental nurseries in proximity to native and cultivated hosts of *M. hirsutus* may be at ongoing risk of infestation. This is a particularly pressing issue for the production of ornamentals in Florida nurseries, where a zero-tolerance policy for pink hibiscus mealybug has been in affect

since 2004 (FDACS 2005) and numerous quarantine actions have occurred (Ranjan 2004, Gaskalla 2006).

Chang (1996) and Moffitt (1999) addressed the economic risks posed by pink hibiscus mealybug to agriculture in the Caribbean and the US, and the factors associated with its continued spread. Chang (1996) evaluated the probability of its spread through human activities (cargo and baggage on boats and aircraft and various sources of mail), but little is known about natural modes of dispersal (windborne, phoresy and drifting debris). Risk analysis to this point has focused exclusively on the spread of pink hibiscus mealybug over long distances, for example between islands or from islands to the mainland, but dispersal on a more local scale has not been addressed (Ranjan 2004).

The study of insect dispersal on a local scale has been somewhat ignored (Pedgley 1993), perhaps due in part to the belief that testing the impact of dispersal on pest populations in agricultural settings is notoriously difficult (Byrne et al. 1996). Studies with aphids suggest that long-range dispersal, defined as more than 5 km (Loxdale et al. 1993), is rare and that more emphasis should be placed on short-range dispersal. Loxdale (1993) stated that, "short-range migration is as important biologically as long-range dispersal, and is probably more important agronomically." Understanding the dispersal capabilities of pest species within the environment, their capacity to locate unexploited hosts and the factors associated with their dispersal is essential for predicting the course of pest infestations and for planning control procedures (Rabkin and Lejeune 1954, Barrass et al. 1994).

Insect dispersal is associated with the location of mates or food resources, escape from deteriorating, crowded or unfavorable environmental or host plant conditions, or to avoid predators (Molles 2002). Immature scale insects (Quayle 1916, Rabkin and Lejeune 1954,

Hoelscher 1967, Greathead 1990), including mealybugs (Debach 1949, Furness 1976, Barrass et al. 1994), typically disperse and colonize new host plants by walking and by traveling passively on the wind. Mealybugs can also disperse by adhering via honeydew to falling leaves, as does the vine mealybug, *Planococcus ficus* Signoret (Daane et al. 2006). Anecdotal observations by Mirsa (1920) and Hall (1921) stated that newly-hatched crawlers of *M. hirsutus* may walk for "considerable" distances on a plant before settling to feed and that first instars are the aerially dispersive stage. Hall (1921) concluded that the rate and direction of spread of *M. hirsutus* around Cairo, Egypt, was directly related to aerial dispersal of nymphs and eggs from infested trees, although the dispersive capabilities of pink hibiscus mealybug and the biotic and abiotic factors that influence its dispersal have not been examined systematically. Understanding these aspects of its biology and their influence on its ability to disperse in the environment is important to those in the nursery and landscape industries. The presence of *M. hirsutus* in southern Florida has resulted in a reduction of hibiscus plantings in the local landscape, which in turn has reduced local hibiscus production.

The ability to effectively and efficiently monitor adult male pink hibiscus mealybug has been enhanced by the recent identification and use of its sex pheromone (Zhang et al. 2004a, Zhang and Amalin 2005, Vitullo et al. 2007). Investigation of the relationships between *M. hirsutus* population density, dispersal and the capture of males in pheromone traps may aid the development of effective management and risk mitigation tactics.

Here we report the results of field studies designed to address basic questions about the effects of biotic and abiotic factors on the aerial dispersal of pink hibiscus mealybug in southern Florida, the role of aerial dispersal in its colonization of new host plants, and the relationship between captures in pheromone traps and the infestation of sentinel plants.

MATERIALS AND METHODS

Research Site. Experiments were conducted at the University of Florida Tropical Research and Education Center (TREC) and at residential sites in Homestead, FL. Environmental data were from the Florida Automated Weather Network (FAWN) database, using instruments located at the TREC. All field experiments were conducted between May and September, 2006 - 2008, corresponding with the annual period of peak *M. hirsutus* abundance in southern Florida (Hall et al. 2008).

Insects and Test Plants. Pink hibiscus mealybug was reared on potatoes in a controlled environment chamber at 26ºC and a 12:12 L:D regime (Serrano et al. 2001), and unparasitized mealybugs from field-collected populations were added to the colony periodically. Hibiscus plants, *Hibiscus rosa-sinensis* L. 'President' with a canopy ~1 m high by 0.5 m wide were grown in 12 liter plastic pots in a greenhouse at 28 ± 1ºC and 40 - 98% RH. Plants were maintained in accordance with standard horticultural recommendations (Ingram and Rabinowitz 1991). For studies requiring different initial mealybug densities, individual adult females with their egg sacs attached were removed from potatoes and placed on apical buds using a micro-pin tool (Fig 2.1). To generate "heavily infested" plants, all mealybug stages were brushed from a potato onto the apical buds, using a fine-tipped soft brush. The potato was then placed on the plant crown and left for 2 wk to allow remaining mealybugs to move onto the plant.

Dispersal Traps. Aerially dispersing *M. hirsutus* were captured using the sticky liner of LDP traps (Suterra Inc., Portland, OR) affixed with binder clips to a 0.2 m^2 piece of pressed wood attached at the top of a 1.5 m wooden stake anchored vertically in a 19 liter plastic pot filled with cement (Fig 3.1). The sticky trapping surface (~18 cm^2) was similar in area to that used in previous studies of scale insect dispersal (Brown 1958, Hoelscher 1967, Barrass et al.

1994). A dissecting stereomicroscope was used to count all individuals captured and identification of their developmental stage was based on their relative size and antennal morphology (Hodges and Hodges 2005).

Dispersal in Relation to Abiotic Variables. On 14 June 2006, five hibiscus plants considered to be heavily infested with PHM and two non-infested control hibiscus plants were placed within a circular hole in the top of 1 m tall wooden stands (Fig 3.2) that were separated by 200 m in an open field at TREC and supported by metal stakes driven into the ground. Each plant was surrounded by a circle (1 m radius) of dispersal traps at the four cardinal and four intermediate compass points, with the sticky trapping surface oriented vertically and facing the plant (Fig 3.2).

Initially, the dispersal traps were replaced and inspected for mealybugs at 2-d intervals. When captures became consistent, the traps were replaced at 4-h intervals from 06:00 on 28 June through 06:00 on 5 July, and the number and stage of all individuals captured in each trap during each interval was recorded.

Environmental variables recorded during each 4-h trapping interval included mean and maximum wind speed (kph), mean relative humidity (%), mean temperature (ºC), mean solar radiation (w/m^2), and total rain fall (mm). Mean values were based on instantaneous readings taken at 1-h intervals.

Prior to all comparisons utilizing analysis of variance, tests for normality (PROC UNIVARIATE, SAS Institute 2001), were conducted. Data that did not meet assumptions of normality were logarithmically transformed. Means of significant effects were separated using Tukey's Honestly Significant Difference (HSD) test, variability was presented as standard error (SE), and all data comparisons were considered significant at $\alpha = 0.05$.

The diel periodicity of aerial dispersal was evaluated using circular statistics. Captures recorded at regular intervals within a 24-h period enabled testing the null hypothesis of uniform distribution around a circle. The time at which a capture was recorded was converted to an angular direction, based on a 360º circle, and these values were used in a chi-square goodness of fit analysis (Zar 1999). Rejection of the null hypothesis indicates that measurements were grouped in time, implying a diel periodicity. Similarly, the numbers of mealybugs captured in traps encircling each plant were converted to angular directions that were used in a chi-square goodness of fit analysis. Stepwise linear regression (PROC REG, SAS Institute 2001) was used to determine which environmental variable(s) best described the daily dispersal pattern.

Dispersal in Relation to Population Density. Beginning on 19 July 2007, five hibiscus plants were infested with 0, 1, 5, 10, or 20 adult female *M. hirsutus* and their egg sac. This was done with a new set of five plants for each of four consecutive weeks. Each group of five plants was held in the greenhouse for 32 d after being infested, at which time the emergence of second generation crawlers would have been predicted (Mani 1989), and then placed in the field. To assess the background population of mealybugs dispersing from other hosts near the site, five dispersal traps were deployed at the site for 1 wk prior to deploying the first group of plants. The infested plants were placed in the wooden stands described previously (Fig 3.2), separated by 100 m and each group of five plants was deployed continuously for 7 d. Due to the prevailing easterly wind direction in southern Florida, three dispersal traps were deployed 1 m downwind from each plant at the northwest, west and southwest compass points, beginning at 06:00 on the first day. Traps were replaced at 24-h intervals for 7 d and the number of crawlers captured was recorded. The number of dispersing mealybugs captured was compared among plants with

different initial populations, blocked by week, using a randomized complete block design ANOVA, (PROC GLM, (SAS Institute 2001).

Dispersal Distance. On 19 June 2007, five hibiscus plants were infested with many PHM adult females and crawlers. For 1 wk prior to placing plants in the field, ten dispersal traps were deployed at the test site to assess the background population of dispersing mealybugs. On 17 July, the infested plants were placed in the field using the wooden stands described previously, separated by 25 m and oriented in a line perpendicular to the prevailing easterly wind direction. Dispersal traps were deployed on a stand at randomly assigned distances of 1, 5, 10, 25 or 50 m from each plant. On each stand, one trap was oriented vertically at 1.5 m above the ground, with the sticky surface facing the plant, and a second trap was oriented horizontally at 0.25 m above the ground, with the sticky surface facing up. Our assumption was that mealybugs captured in vertical traps were being carried further downwind while those in the horizontal traps were dropping out of the air stream. The downwind distance of traps from each plant was rotated daily. Beginning at 06:00 on the first day, traps were replaced at 24-h intervals for ten consecutive days and the number of individuals captured on each was recorded. The capture of mealybugs on vertical and horizontal traps was compared among distances using PROC GLM (SAS Institute 2001).

Sex Pheromone Traps. Vitullo et al. (2007) showed that Jackson traps (Fig 1.2) were most efficient for monitoring pink hibiscus mealybug. These traps (Scentry Biologicals Inc., Billings, MT) were baited with a grey, halo-butyl septum (5 mm; West Pharmaceutical Services, Kearney, NE) loaded with 1 µg of *M. hirsutus* sex pheromone (Zhang et al. 2004a) and suspended ~3 cm above the sticky trap liner in the center of the trap interior. The trap liners were

examined for male *M. hirsutus* using a dissecting stereomicroscope at 60X magnification. Mealybug males were identified using the key in Hodges and Hodges (2005).

Infestation of Sentinel Plants in Relation to Male Captures. This experiment was designed to compare the latency to infestation of sentinel hibiscus plants deployed at residential sites that differed in the relative number of male mealybugs captured during a preliminary survey using pheromone traps. For the preliminary survey, a pheromone-baited Jackson trap was deployed for 2 wk at 50 and 40 residential sites in southern Miami-Dade County in late May 2006 and 2007, respectively. Most sites contained hibiscus plants while others were selected because there were no apparent or known hosts of *M. hirsutus* within 100 m (predominantly fallow agricultural fields). After the 2 wk survey, the total number of males captured at sites with hibiscus plants were divided into 33rd and 66th percentiles, and site ranking was determined based on their location within the percentiles. Sites with values closest to the middle of a percentile were selected and received their rank as follows: low (30-65 males), moderate (105-205 males), and high (>225 males). A fourth ranking included sites with no mealybug host plants present and traps at those sites yielded 0-15 males. Seven sites were selected for each ranking.

Four days after the survey traps were evaluated, a potted sentinel, hibiscus plant was secured to the ground at each site using a metal stake and placed 1-3 m downwind from other plants in the landscape, depending upon the physical constraints at each site. A pheromone trap was suspended just above the canopy of each sentinel plant. The plants were watered and terminals were inspected for feeding injury symptoms from *M. hirsutus* ("bunchy top") three times per week, while the pheromone trap liners were replaced and the capture of males was recorded weekly. Sentinel plants were deployed at the sites for 60 and 78 d in 2006 and 2007, respectively.

At the conclusion of the studies in both years, the presence of the introduced parasitoids, *A. kamali* and *G. indica*, was evaluated by pruning two terminals exhibiting feeding symptoms from each plant and holding them together in a cylindrical clear plastic container (20 cm high x 5 cm diam) topped with No Thrips Insect screen (81 x 81 mesh, International Greenhouse Company, Georgetown, IL) (Fig 3.3) and held for 50 d at 24.5ºC and a 12:12 L:D photo regime. Containers were inspected daily and emerged parasitoids were identified using Michaud's (2000) key.

The number of males captured during the 2 wk survey was regressed against the number of males captured during the trial period when sentinel plants were deployed at each site (PROC REG, SAS Institute 2001). PROC GLM (SAS Institute 2001) was used to compare the number of males captured after sentinel plants were deployed and the latency (d) to first expression of feeding symptoms among sites differing in ranking.

RESULTS

Dispersal in Relation to Abiotic Variables. No mealybugs were captured in traps surrounding non-infested control plants. The total number of *M. hirsutus* captured per infested plant over the 7 d trial ranged from 1,284 to 3,132. Of the 11,571 *M. hirsutus* captured in traps surrounding infested plants, 97.2% and 2.8% were 1st and 2nd instars, respectively.

The capture of aerially dispersing *M. hirsutus* nymphs was not uniformly distributed within a 24-h period ($X^2_{0.05,4}$ = 14,164; $P < 0.001$), but rather showed a diel periodicity that peaked between 14:00 and 18:00 h (Fig. 3.4). Similarly, the capture was not uniformly distributed among the traps encircling each plant ($X^2_{0.05,7}$ = 27,356; $P < 0.001$), showing a

directionality that revealed highest captures in traps to the northwest, west, and southwest (Fig. 3.5).

There was a significant relationship between the number of dispersing *M. hirsutus* nymphs captured and abiotic, environmental variables ($F = 33.73$; df = 1,40; $P < 0.001$). Mean wind speed explained 45.75% of the variation, while mean temperature, maximum solar radiation, mean relative humidity, maximum wind speed, and rain (total = 16 mm during the study) did not significantly affect the model.

Dispersal in Relation to Population Density. No mealybugs were captured in dispersal traps deployed prior to placing infested plants in the field or in traps downwind of non-infested hibiscus plants. Initial infestation level had a significant effect on the mean number of *M. hirsutus* nymphs captured ($F = 24.66$; df = 4,135; $P < 0.001$) (Fig. 3.6). Captures did not differ among plants initially infested with 5, 10 or 20 females, or between plants infested with one and five females, but were significantly higher from plants with 10 and 20 females than with one female.

Dispersal Distance. No mealybugs were captured in traps deployed prior to placing infested plants in the field. There was no significant difference among plants in the mean number of *M. hirsutus* nymphs captured per day ($F = 2.36$; df = 4,95; $P = 0.059$). Significantly more *M. hirsutus* nymphs were captured in vertical (18.4 ± 6.0a) than in horizontal (3.0 ± 1.2b) sticky traps ($F = 4.58$; df = 1,98; $P = 0.035$). There was a significant effect of trap distance from the infested source plant on the capture of dispersing *M. hirsutus* nymphs ($F = 8.42$; df = 9,90; $P < 0.001$). Nymphs were captured in traps at all distances from the infested source plant and in significantly greater numbers in the vertical trap at 1 m than in all others (Fig. 3.7). As the distance a trap is placed away from an infestation point source increases, the width of the cone of

dispersal also increases, decreasing the likelihood of capturing a dispersing *M. hirsutus* nymph in a same sized trap when compared to shorter distances. The fact that a few nymphs were captured at 50 m suggests that many more dispersed up to 50 m and were not trapped.

Infestation of Sentinel Plants in Relation to Male Captures. During the 2 wk survey, captures of male *M. hirsutus* in pheromone traps at residential sites with hibiscus hosts ranged from 11 – 1,320 in 2006 and from 14 – 4,751 in 2007 (Table 3.1).

Sentinel plants were lost from four and five sites in 2006 and 2007, respectively, and data from those sites were not used in analyses. None of the plants at sites where there were not hosts of *M. hirsutus* exhibited feeding symptoms and those were not included in analyses of the latency to first expression of feeding symptoms.

In 2006, the number of males captured in pheromone traps during the 2 wk survey was positively correlated with captures during the subsequent trial period when sentinel plants were deployed ($F = 7.61$; df = 1,22; $P < 0.001$) and explained 71.24% of the variation. In 2007, one site initially ranked as low yielded 50 and 938 males during the survey and trial periods, respectively. The number of males captured in pheromone traps during the 2 wk survey in 2007 was not positively correlated with captures during the subsequent trial period when sentinel plants were deployed ($F = 0.41$; df = 1,21; $P = 0.531$) and only 1.90% of the variation was explained. The inconsistent relationship between the numbers of males trapped during the trial is further supported by the relationship among site ranking and the number of males trapped during the trial. The number of males captured in pheromone traps during the trial period differed significantly among the four different site rankings in 2006 ($F = 34.81$; df = 3,20; $P < 0.001$) and 2007 ($F = 16.06$; df = 3,19; $P < 0.001$) (Table 3.2). In both years, pheromone traps at no-host ranked sites captured fewer males than traps at all other sites.

The percentage of plants that became infested at sites ranked no-host, low, medium and high was 0.0, 42.9, 85.7, and 66.7% in 2006, and 0.0, 71.4, 66.7, and 83.3% in 2007, respectively. Latency (d) to the first shoot exhibiting feeding symptoms did not differ significantly among sites ranked differently in 2006 ($F = 0.33$; df = 2,10; $P = 0.727$) or 2007 ($F = 0.07$; df = 2,11; $P = 0.930$) (Table 3.2).

Of 13 and 14 sentinel plants that exhibited feeding injury in 2006 and 2007, terminals from eight plants yielded parasitoids each year. In 2006, 76 *A. kamali* (73% male) were collected and the mean total number of parasitoids from plants at sites ranked low (n = 2), moderate (n = 3) and high (n = 3) were 7.6 ± 2.8, 16.3 ± 11.9 and 2.0 ± 1, respectively. In 2007, 61 *A. kamali* (57% male) and 28 *G. indica* (60% male) were collected and the mean total number of parasitoids from plants at sites ranked low (n = 2), moderate (n = 3) and high (n = 3) were 1.6 ± 1.6, 12.6 ± 5.5 and 23.0 ± 21, respectively.

DISCUSSION

Our studies have documented the aerial dispersal of pink hibiscus mealybug and provide new information on some of the factors associated with its local dispersal. First instars were the predominant dispersive stage, representing 97% of the individuals captured, concurring with the results of Barrass et al. (1994), who showed that first instars of *Pseudococcus longispinus* (Targioni-Tozzetti) represented 89% of the aerially dispersing individuals captured. The diel periodicity of aerial dispersal of *M. hirsutus* crawlers suggested that dispersal is not a passive process but is evoked by some factor(s). Of the abiotic factors measured, I showed that dispersal of *M. hirsutus* was most strongly influenced by mean wind speed. Aerial dispersal of *Aonidiella auranti* (Maskell) (Greathead 1990) and *P. longispinus* (Barrass et al. 1994) was also positively

correlated with wind speed, as well as temperature. Once aerial dispersal was initiated, crawlers traveled passively on air currents, indicated by the correspondence between the directionality of captures and the direction of the prevailing wind in southern Florida, supporting Hall's (1921) observations regarding the spread of an infestation by pink hibiscus mealybug in Cairo, Egypt.

Under the study conditions, plants initially infested with 5, 10 or 20 adult female mealybugs and their egg sacs did not differ significantly in the number of aerially dispersing crawlers. At the onset of trapping, plants showed varying degrees of feeding symptoms that increased with initial infestation density corresponding with the results from Chapter 2 (Fig 2.4), although the effects of differing biotic factors that may have evoked dispersal (e.g. crowding, deteriorating plant conditions) were not apparent and the effects of abiotic factors (e.g. weather conditions) may have been most influential.

Maconellicoccus hirsutus crawlers were captured in vertically oriented traps 50 m downwind of infested source plants. Captures in horizontally oriented traps at all distances showed that crawlers settled out of the air stream at distances as close as 1 m from the plant. The distance over which small organisms can be carried passively on air currents is influenced by the height from which they disperse, wind speed, and the velocity at which they settle out of the airstream (Frost 1997). Given that our source plants were at an elevation of only 1.5 m above the ground, it appears likely that crawlers leaving host plants taller than those used here traveled for distances considerably further than 50 m. Armored scale crawlers of *A. auranti* have been carried over 300 m and *Aulacaspis tegalensis* (Zehnter) can be carried vertically by temperature inversions and then moved long distances downwind (Greathead 1990). Soft scale, *Pulvinariella mesembryanthemi* (Vallot) were captured 50 m above the ground, and Greathead (1997) calculated that crawlers could be spread over 190 km in 24 h on winds moving 8 km/h.

Male *M. hirsutus* were captured in pheromone traps at 45 of 48 and 38 of 40 sites in 2006 and 2007, respectively, with substantial variation among sites. Captures at non-residential sites, selected because of the absence of mealybug host plants nearby, were much less variable and typically much lower than at residential sites. The data suggest that captures of males in pheromone traps during an initial 2 wk survey were not an accurate predictor of future captures. In 2006, initial ranking of sites corresponded with subsequent captures and the relationship explained 71% of the variation, but this relationship is influenced by two highly leveraged points in the high ranking. In 2007, the relationship between initial and subsequent captures was not significant, explaining only 1.9% of the variation. Given that numerous variables could have affected the relationship between our initial and subsequent captures, the use of sex pheromone traps as a predictor of future numbers of males trapped has yet to be resolved. However, these data further support the utility of pheromone traps for monitoring the number of male mealybugs present in the environment, as their numbers were quite variable at all but the no-host ranked sites.

Relationships that may have been associated with the infestation of sentinel plants include those between population density, the number of males captured and the number of crawlers dispersing. The data do not allow interpretation of the relationship between captures in traps and mealybug population densities or between male captures and dispersing crawlers. My attempts to develop these relationships under controlled conditions using plants infested with different numbers of mealybugs and deployed in an open field were not successful (Vitullo, unpublished data). However, I have indications that the number of aerially dispersing crawlers is related to population density. Nevertheless, neither the number of sentinel plants that became infested nor the latency to their expression of feeding symptoms differed among sites differing in

the relative number of males captured, with the exception of sites with no mealybug host plants where few males were captured and no sentinel plants became infested. Most sentinel plants at residential sites ranked low, moderate and high showed feeding injury symptoms after about one month. The expression of feeding injury by similar numbers of sentinel plants at sites ranked as low, moderate and high may have been due to the susceptibility of the cultivar used. Vitullo et al. (2009) showed that plants of this cultivar ('President') expressed injury symptoms from mealybugs within 7 to 15 d after being infested with densities of 1, 5, 10, or 20 adult females and egg sac per plant. There did not appear to be a relationship among sites differing in the relative numbers of male mealybugs captured and the numbers of parasitoids in the area. *Anagyrus kamali* and *G. indica* were generally found in relatively low numbers and were highly variable among sites with the same infestation ranking.

Millar et al. (2002) demonstrated significant correlations among *Planococcus ficus* (Signoret) captures in pheromone traps, visual sampling and economic damage, although Walton et al. (2004) could not develop a simple model to estimate *P. ficus* infestation levels because of the lure's high level of attractiveness. The traps often captured males in vineyards where no female *P. ficus* were located during visual searches (Walton et al. 2004). Monitoring *P. ficus* populations using pheromones in monoculture crops is different from that for *M. hirsutus* populations in urban landscapes and nurseries that are heterogeneous in their composition and distribution of host plants. Under these heterogeneous conditions, monitoring becomes much more complex (Hanski 1998).

While these studies have revealed new information about the dispersal of *M. hirsutus* and the risk that this may pose to the production of susceptible host plants in commercial nurseries and managed landscapes, our results also highlight much that remains unknown, particularly

with respect to the use of pheromone traps for monitoring populations and measuring risk. Commercial production of hibiscus and other ornamental plants in southern Florida occurs in heterogeneous urban landscapes in which nurseries are interspersed with both residential and undeveloped areas containing many potential hosts, creating a situation of ongoing risk of infestation of nurseries. Ultimately I seek to provide commercial ornamental producers with a tool to help manage the risk associated with the dispersal of pink hibiscus mealybug in the local environment. While the sex pheromone of pink hibiscus mealybug is a sensitive and species-specific tool for monitoring the pest, the variables that influence the number of males captured are numerous. The results of the previous studies have added valuable information toward the optimization of sex pheromone based monitoring programs and will help make it possible to recommend trapping programs to nursery growers and to mitigate risk from *M. hirsutus*. However, further understanding of the relationship between pheromone trapping and other varying aspects of *M. hirsutus* population dynamics is needed to realize this goal.

Table 3.1. The range of male pink hibiscus mealybugs captured in pheromone traps at residential sites containing host plants and at non-residential sites without host plants in Homestead, FL.

Frequency distribution of the number of males captured	Number of sites			
	2006[1]		2007	
	No host plants	Host plants	No host plants	Host plants
0-15	8	1	7	1
16-100	1	11	3	13
101-200	0	12	0	8
201-300	0	4	0	3
301-400	0	3	0	3
401-500	0	6	0	1
>1000[2]	0	2	0	1

[1] 2-wk survey during May 2006 and 2007.

[2] 1030 and 1320 males captured at two sites in 2006, and 4751 males captured at one site in 2007.

Table 3.2. Male pink hibiscus mealybugs captured in pheromone-baited traps and the latency (d) to first feeding symptom on sentinel hibiscus plants deployed at residential and non-residential sites ranked according to preliminary surveys of mealybug populations.

Ranking	No. males captured during initial 2-wk survey		Mean ± SE total no. males trapped subsequently (no. sites)		Mean ± SE latency (d) (no. infested plants)	
	2006	2007	2006	2007	2006	2007
High	403 - 1320	225 - 441	954.3 ± 189.0a (6)	193.2 ± 49.0a (6)	36.23 ± 2.8a (4)	37.8 ± 7.9a (5)
Moderate	133 - 202	108 - 165	547.0 ± 125.1ab (7)	149.3 ± 37.4a (6)	29.5 ± 7.1a (6)	35.0 ± 4.5a (4)
Low	31 - 64	35 - 64	254.4 ± 68.6b (7)	275.4 ± 112.7a (7)	33.0 ± 3.2a (3)	34.8 ± 5.4a (5)
No-host	0 - 5	0 - 14	26.8 ± 7.8c (4)	12.0 ± 4.1b (4)	0	0

Means followed by the same letter are not significantly different ($P<0.05$) using logarithmically transformed data, according to Tukey's HSD test.

Figure 3.1. Sticky liner of LDP traps positioned vertically 1.5 m from the ground and horizontally 0.25 m from the ground, affixed with binder clips to a wooden stake anchored vertically in a 19 liter plastic pot filled with cement.

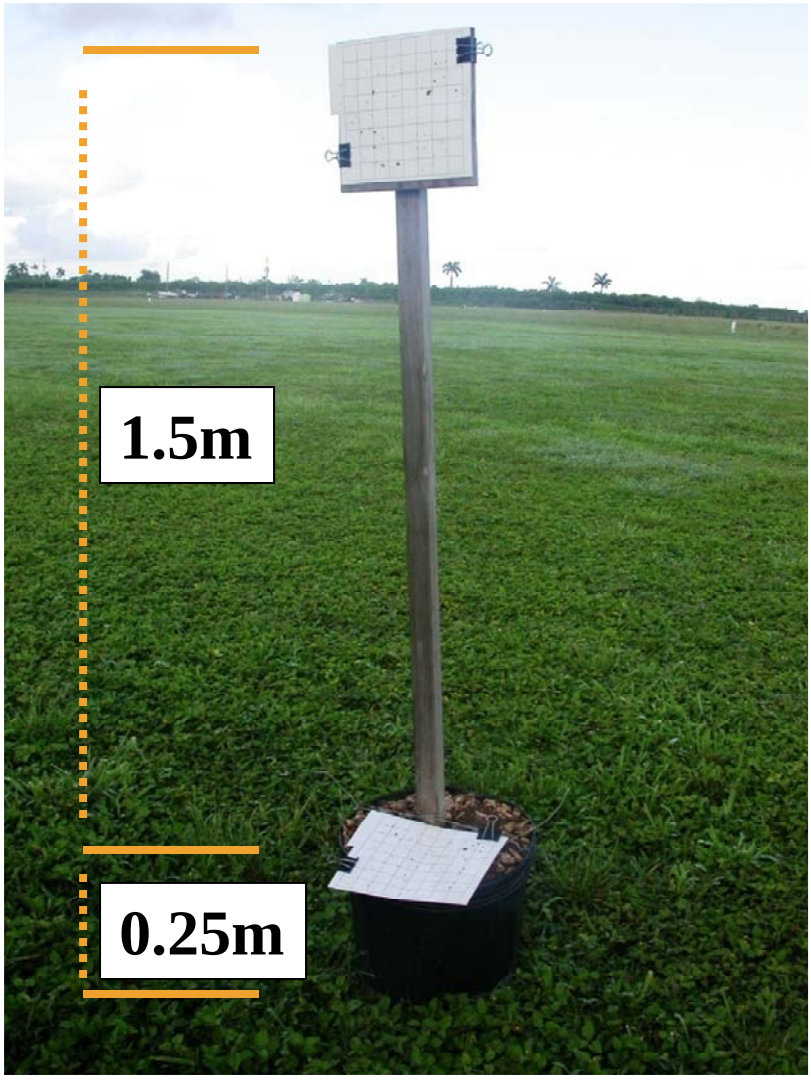


Figure 3.2. Hibiscus within a circular hole in the top of 1 m tall wooden stand, surrounded by a circle (1 m radius) of dispersal traps at the four cardinal and four intermediate compass points, with the sticky trapping surface oriented vertically and facing the plant.

Figure 3.3. Terminals exhibiting feeding symptoms held together in a cylindrical clear plastic container (20 cm high x 5 cm diam) topped with No Thrips Insect screen.

Figure 3.4. The number of pink hibiscus mealybug crawlers captured in dispersal traps encircling individual hibiscus plants (n = 5) at 4-h intervals over 7 consecutive days. Each line represents a plant.

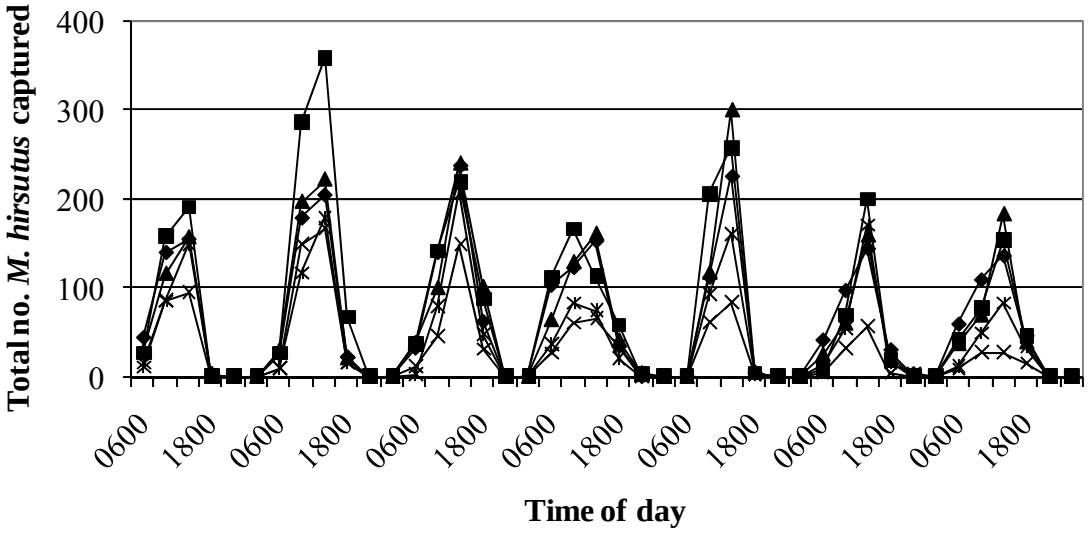


Figure 3.5. The mean (±SE) number of pink hibiscus mealybug crawlers captured in dispersal traps encircling infested hibiscus plants.

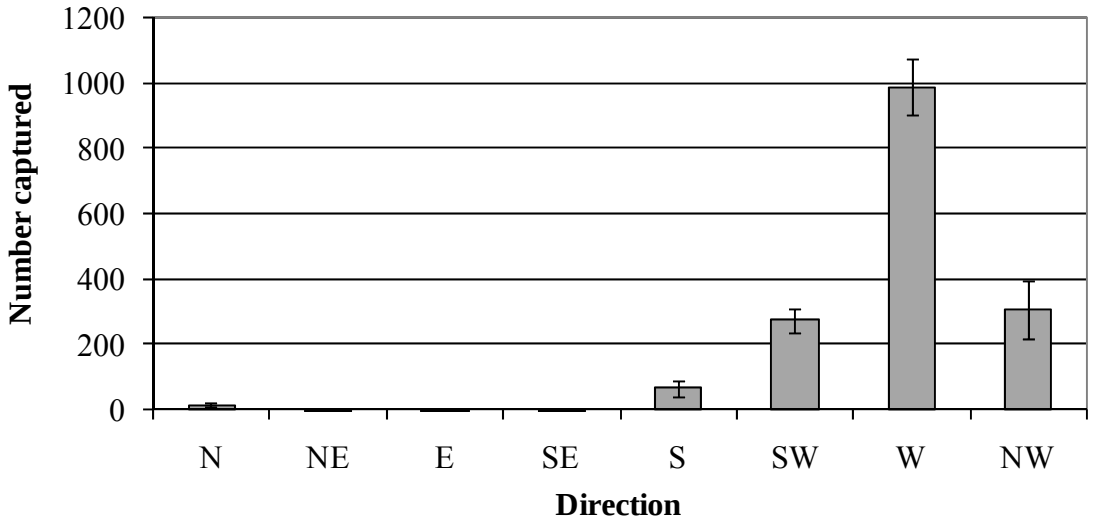


Figure 3.6. The mean (±SE) number of pink hibiscus mealybug crawlers captured per day (n = 7 d) in dispersal traps downwind of hibiscus plants differing in initial density of females.

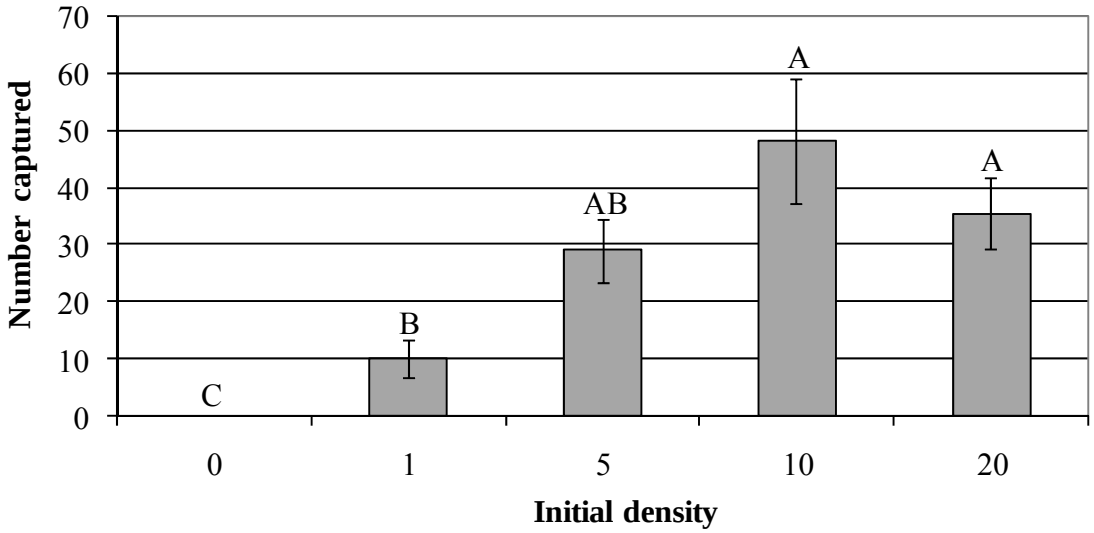
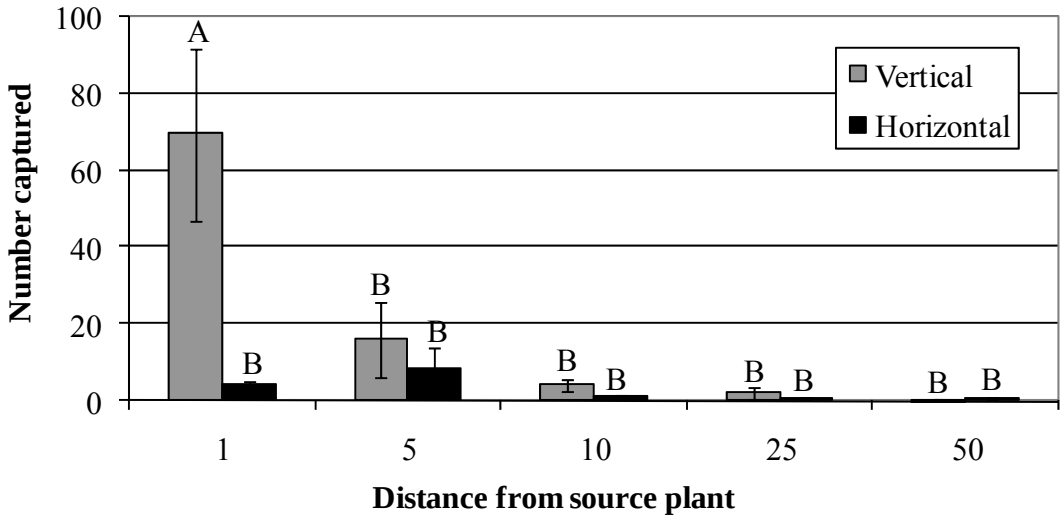


Figure 3.7. The mean (±SE) number of pink hibiscus mealybug crawlers captured in vertically- and horizontally-oriented dispersal traps at distances (m) downwind of infested hibiscus.



CHAPTER 4: THE RESPONSE OF PINK HIBISCUS MEALYBUG (HEMIPTERA: PSEUDOCOCCIDAE) TO CHEMICAL AND BIOLOGICAL MANAGEMENT TACTICS UNDER SEMI-FIELD CONDITIONS

ABSTRACT

A study assessing the ability of sticky band traps to reflect different densities of pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) on potted hibiscus plants showed that captures consisted predominantly of 1st instars (89.1%) and adult females (9.7%) and that total captures were directly dependent on the number of females initially transferred to the apical buds on shoots. The effects of mating disruption by using sex pheromone, soil-applied insecticide (dinotefuran), the parasitoid, *Anagyrus kamali* (Moursi), and the predator, *Cryptolaemus montrouzieri* (Mulsant) on *M. hirsutus* populations on hibiscus plants in screened field cages were evaluated. Treatment effects were compared via differences in the number of nymphs and adult females captured in sticky band traps and adult males captured in pheromone traps during the studies and the number of individuals recovered from hibiscus shoots at the conclusion. In a field cage study extending over 7 wk, the total number of mealybugs captured in sticky band traps and pheromone traps during the study was significantly reduced by dinotefuran and the predator, but not by mating disruption or the parasitoid, relative to the untreated controls. At the end of the study, the number of nymphs recovered from hibiscus terminals was significantly reduced relative to the controls, by the dinotefuran, predator and parasitoid treatments while only

the predator treatment significantly reduced the number of adult females and egg sacs recovered. In a second field cage study, a higher rate of mating disruption alone and in combination with parasitoids was evaluated and compared with dinotefuran and the predator. Due to tropical storm Fay, this study was ended at 4 wk after treatments were initiated but yielded similar results. Four weeks after treatment, dinotefuran reduced the number of mealybugs captured in sticky band traps, relative to the untreated controls.

INTRODUCTION

The pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae), is considered an economic pest in many of the world's tropical and semi-tropical regions (CABI/EPPO 2004). Since its initial detection on hibiscus plants in southern Florida in 2002 (Amalin et al. 2003), *M. hirsutus* has been detected in Louisiana (LDAF 2006), Texas (Bogran and Ludwig 2007), and Georgia (Horton 2008). *Maconellicoccus hirsutus* has a broad host range that includes both economically important and native plants (Persad 1995, Stibick 1997, Hodges and Hodges 2005), and typically establishes colonies in sheltered locations on plants. Colonies are further protected by hydrophobic wax filaments, making control using foliar insecticides difficult. In the Caribbean, the use of pesticides and the destruction of infested host plants did not provide adequate control of *M. hirsutus* (Sagarra and Peterkin 1999). In forests and urban landscapes, the diversity of *M. hirsutus* hosts and/or the size of some host plants make insecticide spray programs financially and logistically unrealistic (Sagarra and Peterkin 1999). In Florida nurseries, prophylactic foliar sprays and drenches to avoid quarantine actions due to pink hibiscus mealybug have been recommended by the University of Florida (FDACS 2005).

In response to the severe impact of *M. hirsutus* in the Caribbean, a biological control program was implemented, involving the importation and/or mass-rearing and release of the encyrtid parasitoids, *Anagyrus kamali* (Moursi) and *Gyranusoidea indica* (Shafee, Alam and Agarwal). Their establishment, in combination with the effects of predation by the coccinellid, *Cryptolaemus montrouzieri* (Mulsant), significantly reduced *M. hirsutus* populations on many islands (Kairo et al. 2000) and slowed its spread in Puerto Rico (Michaud and Evans 2000). Within a week of detecting *M. hirsutus* in southern Florida, state and federal agencies responded with a biological control program involving release of the aforementioned parasitoids and predator (Amalin et al. 2003) and while natural enemies have greatly reduced its populations in Florida (Amalin et al. 2003), the pest has spread to 37 counties since 2002 (G. Azore, PHM Field Supervisor, FL Department of Agriculture & Consumer Services (FDACS), Division of Plant Industry (DPI), pers. comm.). Since new infestation sites continue to be identified (G. Azore, pers. comm.) and re-infestation of previously infested sites is possible (Ranjan 2004), *M. hirsutus* remains an ongoing threat to ornamental nursery production and landscape management in Florida.

Identification and synthesis (Zhang et al. 2004a) of the pink hibiscus mealybug sex pheromone was followed by tests comparing the effects of pheromone blends and source concentrations on the response of males under field conditions in Florida (Zhang and Amalin 2005). Traps baited with 1 and 10 μg pheromone lures captured significantly more males than those baited with a 0.1 μg lure. However, captures were reduced significantly in traps baited with a 100 μg lure and equivalent to those containing a 0.1 μg lure (Zhang and Amalin 2005), suggesting that the higher pheromone source concentration was inhibitory to the response of male *M. hirsutus* and that pheromone-based mating disruption may have some utility under

certain circumstances. Zhang and Amalin (2005) also reported that parasitoids of *M. hirsutus* captured in baited pheromone traps were not different from unbaited traps, indicating the compatibility of pheromone-based monitoring and management with biological control programs. Walton et al. (2006) evaluated management of vine mealybug, *Planococcus ficus* (Signoret), in California vineyards using a sprayable formulation of its sex pheromone alone and in combination with applications of the insect growth regulator, buprofezin, and observed a reduction in the number of males caught in traps. Although reduced captures may be a useful indicator of the effects of pheromone on male orientation to pheromone baited traps, they do not necessarily correlate with reduced mating or population size. Walton et al. (2006) also showed that while mating disruption alone did not reduce the size of vine mealybug populations, mating disruption in combination with insecticide applications significantly reduced populations and that crop damage was reduced by about 6%.

Due to the pest status and distribution of pink hibiscus mealybug there has been considerable research on the effectiveness of management tactics, especially biological (Mani 1987, Sagarra & Peterkin 1999, Michaud & Evans 2000, Kairo et al. 2000, USDA 2001, Amalin et al. 2003, Roltsch et al. 2006) and chemical control (Stibick 1997, Sagarra & Peterkin 1999, Osborne 2005, Cloyd & Dickinson 2006). Mating disruption for *M. hirsutus* has not been evaluated alone or in combination with other tactics. Aside from measuring the effects of mating disruption, pheromone traps may be a useful tool for monitoring and measuring the effects of other control tactics for pink hibiscus mealybug. Here, we report the results of field cage trials in which the effects of several management tactics for pink hibiscus mealybug were compared, based on changes in the numbers of individuals captured in pheromone and sticky band traps.

MATERIALS AND METHODS

Insects, Test Plants, and Field Cages. Experiments were conducted at the University of Florida Tropical Research and Education Center (TREC) in Homestead, FL. Following Serrano et al. (2001), pink hibiscus mealybug was reared on potatoes in a controlled environment chamber at 26ºC and a 12:12 L:D photo regime. *Hibiscus rosa-sinensis* L. (cultivar 'President') plants with a canopy ~1 m high and 0.5 m wide were maintained in accordance with standard horticultural recommendations (Ingram and Rabinowitz 1991) in 12 L plastic pots in a greenhouse at the TREC at 28 ± 1ºC and 40 - 98% RH. Plants were infested by removing individual adult female *M. hirsutus* and their egg sac (Fig. 2.1) from colonies on potatoes using a micro-pin tool and transferring them to apical buds. Periodic releases of *Aphidius colemani* (Viereck) (IPM Laboratories, Inc., Locke, NY) kept aphid levels low in the greenhouse.

Wood frame cages (1.5 m tall x 1 m wide x 1 m deep) for isolating individual plants in the field were enclosed by 0.5 m tall plywood panels at the bottom and No Thrips Insect screen (81 x 81 mesh, hole opening = 0.15 mm x 0.15 mm, International Greenhouse Company, Georgetown, IL) on the remaining portions of the sides and the top of each (Fig. 4.1). A hinged door allowed access to the cage interior.

Mealybug Traps. Treatment effects were compared via changes and differences in the number of mealybugs captured in traps. Sticky band traps were used to capture juveniles and adult females walking on branches while pheromone traps were used to capture adult males. Sticky band traps were constructed by wrapping a piece of electrician's tape (2.0 cm wide) snugly around a branch. A 1.5 cm wide strip of Parafilm (American National Can, Greenwich, CT) was wrapped tightly around the electrician's tape and a 1.0 cm wide band of Tangle-Trap (The Tanglefoot Company, Grand Rapids, MI) was created around the middle of each Parafilm

band (Fig. 4.2). To remove these traps, the Parafilm band was cut vertically and placed on a piece of double-sided tape in the bottom of a Petri dish, labeled so that the upper and lower trapping surfaces on each band were identifiable. The numbers of *M. hirsutus* captured in the upper and lower trapping surfaces (i.e. those moving away from and toward each colony, respectively) were counted using a dissecting stereomicroscope at 50X magnification and identification of their developmental stage was based on antennal morphology and size (Hodges and Hodges 2005).

Jackson traps (Scentry Biologicals Inc., Billings, Montana) were shown to be preferable for monitoring male *M. hirsutus* (Vitullo et al. 2007) (Fig 1.2). These were baited with a grey, halo-butyl septum (5 mm; West Pharmaceutical Services, Kearney, NE) loaded with 1 µg of *M. hirsutus* sex pheromone (Zhang et al. 2004b) and suspended ~3 cm above the trap liner in the center of the trap. The mealybugs captured in traps were counted using a dissecting stereomicroscope at 60X magnification and identification was based on Hodges and Hodges (2005).

Capture of Mealybugs in Relation to Initial Infestation Density. Since we intended to use changes or differences in the number of mealybugs captured in sticky band traps on plant branches as one measure of the effects of management tactics on mealybug populations, an initial experiment was conducted to determine whether captures reflected different mealybug densities. Five hibiscus plants were placed in the greenhouse on 18 May 2006. Each plant was pruned to four lateral branches with an apical bud, with no contact among branches, and a sticky band trap was placed at the base of each branch. Initial infestation densities of 0, 1, 5, or 10 adult females with egg sac were randomly assigned to the apical buds on branches of each plant and mealybugs were transferred on 18 June. The traps were inspected daily using a 10X hand lens

until 10 July, when the first crawler was recorded, after which traps were removed and replaced at 2-d intervals from 17 July until 11 August.

The total number of *M. hirsutus* captured in traps was compared among the four initial infestation densities and between the upper and lower trapping surfaces on band traps using a 4x2 fixed factor randomized complete block ANOVA with plants serving as replicated blocks (n = 5) (PROC GLM, (SAS Institute 2001).

Effectiveness of Individual Management Practices. Based on the seasonal phenology of *M. hirsutus* in southern Florida, where populations peak during the summer and are abundant through November (Hall et al. 2008), field cage trials were conducted between May and August, 2008. Twenty-five hibiscus plants were infested with five adult females with egg sac on each of 3, 10, 17, and 24 April, held in a greenhouse until 1 May and then placed individually in cages. Cages were separated by 5 m in a line parallel to the prevailing easterly wind direction. On each plant, a sticky band trap was placed 10 cm below the apical bud on three branches showing *M. hirsutus* feeding symptoms and a pheromone trap was suspended just above the canopy.

After 7 d, five replicates of each of the following treatments were randomly assigned to blocks of five plants within the array of cages; 1) Insecticide: Dinotefuran (Safari 20SG, Valent USA Corp, Walnut Creek, CA) applied as a soil drench at 0.425 g formulated product in 237 ml water (maximum labeled rate for a 12 L pot), 2) Parasitoid: *A. kamali* obtained from the FDACS-DPI were released at a density of 75 wasps per cage, based on FDACS guidelines, 3) Predator: *C. montrouzieri* obtained from FDACS were released at a density of 15 adult beetles per cage, based on FDACS guidelines, 4) Mating disruption: Grey, halobutyl septa (n = 5 per cage) loaded with 50 μg of *M. hirsutus* pheromone were attached to the sides of each cage and to a stake within the canopy of each plant at mid-plant height, and 5) untreated control.

Pheromone trap liners and sticky band traps were replaced at 7 d intervals from 1 May – 26 June and the number of individuals captured in each was recorded. At the end of the trial (26 June), three 15 cm long terminals showing *M. hirsutus* feeding symptoms were pruned from each plant and the number of *M. hirsutus* and *C. montrouzieri* on each were counted. To measure the presence and abundance of parasitoids, two terminals exhibiting feeding symptoms were pruned from each plant, placed in a clear plastic container (20 cm high x 5 cm diam) topped with No Thrips Insect screen and held at 24.5ºC and a 12:12 L:D photo regime. Containers were inspected daily for 28 d and emerged parasitoids were identified following Michaud (2000).

Effectiveness of Combined Management Practices. Twenty-five hibiscus plants were infested with five adult females with egg sac on each of 5, 12, 19, and 26 June, held in a greenhouse until 3 July and then placed individually in cages. Pheromone and sticky band traps were deployed as described previously and replaced at 7 d intervals from 3 July – 7 August. The treatments compared were based on the outcome of the previous experiment. After 7 d, five replicates of each of the following treatments were randomly assigned to blocks of five plants within the array of cages; 1) Insecticide: As described previously, 2) Parasitoid: As described previously, 3) Mating disruption: Grey, halobutyl septum (n = 5 per cage) loaded with 100 μg of *M. hirsutus* pheromone and deployed as described previously, 4) Parasitoid and mating disruption: *A. kamali* at 75 wasps per cage and mating disruption at 5 x 100 μg dispensers per cage, and 5) untreated control.

On August 12, the impending arrival of tropical storm Fay required that cages and plants be moved, exposing plants to rain and 40 kph winds. Due to this interruption, only data from the first 5 wk of this study (3 July – 7 August) were used in the analyses.

Data Analysis. Repeated measures ANOVA (PROC MIXED, SAS Institute 2001) based on compound symmetry and first-order autoregressive models (Ott and Longnecker 2001) was used to determine the effects of treatment, week after infestation, and their interaction on the number of nymphs captured in sticky band traps. The number of nymphs captured in band traps each week and in total was then compared among treatments using PROC GLM (SAS Institute 2001). The number of adult males captured in pheromone traps was evaluated via the same tests. The total number of adult females captured in band traps and the total number of nymphs, adult females, and unhatched egg sacs recovered from terminals at the end of the study were compared using PROC GLM (SAS Institute 2001). Percentage reductions in the number of mealybugs trapped among significantly different treatments were calculated, based on Abbott's formula (). The relationship between the number of males captured in pheromone traps and mealybugs in band traps was evaluated using linear regression (PROC REG, SAS Institute 2001).

Prior to ANOVA, tests for normality using PROC UNIVARIATE (SAS Institute 2001) were performed and data not meeting that assumption were logarithmically transformed. Means of significant effects using ANOVA were separated using Tukey's HSD test and analyses of data from all experiments were considered significant at $\alpha = 0.05$.

RESULTS

Capture of Mealybugs in Relation to Initial Infestation Density. There was no block effect from plants on the number of mealybugs captured ($F = 0.05$; df = 4,35; $P = 0.995$). In total, 19,606 *M. hirsutus* were captured in sticky band traps, comprised of 89.1% 1st instars, 0.7% 2nd instars, 0.5% 3rd instars and 9.7% adult females. There was a significant effect of initial infestation density on the number of mealybugs captured ($F = 9.59$; df = 3,32; $P < 0.001$). Mean

(± SE) total captures over the 21 d trial from terminals initially infested with 0, 1, 5 and 10 females with egg sac were 30 ± 9, 114 ± 91, 349 ± 263 and 1468 ± 551 mealybugs, respectively. Captures from plants infested with 10 females with egg sacs were significantly higher than all other initial infestation densities, with no statistical separations among the others. Significantly more ($F = 16.31$; df = 1,32; $P < 0.001$) mealybugs were captured in the upper (924.3 ± 324.5) versus the lower trapping surface of sticky bands (56.0 ± 9.8) and there was a significant interaction between initial infestation density and trapping surface ($F = 8.63$; df = 3,32; $P < 0.001$) (Fig. 4.3).

Effectiveness of Individual Management Practices. There was no significant block effect on the total number of nymphs ($F = 0.20$; df = 4,20; $P = 0.933$) or adult males ($F = 1.60$; df = 4,20; $P = 0.213$) trapped during the study. During the first week of sampling, prior to the imposition of treatments, neither the number of mealybug nymphs ($F = 1.23$; df =4,20; $P = 0.328$) or adult males ($F = 0.47$; df =4,20; $P = 0.755$) captured differed significantly among treatments (Pre-treatment, Table 4.1). Repeated measures comparisons of the effects of treatment, week after treatment and their interaction on the number of nymphs captured in sticky band traps and adult males captured in pheromone traps showed negligible differences between compound symmetry and first-order autoregressive analysis for all analyses. Compound symmetry analysis of the number of nymphs captured showed significant effects of treatment ($F = 26.21$; df = 4, 140; $P < 0.001$), week after treatment ($F = 17.83$; df = 6, 140; $P < 0.001$), and their interaction ($F = 6.43$; df = 24,140; $P < 0.001$).

The mean number of nymphs captured increased in all treatments in weeks 1 and 2 and there were not significant differences among them (Table 4.1). Captures of nymphs from the untreated control plants showed a relatively steady increase through week 6 and generally similar

trends were exhibited by plants exposed to mating disruption or parasitoids, with no significant differences among those treatments for the duration of the study. By week 3, captures of nymphs in the predator treatment were significantly reduced relative to all treatments except the insecticide. Thereafter, the predator treatment showed a steady increase in the number of nymphs captured through week 6, at which point there were no differences between it, the untreated controls, mating disruption or parasitoids. Captures of nymphs from insecticide treated plants showed a pronounced decline on week 4 and captures were significantly lower than from all other treatments through week 6. In week 7, captures of nymphs decreased in all treatments except the insecticide, which showed increased captures (Table 4.1).

The total number of nymphs captured during the study differed significantly among treatments ($F = 10.37$; df = 4,20; $P < 0.001$), with considerable variability among plants within some treatments (Table 4.2). Total captures did not differ significantly among controls, mating disruption or parasitoids or between the parasitoid and predator treatments but were significantly lower in the insecticide treatment than in all but the predator treatment (Table 4.2). There was no significant treatment effect on the total number of adult females captured in sticky band traps ($F = 2.39$; df = 4,20; $P = 0.086$) (Table 4.2). Relative to the untreated controls, the total number of nymphs captured was reduced 9% by mating disruption, 45% by the parasitoid, 67% by the predator, and 87% by the insecticide.

Compound symmetry analysis of the number of males captured in pheromone traps revealed significant effects of treatment ($F = 32.53$; df = 4,140; $P < 0.001$), week after treatment ($F = 57.70$; df = 6,140; $P < 0.001$) and their interaction ($F = 7.27$; df = 24,140; $P < 0.001$). Male captures remained generally low through week 3, then showed a pronounced increase in all treatments except the insecticide on week 4 (Table 4.1). Substantial numerical differences among

plants in the mean weekly number of males captured resulted in no statistical separations among the controls, mating disruption and parasitoid treatments throughout the remainder of the study. Male captures in cages with the predator were significantly lower than in control cages in weeks 5 - 7. The insecticide treatment resulted in the lowest weekly captures, relative to all others, and was significantly different from the controls, mating disruption and parasitoids from weeks 4 – 7 (Table 4.1).

There was a significant treatment effect on the total number of males captured during the study ($F = 9.60$; df = 4,20; $P < 0.001$). Significantly higher captures were recorded from the untreated controls, mating disruption and parasitoid treatments than from the insecticide treatment, with no difference between mating disruption and predator or between predator and insecticide (Table 4.2). Relative to the untreated controls, the total number of males captured was reduced by 66% by mating disruption, 29% by the parasitoid, 90% by the predator, and 96% by the insecticide.

Weekly captures of males in pheromone traps and mealybugs in band traps were significantly correlated for pooled data regardless of treatment ($F = 45.12$; df = 1,198; $P < 0.001$; $r^2 = 0.19$), for untreated ($F = 4.26$; df = 1,38; $P = 0.046$; $r^2 = 0.10$), mating disruption ($F = 22.07$; df = 1,38; $P < 0.001$; $r^2 = 0.37$), parasitoid ($F = 56.14$; df = 1,38; $P < 0.001$; $r^2 = 0.60$), and predator ($F = 6.12$; df = 1,38; $P = 0.018$; $r^2 = 0.14$) treatments, but not insecticide ($F = 0.99$; df = 1,38; $P = 0.326$; $r^2 = 0.03$) treatment.

There was a significant effect of treatment on the number of nymphs ($F = 5.94$; df = 4,20; $P = 0.003$), adult females ($F = 8.20$; df = 4,20; $P < 0.001$), and unhatched egg sacs ($F = 4.13$; df = 4,20; $P = 0.0014$) recovered from terminals at the end of the study (Table 4.2), but not a significant block effect for nymphs ($F = 0.78$; df = 4,20; $P = 0.553$), adult females ($F = 0.12$; df =

4,20; $P = 0.973$) or unhatched egg sacs ($F = 0.05$; df = 4,20; $P = 0.996$). Significantly more nymphs were recovered from untreated controls and the mating disruption treatment than from plants treated with the parasitoid, predator or insecticide, which did not differ (Table 4.2). The number of adult females and unhatched egg sacs recovered was significantly higher in the controls than from plants treated with predators, from which 0 adult females were recovered. Upon completion of the study, *A. kamali* (total no. male, female) emerged from mealybugs on sampled terminals from 1 untreated cage (1, 1), 4 mating disruption cages (21, 22), 4 parasitoid cages (23, 44), and 1 predator cage (1, 4). *C. montrouzieri* (total no. larvae, adults) were only found in 4 predator cages (25, 3). The method by which parasitoids entered cages in which they were not intentionally released cannot be determined.

Effectiveness of Combined Management Practices. There was no significant block effect on the total number of nymphs ($F = 1.32$; df = 4,20; $P = 0.295$) or males ($F = 1.51$; df = 4,20; $P = 0.236$) trapped during the study. During the first week of sampling, prior to the imposition of treatments, the number of mealybug nymphs ($F = 0.42$; df =4,20; $P = 0.792$) or adult males ($F = 1.44$; df =4,20; $P = 0.257$) captured did not differ significantly among treatments (Pre-treatment, Table 4.3). Compound symmetry analysis of the number of nymphs captured showed significant effects of treatment ($F = 2.53$; df = 4,100; $P = 0.045$), week after treatment ($F = 67.09$; df = 4,100; $P < 0.001$), and their interaction ($F = 3.68$; df = 16,100; $P < 0.001$).

The mean number of nymphs captured increased in all treatments in weeks 1 and 2 with significantly fewer trapped from mating disruption than insecticide treated plants during week 1 (Table 4.3). Captures of nymphs from the untreated control plants showed a relatively steady increase through week 4 and generally similar trends were exhibited by plants exposed to mating disruption, parasitoids, or parasitoids in combination with mating disruption, with no significant

differences among those treatments for the duration of the study. By week 4, captures of nymphs in the insecticide treatment were significantly reduced relative to untreated controls and mating disruption treatments (Table 4.3).

The total number of nymphs captured during the study did not differ significantly among treatments ($F = 1.65$; df = 4,20; $P = 0.201$), although there was considerable variability among treatments (Table 4.4). There was not a significant effect of treatment on the total number of adult females captured in sticky band traps ($F = 2.28$; df = 4,20; $P = 0.087$) (Table 4.4).

Compound symmetry analysis of the number of males captured in pheromone traps revealed significant effects of treatment ($F = 8.49$; df = 4,100; $P < 0.001$), week after treatment ($F = 7.43$; df = 4,100; $P < 0.001$) and their interaction ($F = 3.07$; df = 16,100; $P < 0.001$). Male captures in cages with untreated control plants showed a relatively steady increase through week 4 (Table 4.3). The insecticide treatment resulted in the lowest weekly captures, relative to all other treatments, and was significantly different from the control from weeks 3 – 4.

There was not a significant treatment effect on the total number of males captured during the study ($F = 2.31$; df = 4,20; $P = 0.092$). Numerically more males were captured from untreated plants over the course of the trial, due to high variability within treatments, there was no statistical separation between means (Table 4.4).

Weekly captures of males in pheromone traps and mealybugs in band traps were significantly correlated for pooled data regardless of treatment ($F = 32.52$; df = 1,123; $P < 0.001$; $r^2 = 0.21$), for untreated ($F = 8.28$; df = 1,23; $P = 0.009$; $r^2 = 0.26$), mating disruption ($F = 13.34$; df = 1,23; $P < 0.001$; $r^2 = 0.37$), parasitoid ($F = 7.06$; df = 1,23; $P = 0.014$; $r^2 = 0.23$), and parasitoid plus mating disruption ($F = 14.28$; df = 1,23; $P < 0.001$; $r^2 = 0.38$) treatments, but not insecticide ($F = 2.33$; df = 1,23; $P = 0.141$; $r^2 = 0.09$) treatment.

DISCUSSION

The ability of *M. hirsutus* to disperse via walking has been noted previously (Misra 1920, Hall 1921). Sticky band traps on plants initially infested with different numbers of adult female *M. hirsutus* captured a total of 17,473 1st instars, 130 2nd instars, 96 3rd instars, and 1,907 adult female *M. hirsutus*. The relative numbers of individuals in different developmental stages captured were reflective of the behavior of coccoidea; 1st instars disperse in search of a suitable feeding site, adult females disperse to find an oviposition site and most 2nd and 3rd instars have settled at a feeding site and are non-dispersive. Although there was considerable variability in the number of mealybugs captured in sticky band traps beneath terminals initially infested with different numbers of adult females with egg sac, captures reflected differences in the initial infestation density. Most captures occurred in the upper trapping surface, representing the dispersal of individuals from colonies that had been initiated in the apical bud of the terminals. The results from this experiment indicated the usefulness of sticky band traps in experiments designed to measure differences or changes in *M. hirsutus* populations as a function of the effects of management practices imposed.

In both field cage trials, nymphs were captured in band traps and males were captured in pheromone traps as soon as infested plants were placed in cages, and captures from untreated plants were similar between studies through week 4. During the first trial, significant differences were measured in the number of nymphs captured in sticky traps beginning on wk 3 after treatment throughout the duration. For the duration of this trial, the number of nymphs captured among plants exposed to 250 µg pheromone or parasitoids did not differ from untreated plants. However, there was a reduction in crawlers on predator and insecticide treated plants, 4 and 3

weeks after treatment, respectively. *C. montrouzieri* larvae are capable of eating an average of 881 eggs, 259 nymphs or 27 adult *M. hirsutus* prior to pupation (Mani and Thontadarya 1987). In the field, *M. hirsutus* infested sapota (*Manilkara zapota* Forberg) inoculated with *C. montrouzieri* at 20 larvae per plant, resulted in mealybug population decline from an average of 54.2 mealybugs (developmental stage not indicated) per plant to 1.5 per plant in two months (Mani and Krishnamoorthy 2008). However, Kairo et al. (2000) stated that predators like *C. montrouzieri* are high density feeders and do not maintain "low" pest populations, additionally they can delay the effective establishment and impact of parasites by feeding on parasitized mealybugs. While dinotefuran is labeled for mealybug control, there is little published information on its efficacy against *M. hirsutus*, other than recommendation for quarantine treatments (Osborne 2005). Although dinotefuran used as a soil drench to control citrus mealybug, *Planococcus citri* (Risso) on Coleus (*Solenostemon x hybridus*) showed no significant effect (Parrella et al. 2006), it was quite effective for *M. hirsutus* on *H. rosa-sinensis*. Use of dinotefuran as a soil applied systemic insecticide is highly preferable to using it as a foliar spray, which resulted in 100% mortality of the citrus mealybug parasitoid *Leptomastix dactylopii* (Howard) (Hymenoptera: Encyrtidae) after 24 h (Cloyd and Dickinson 2006).

Total mealybugs counted from terminals sampled upon completion of the study were similar in number to the total number captured in band traps with the exception of the parasitoid treatment. Nymphs sampled from untreated and pheromone treated plants were almost identical. When compared with untreated plants, nymphs were reduced by 69% by the parasitoid, 85% by the predator, and 87% by the insecticide. While the predator and insecticide treatments reduced the number of crawlers captured during the trial and on terminals sampled upon conclusion of the trial, significant effects of parasitoids did not occur until the completion of the trial. Our

findings are supported by field observations in southern California by Roltsch et al. (2006) that reported a 95% reduction in *M. hirsutus* population densities within 1 year of treatment with parasitoids, of which *A. kamali* was found responsible for >50% of the parasitism. The emergence of 21 male and 22 female *A. kamali*, and 23 male and 44 female *A. kamali* from mealybugs on terminals taken from four mating disruption cages and four parasitoid cages, respectively, suggests either that mealybugs on some were parasitized prior to placement in cages or that parasitoids entered cages during the study.

Predator and insecticide treatments were most effective at reducing dispersing nymphs, but neither treatment completely eradicated the pest to the 0 tolerance level necessary for quarantine areas. The addition of a foliar insecticide to dinotefuran soil treated plants is recommended (Osborne 2005) and may be sufficient to eradicate *M. hirsutus* on a single plant.

Significant differences occurred among treatments in the number of males captured in sex pheromone traps during weeks 1 and 2, then from week 4 onward. Significant effects in weeks 1 and 2 were due to more males captured from insecticide treated plants. In weeks 4 to 7 after treatment, the number of males captured was lowest for predator and insecticide treatments, corresponding with nymphs captured in the band traps. The pheromone treatment did not significantly reduce the number of males or nymphs captured. The males captured in pheromone traps were a reasonably good indicator of nymphs captured in band traps, exhibiting a linear relationship for all treatments except insecticide.

During the second field cage trial which evaluated the effectiveness of biocontrol agents and mating disruption in combination, significant differences occurred among treatments and the number of nymphs captured in band traps during week 4. Only the insecticide treatment reduced the number of dispersing nymphs. Like nymphs captured in band traps, the number of adult

males captured was only reduced by the insecticide treatment. As in trial 1, males captured were a reasonably good indicator of nymphs captured in band traps, exhibiting a linear relationship for all treatments except insecticide.

An ability of pheromone to disrupt mating and suppress pink hibiscus mealybug populations was not established in field cages at low rates (250 μg/cage) in the 1st trial, high rates (500 μg/cage) in the 2nd trial, or in conjunction with the release of parasitoids. Walton et al. (2006) demonstrated that mealybug density influenced the impact of mating disruption of vine mealybug. Vines categorized as having high, medium, or low mealybug densities prior to application, only showed a reduction in density for the low category after treatment. If populations are high, other cues could facilitate mate location and *M. hirsutus* may not be suitable for mating disruption when populations are high.

The relationship between the number of males captured in pheromone traps and the ability to make management decisions is complex and made more so when management tactics are involved. A study of the Comstock mealybug found no correlation between the number of males attracted to 10 or more virgin females in pheromone traps and the relative population density on leaves of mulberry trees (Meyerdirk et al. 1981). Millar et al. (2002) monitored *Planococcus ficus* in California vineyards, demonstrating that visual sampling methods and sampling of males using pheromone baited traps were significantly correlated with economic damage. However, Walton et al. (2004) attempted to develop a model to estimate infestation levels of *P. ficus* using pheromone traps in South African vineyards and concluded that "…the model's use may be limited because of the lure's high level of attractiveness, which often resulted in positive trap catches in vineyards where no females were located during visual searches". Accuracy in correlating males captured in sex pheromone traps with surrounding

populations or risk of infestation needs to take into account the suppressive effects of management tactics on *M. hirsutus* populations and consequent effects on males captured in sex pheromone traps. These studies elucidate the relationship between males and nymphs captured, and population density under different management tactics. Further investigation of management tactics under field conditions for a period longer than seven weeks would be appropriate. Field trials will help determine if the lack of efficacy in using pheromones for mating disruption was due to confinement in field cages, population density, or reproductive biology of this pest.

Table 4.1. The effects of management tactics on the mean ± SE weekly captures of pink hibiscus mealybug nymphs in sticky band traps and adult males in pheromone traps from 1 May to 26 June 2008.

			Week after treatment						
Stage	Treatment	Pre-treatment	1	2	3	4	5	6	7
Nymph	Untreated	0.2 ± 0.2a	2 ± 2a	67 ± 37a	167 ± 43a	434 ± 69a	431 ± 54a	508 ± 124a	324 ± 89a
	Pheromone	7 ± 7a	15 ± 8a	73 ± 26a	138 ± 22a	323 ± 68a	321 ± 55a	521 ± 64a	364 ± 82a
	Parasitoid	7 ± 6a	44 ± 22a	76 ± 35a	126 ± 24a	230 ± 43ab	166 ± 22ab	289 ± 104a	129 ± 26ab
	Predator	0.2 ± 0.2a	34 ± 12a	58 ± 11a	47 ± 17b	79 ± 40b	96 ± 41b	192 ± 135a	131 ± 107ab
	Insecticide	3 ± 2a	83 ± 52a	100 ± 43a	79 ± 24ab	13 ± 6c	7 ± 2c	17 ± 10b	53 ± 38b
Male	Untreated	7 ± 2a	6 ± 2ab	9 ± 4ab	6 ± 3a	219 ± 70a	1234 ± 630a	842 ± 155a	190 ± 73a
	Pheromone	9 ± 3a	3 ± 1b	3 ± 1b	3 ± 1a	128 ± 50a	358 ± 113ab	282 ± 54ab	71 ± 16a
	Parasitoid	8 ± 3a	5 ± 1ab	2 ± 1b	36 ± 22a	362 ± 144a	632 ± 279ab	654 ± 283a	86 ± 43ab
	Predator	9 ± 2a	4 ± 1ab	4 ± 1b	3 ± 2a	62 ± 30ab	106 ± 49b	58 ± 28bc	11 ± 7bc
	Insecticide	13 ± 3a	20 ± 6a	20 ± 2a	0.4 ± 0.2a	8 ± 6b	9 ± 6c	22 ± 16c	8 ± 4c

Means within columns for nymphs and adult males followed by the same letter are not significantly different ($P<0.05$) using logarithmically transformed data, according to ANOVA (df = 4,20) for nymphs during week 1 ($F = 2.54$; $P = 0.072$), 2 ($F = 0.37$; $P = 0.830$), 3 ($F = 4.79$; $P = 0.007$), 4 ($F = 12.27$; $P < 0.001$), 5 ($F = 36.79$; $P < 0.001$), 6 ($F = 10.65$; $P < 0.001$), and 7 ($F = 5.43$; $P = 0.004$) after treatment, and for males during week 1 ($F = 3.05$; $P = 0.041$), 2 ($F = 6.18$; $P = 0.002$), 3 ($F = 2.74$; $P = 0.058$), 4 ($F = 7.75$; $P < 0.001$), 5 ($F = 12.93$; $P < 0.001$), 6 ($F = 12.53$; $P < 0.001$), and 7 ($F = 9.31$; $P < 0.001$) after treatment, using Tukey test.

Table 4.2. The effects of management tactics on the mean ± SE total number of pink hibiscus mealybugs captured in sticky band and pheromone traps from 1 May to 26 June 2008 and recovered from post trial samples.

	Number captured during study			Number recovered from plant (n = 3 terminals per plant)		
Treatment	Nymph	Adult female	Adult male	Nymph	Adult female	Egg sac
Untreated	1933 ± 184a	19 ± 3a	2513 ± 750a	2733 ± 587a	89 ± 20a	36 ± 11a
Pheromone	1762 ± 112a	18 ± 7a	855 ± 211ab	2950 ± 648a	36 ± 10a	14 ± 9ab
Parasitoid	1068 ± 234ab	18 ± 3a	1784 ± 765a	847 ± 230b	24 ± 15ab	14 ± 10ab
Predator	637 ± 256bc	10 ± 6a	258 ± 110bc	400 ± 376b	0 ± 0 b	0.2 ± 0.2b
Insecticide	354 ± 229c	9 ± 5a	101 ± 35c	348 ± 143b	22 ± 11ab	17 ± 9ab

Means within columns followed by the same letter are not significantly different ($P<0.05$) using logarithmically transformed data, according to ANOVA using Tukey's HSD test.

Table 4.3. The effects of management tactics on the mean ± SE weekly captures of pink hibiscus mealybug nymphs in sticky band traps and adult males in pheromone traps from 3 July to 7 August 2008.

			Week after treatment			
Stage	Treatment	Pre-treatment	1	2	3	4
Nymph	Untreated	6 ± 3a	19 ± 7ab	106 ± 31a	209 ± 59a	288 ± 84a
	Pheromone	7 ± 2a	15 ± 4b	89 ± 23a	156 ± 48a	171 ± 60a
	Parasitoid	2 ± 1a	16 ± 6ab	55 ± 19a	100 ± 21a	83 ± 35ab
	Parasitoid & Pheromone	3 ± 2a	51 ± 24ab	117 ± 47a	147 ± 34a	139 ± 71ab
	Insecticide	6 ± 2a	89 ± 27a	120 ± 53a	53 ± 14a	14 ± 2b
Male	Untreated	9 ± 3a	5 ± 1a	37 ± 24a	72 ± 25a	133 ± 70a
	Pheromone	3 ± 1a	4 ± 3a	23 ± 20a	52 ± 40ab	18 ± 7a
	Parasitoid	4 ± 2a	3 ± 2a	4 ± 3a	28 ± 18ab	98 ± 70a
	Parasitoid & Pheromone	5 ± 2a	2 ± 1a	5 ± 3a	17 ± 7ab	23 ± 7a
	Insecticide	2 ± 1a	4 ± 2a	16 ± 5a	0 ± 0b	0.2 ± 0.2b

Means within developmental stage rows and weekly column followed by the same letter are not significantly different ($P<0.05$) using logarithmically transformed data, according to ANOVA (df = 4,20) for nymph during week 1 ($F = 3.51$; $P = 0.025$), 2 ($F = 0.59$; $P = 0.670$), 3 ($F = 2.74$; $P = 0.058$), and 4 ($F = 6.26$; $P = 0.002$) after treatment, and for male week 1 ($F = 0.82$; $P = 0.528$), 2 ($F = 1.98$; $P = 0.136$), 3 ($F = 2.99$; $P = 0.046$), and 4 ($F = 7.45$; $P < 0.001$) after treatment, using Tukey's HSD test.

Table 4.4. The effects of management tactics on the mean ± SE total number of pink hibiscus mealybugs captured in sticky band and pheromone traps from 3 July to 7 August 2008.

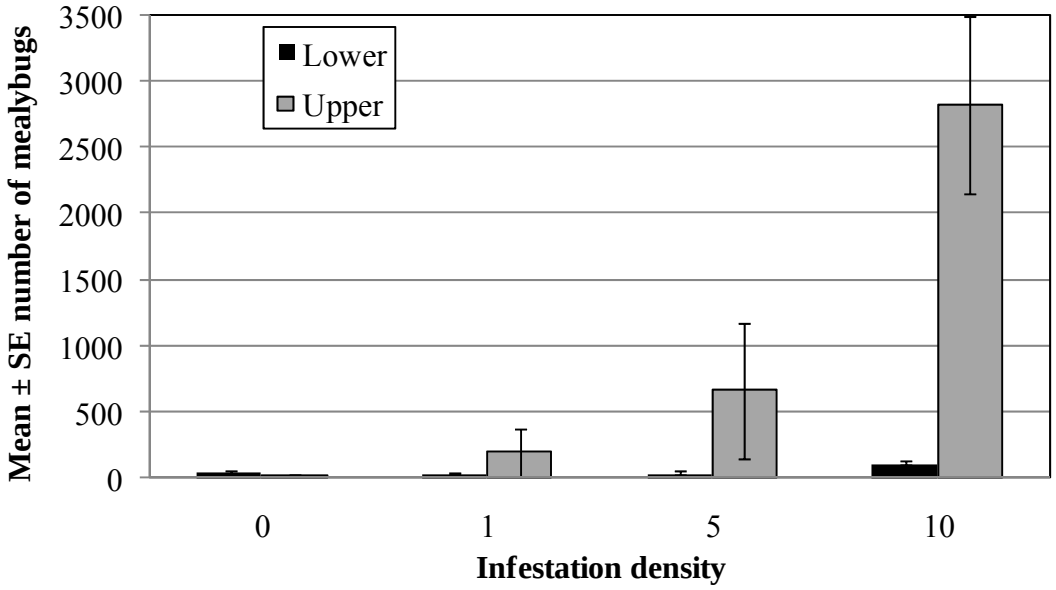
Treatment	Nymph	Adult female	Adult male
Untreated	628 ± 155a	4 ± 1.6a	256 ± 73a
Pheromone	438 ± 105a	1 ± 0.3a	100 ± 69a
Parasitoid	257 ± 71a	1 ± 0.6a	136 ± 87a
Parasitoid & Pheromone	447 ± 149a	4 ± 1.5a	50 ± 16a
Insecticide	281 ± 76a	1 ± 0.5a	22 ± 5a

Means within columns followed by the same letter are not significantly different ($P<0.05$) using logarithmically transformed data, according to ANOVA using Tukey's HSD test.

Figure 4.1. Wood frame cage (1.5 m tall x 1 m wide x 1 m deep) enclosed by 0.5 m tall plywood panels at the bottom and No Thrips Insect screen on the remaining portions of the sides and the top, with a hinged door to allow access to the cage interior.

Figure 4.2. Sticky band trap constructed by wrapping a piece of electrician's tape (2.0 cm wide) snugly around a branch, a 1.5 cm wide strip of Parafilm around the electrician's tape, with a 1.0 cm wide band of Tangle-Trap created around the middle of each Parafilm band.

Figure 4.3. The capture of pink hibiscus mealybug nymphs and adult females over 5 weeks in the upper and lower trapping surfaces of sticky band traps below hibiscus terminals initially infested with 0, 1, 5, or 10 adult females and their egg sac.



CHAPTER 5: SPATIAL AND TEMPORAL RESPONSE OF PINK HIBISCUS MEALYBUG TO SEX PHEROMONE TRAPS

ABSTRACT

Field experiments evaluating the factors that influence the response of pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green), adult males to sex pheromone traps were conducted in southern Florida. *Maconellicoccus hirsutus* captures in pheromone traps in May and September was crepuscular, with most captures occurring from 18:00 to 21:00 h. Significantly more males were captured in traps placed in non-host trees at an elevation of 2 m above the ground than 6 m and more males were captured in traps placed within hibiscus plants than in those 3 m upwind, suggesting that male *M. hirsutus* are weak fliers. Pheromone traps placed in hibiscus plants treated with soil applied dinotefuran or left untreated captured equal numbers of males during the 3 wk prior to treatment and during the 12 wk after treatment. Release of parasitoids at residential sites did not have a significant effect on the total number of males captured in sex pheromone traps over 18 mo. The mean ± SE number of mealybugs on sampled terminals in May and September 2008 was 131 ± 74 and 184 ± 162 at treated sites and 31 ± 14 and 340 ± 380 at untreated sites, respectively. By the second year after release, parasitoids kept mealybugs at a consistent level through the peak period of abundance. Parasitoids were found at both treated and untreated sites with a maximum percent parasitism of 77% and 31%, respectively. The numbers of pink hibiscus mealybug nymphs and adult females

found at both treated and untreated sites were highly variable and corresponded with males captured in sex pheromone traps. Development of optimally efficient and standardized trapping protocols for *M. hirsutus* is warranted. Proper trap placement and knowledge of the effects of management tactics on sex pheromone trapping are discussed.

INTRODUCTION

The pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae) has been recorded from more than 300 species of economically important and native host plants (Meyerdirk et al. 2001) in over 50 countries (CABI/EPPO 2004) and has long been considered a significant agricultural pest in countries such as India, Egypt and Australia (Das and Singh 1986, Williams 1996, Mani and Krishnamoorthy 2008). In the United States, *M. hirsutus* invaded California in 1999 (Anonymous 1999), southern Florida in 2002 (Amalin et al. 2003), Louisiana in 2006 (LDAF 2006), Texas in 2007 (Borgan and Ludwig 2007), and Georgia in 2008 (Horton 2008).

Although biological control programs involving the release of introduced parasitoids and a native predator have helped to manage pink hibiscus mealybug in the Caribbean (Kairo et al. 2000, Michaud and Evans 2000), California (Roltsch et al. 2006) and Florida (Amalin et al. 2003), they are costly (Amalin et al. 2003) and do not eradicate infestations (Ranjan 2004). In Florida, pink hibiscus mealybug has spread to 37 counties and new infestation sites continue to be identified (G. Azore, Florida Department of Agriculture and Consumer Services (FDACS), Division of Plant Industry (DPI), pers. comm.). Identification of new infestations for targeting the release of biological control agents is based on visual scouting of 8 to 10 randomly selected sites within a 1.6 x 1.6 km section, township, and range found on standard maps. This scouting is

partially reliant on detection of feeding damage on host plants, especially cultivars of *Hibiscus rosa-sinensis* (G. Azore, pers. comm.), although Vitullo et al. (2009) showed that commercially important hibiscus cultivars vary considerably in their expression of feeding symptoms from *M. hirsutus*, potentially affecting the outcome of scouting based on the presence or severity of these symptoms.

An ability to detect and monitor *M. hirsutus* is especially important for producers of ornamental plants in southern Florida nurseries, as the pest is widely distributed throughout the main production area (Vitullo, Chapter 3) and nurseries continue to be at an ongoing risk of infestation from populations on native hosts and plants in managed landscapes. A zero-tolerance policy for pink hibiscus mealybug in Florida nurseries has been in effect since 2004 and numerous quarantine actions have occurred (Ranjan 2004, Gaskalla 2006).

Detection and monitoring capabilities for *M. hirsutus* have been enhanced greatly by the identification, synthesis, and field testing of its sex pheromone (Zhang et al. 2004a, Zhang and Amalin 2005, Zhang and Nie 2005, Vitullo et al. 2007). Zhang and Amalin (2005) showed that the hymenopteran parasitoids of *M. hirsutus* captured in unbaited control traps were equal to those baited with sex pheromone, demonstrating the compatibility of pheromone-based monitoring with biological control programs and Vitullo et al. (2007) concluded that Jackson style traps were most efficient for trapping *M. hirsutus*. In addition to improving the ability to detect and target *M. hirsutus* infestations, the use of pheromone traps may provide important information about relative levels of risk to commercial nurseries and the effectiveness and persistence of biological control and other management efforts in urban landscapes. However, the information content from and utility of pheromone traps for monitoring *M. hirsutus* have not

been fully realized or exploited and there remain a number of key questions that influence our ability to interpret trapping data.

Realization of a standardized pheromone monitoring program necessitates a large amount of preliminary investigation to optimize trap use. Little is known about the response of *M. hirsutus* to sex pheromone baited traps and the factors that affect that response. Factors that could influence captures include height of trap placement as well as their proximity to active colonies. Pink hibiscus mealybug hosts vary considerably in size and distribution in the environment, the location of trap placement may be quite influential on captures of males in traps, especially given that the closely-related male long-tailed mealybug, *Pseudococcus longispinus* (Targioni-Tozzetti) has been shown to be a weak flier (Barrass et al. 1994). The effect of management tactics on the number of males captured in pheromone traps has not been determined under field conditions. Furthermore, although natural enemies do suppress *M. hirsutus* populations, quantification of the rate at which this occurs and the extent and persistence of such control under Florida conditions is lacking. Proper trap placement and knowledge of the effects of management tactics on pheromone trap based monitoring will aid in standardizing trapping protocols.

Here, we report the results of studies conducted in southern Florida that addressed the effects of several biotic and abiotic factors on the capture of male pink hibiscus mealybugs in pheromone-baited traps.

MATERIALS AND METHODS

Research Site. Experiments were conducted at the University of Florida Tropical Research and Education Center (TREC) and surrounding residential sites in Homestead, FL,

where *M. hirsutus* exhibits an annual period of peak abundance between May and September (Hall et al. 2008).

Pheromone Traps. Jackson traps (Scentry Biologicals Inc., Billings, MT) (Fig 1.2) were baited with a grey, halo-butyl septum (5 mm; West Pharmaceutical Services, Kearney, NE) containing 1 µg *of M. hirsutus* sex pheromone (Zhang et al. 2004a) and suspended within the center of the trap. The half-life of these lures is 3.5 months (Francis et al. 2007) and individual lures were used for ≤3 mo. The mealybugs captured were counted using a dissecting stereomicroscope at 60X magnification and identified following Hodges and Hodges (2005).

Diel Periodicity of Captures. Beginning on 26 May and 15 September 2008, a trap was deployed at three residential sites with a hibiscus hedge from which *M. hirsutus* had been captured during a 5-d survey in April (15 - 41 males captured per site). Traps were placed within the hedge at 1.2 m above the ground and their liners were replaced at 3-h intervals from 06:00 to 21:00 h and then at 06:00 h the next day, for five consecutive days. Using sites as replicates, the total number of mealybugs captured was compared among trapping intervals in May and September, using PROC GLM (SAS Institute 2001).

Prior to all comparisons utilizing analysis of variance, tests for normality, PROC UNIVARIATE (SAS Institute 2001) were conducted. Data that did not meet assumptions of normality were logarithmically transformed. Means of significant effects were separated using Tukey's Honestly Significant Difference (HSD) test and all data comparisons were considered significant at $\alpha = 0.05$.

Captures in Relation to Trap Elevation. Fifteen residential sites containing a deciduous tree ≥10 m tall were surveyed by deploying a pheromone trap from the lowest branch for 2 wk beginning on 28 May 2008. Seven sites at which similar numbers of mealybugs were captured

(range = 173-291 males per site) were selected for subsequent trapping. Trees from which traps were suspended included; Caribbean mahogany, *Swietenia mahogani* L. (3 sites), Javanese bishopwood, *Bischofia javanica* (Blume) (2 sites), black olive, *Bucida buceras* L. (one site) and tropical almond, *Terminalia catappa* L. (one site). Of these trees, only *T. catappa* has been reported as a host of *M. hirsutus* (Williams 1986). On 22 July, three pheromone traps were deployed from each tree at 2, 4, and 6 m above the ground, via a pulley attached to each plant. Trap liners were replaced weekly for 10 wk and the number of males captured per week at each elevation was recorded. The effect of trap elevation on total captures was compared using PROC GLM (SAS Institute 2001), using sites as replicates.

Captures in Relation to a Pesticide Treatment. Sixteen residential sites with a hibiscus plant from which similar numbers *of M. hirsutus* had been captured during a 2 wk survey in May-June 2008 (112-398 males per site) were randomly assigned to receive an insecticide application or to be left untreated. On 1 July, a pheromone-baited trap was placed in the canopy of the hibiscus plant at 1.2 m above the ground and a second trap was suspended from a metal stake 3 m upwind (east, based on the prevailing wind direction in southern Florida) of the host at 1.2 m above the ground. Trap liners were replaced and the number of mealybugs captured was recorded weekly through 24 October. On 21 July, dinotefuran (Safari 20SG, Valent USA Corp., Walnut Creek, CA) at 9.84 g per meter of plant height and mixed with 1 L of water per 3 g was applied as a drench to the base of plants at eight sites. The total number of mealybugs captured was compared using a 2x2 factorial design including insecticide treatment and trap placement, prior to and after treatment using PROC GLM (SAS Institute 2001).

Captures in Relation to the Release of Biological Control Agents. Ten residential sites were identified as new infestation sites in April 2007 by FDACS-DPI. Although none of the sites

had received releases of biological control agents previously, all were in the general vicinity of previous release sites. The sites were randomly assigned to those at which parasitoids would be released and those to be left untreated by FDACS until the conclusion of the study. At each site, a pheromone-baited trap was deployed within the canopy of the infested hibiscus plant at 1.2 m from the ground on 18 May 2007. Trap liners were changed weekly through 19 October, then at 2-wk intervals through 4 April 2008, when weekly changes were resumed through 28 November 2008. Pheromone lures were replaced at 3-mo intervals.

On 13 June and 5 July 2007 the parasitoids, *Anagyrus kamali* (Moursi) and *Gyranusoidea indica* (Shafee, Alam and Agarwal), were released at five sites by FDACS personnel, according to their discretion and protocols, which specify "roughly 1 vial (400 parasitoids/vial) per 5 or 6 plants and to release 3 vials of *A. kamali* or *G. indica* per property." The number of each species released at each site on each date is provided in Fig. 5.2.

At 3-mo intervals beginning on 18 May 2007 six terminals (15 cm long) were pruned from the hibiscus plant identified as infested by FDACS at each site and the number of *M. hirsutus* nymphs and adult females on each were counted using a dissecting microscope at 120X. As hibiscus terminals were destructively sampled, depending on their availability in a sample, a maximum of 100 live mealybugs were placed in gelatin capsules (size "0"; Capsuline Inc., Pompano Beach, FL) using a micro-pin tool, following Amalin et al. (2003) and Roltsch et al. (2006). The delicate body of the mealybugs and dissection of terminals added to the difficulty of transferring them to capsules. Only live, intact mealybugs from 1st instar through adult female were placed in capsules and mean number sampled for each period is provided in Table 5.2. After 35 d, parasitoids that had emerged were counted and the percentage parasitism was calculated (Roltsch et al. 2000, Goolsby et al. 2002, Amalin et al. 2003). In August 2007, the

homeowner at one untreated site denied our access to the hibiscus plant on their property, but permitted deployment of the pheromone trap on a fence at about 1.5 m upwind of the hibiscus. Consequently, analyses of the total number of mealybugs present on sampled terminals and percent parasitism per plant were based on a sample size of n = 5 for treated sites over the duration of the study and n = 4 for untreated sites from 31 August 2007 onward.

The total number of male mealybugs captured and the numbers captured during the periods of peak abundance each year (May to September) were compared between treatments using PROC GLM (SAS Institute 2001). The total number of mealybugs present on sampled terminals and percent parasitism was compared between treatments using PROC TTEST (SAS Institute 2001) at 3 mo sample intervals. When equality of variance was significantly different, t-test data were presented using the Satterthwaite approximation value. Percent parasitism analyses were conducted on arcsine square-root transformed data (Zar 1999).

RESULTS

Diel Periodicity of Captures. Male pink hibiscus mealybug adults were captured at all sites during the trials in May and September and trapping interval had a significant effect on the number of males captured in May ($F = 4.72$; df = 5,12; $P = 0.013$) and September ($F = 46.33$; df = 5,12; $P < 0.001$). In May, significantly more males were recorded at 18:00 h than at 12:00 or 15:00 h, with no difference among the other intervals (Fig. 5.1). In September, significantly more males were recorded at 18:00 h than at all other intervals, while 06:00 and 09:00 h were not different, but were different from 12:00, 15:00, and 21:00 h, which were not different (Fig. 5.1).

Captures in Relation to Trap Elevation. *M. hirsutus* was captured in traps deployed in association with all trees. Mean total captures during the 10 wk trapping period were 355 ± 65

SE per site. There was a significant effect of trap elevation on the total number of males captured ($F = 3.73$; df = 2,18; $P = 0.044$). Significantly more mealybugs were captured in traps at 2 m (100 ± 32a SE) than at 6 m (27 ± 3b SE), with no difference between 2 m and 4 m (49 ± 8ab SE) or between 4 m and 6 m.

Captures in Relation to a Pesticide Treatment. Adult male *M. hirsutus* were captured at all sites. During the 3-wk period prior to the insecticide treatment, there was a significant effect of trap placement ($F = 6.96$; df = 1,28; $P = 0.013$), but not treatment ($F = 0.68$; df = 1,28; $P = 0.415$) or their interaction ($F = 0.01$; df = 1,28; $P = 0.926$) on the total number of males captured (Table 5.1). Similarly, the total number of males captured during the 12 wk period following the insecticide treatment was significantly affected by trap placement ($F = 19.69$; df = 1,28; $P < 0.001$), but not insecticide treatment ($F = 0.28$; df = 1,28; $P = 0.604$) or their interaction ($F = 0.21$; df = 1,28; $P = 0.651$) (Table 5.1).

Captures in Relation to the Release of Biological Control Agents. During the three weeks prior to the release of parasitoids, the total number of males captured did not differ significantly ($F = 2.91$; df = 1,8; $P = 0.132$) between treated (418 ± 125 SE) and untreated sites (194 ± 48 SE). Similarly, the total number of males trapped over 18 mo following the release of parasitoids did not differ significantly ($F = 0.72$; df = 1,8; $P = 0.422$) between treated (7,819 ± 2,354) and untreated sites (5,308 ± 1,780). During the periods of peak abundance from May through September, the total number of males trapped did not differ significantly between treated and untreated sites in 2007 ($F = 0.02$; df = 1,8; $P = 0.884$) or 2008 ($F = 1.54$; df = 1,8; $P = 0.250$). Mean ± SE total captures during those periods at treated and untreated sites, respectively, were 3,184 ± 1,028 and 1,683 ± 640 in 2007 and 2,478 ± 957 and 2,775 ± 1,722 in 2008. Captures were consistently low from January to May and highest from May to September (Fig. 5.2).

Prior to the release of parasitoids, there was no significant difference between treated and untreated sites in the total number of nymphs and adult female mealybugs collected from terminals or the percentage of parasitized mealybugs (Table 5.2). Similarly, after the release of parasitoids there was no significant difference between treatments for the total number of nymphs and adult female mealybugs present or percent parasitism in all 3-mo intervals (Table 5.2). On 28 November 2008, no mealybugs were present on any terminals sampled.

The total number of *M. hirsutus* present on all sampled hibiscus was <10 during the winter after treatment at all sites, while *M. hirsutus* present on infested hibiscus and percent parasitism exhibited high variance during peak abundance sample periods. At biocontrol sites in May 2008, nymphs and adult females ranged from 10 to 417 with percent parasitism from 0 to 77%. Similar numbers of *M. hirsutus* were found at biocontrol sites in August 2008, and nymphs and adult females ranged from 4 to 833 with percent parasitism from 0 to 60% (Table 5.2). Pink hibiscus mealybugs at untreated sites in May 2008 ranged from 5 to 69 with percent parasitism from 0 to 15%. In August 2008, mealybugs were only found at two untreated sites, having 1 and 1701 individuals and a percent parasitism of 0 and 31%, respectively (Table 5.2). Traps at the biocontrol site where 417 mealybugs were present in May 2008 captured ≈5,000 males over the following 3-mo interval.

DISCUSSION

There are many factors that influence the number of male pink hibiscus mealybugs captured in sex pheromone traps at a given location. Our studies have documented the influence of time of day, height and location of trap placement, and effects of management tactics on subsequent captures of males in pheromone traps. The time of day at which males were captured

in pheromone traps was crepuscular, though most captures occurred at dusk. A previous study of the time of day at which males located traps (Francis et al. 2007) reported the capture of very few males (<10 males for all data points), but also found that more males were captured at dusk. Male flight activity of the citrus mealybug, *Planococcus citri* (Risso) was greatest at 10:00 h, and catches were directly related to maximum daily temperatures (Ortu and Delrio 1982).

The height and location of trap placement significantly influenced the number of *M. hirsutus* males captured. Significantly more males were captured in traps placed at 2 m from the ground than those placed at 6 m. More males were captured in traps placed within a hibiscus host than those 3 m upwind from the host at the same height from the ground. Studies in citrus groves trapping *P. citri* demonstrated that more males were caught by traps suspended inside trees than by those suspended between trees or by those put further away from the study plot (Gross et al. 2001). Gross et al. (2001) stated, "male *P. citri* would fly toward the tree crown and only then start looking for the pheromone source, and that the contribution of individual trees to the level of the mealybugs trapped was insignificant." Of the four tree species utilized in the trap elevation study, only *T. catappa* was a known host, although similar numbers of mealybugs were captured in traps within all trees. Male mealybugs, such as *P. longispinus* are considered to be poor fliers (Barrass et al. 1994). Serrano (2001) stated that, "volatiles produced by virgin female *M. hirsutus* seem to be most effective at short distances" and showed that the female sex pheromone was capable of attracting males at a distance of 50 m, though more were captured at shorter distances. Based on our trap placement studies showing that more males captured in traps suspended in hosts and those closest to the ground in non-host trees, the data suggest that male *M. hirsutus* are poor fliers, in accordance with anecdotal observations. Short adult male life span of 3.5 (20ºC) to 1.4 (30ºC) days (Chong et al. 2008), in addition to poor flight ability, suggests that the accuracy

between infestations and males captured in traps would be relatively strong as trapped males are not likely to have traveled long distances. However, captured males may not all be from local populations as adult males have the potential to be blown by wind currents in the upper-atmosphere for hundreds of miles (Stibick 1997).

The addition of management tactics further complicates the ability to correlate males captured in pheromone traps with surrounding infestation levels. Traps within hibiscus that showed no difference in the number of males trapped during the 3-wk period prior to the insecticide treatment, captured ≈500 more males from dinotefuran treated plants the first 4 wk after treatment compared to untreated. The number of males trapped at untreated sites was relatively consistent, the number of males captured from insecticide treated plants were higher immediately following treatment. Vitullo (Chapter 4) showed that traps in cages with *M. hirsutus* infested hibiscus treated with dinotefuran captured the most males the first 2 wks following application, and least males from week 4-7. A study of yellow scale, *Aonidiella citrina* (Coquillett), showed that insecticide application suppressed the number of males captured on pheromone cards (Grafton-Cardwell et al. 2000), supporting Vitullo (Chapter 4) exclusion cage results, but there was no significant suppression of males trapped in the landscape after 12 wk.

Parasitoids were found at sites in the biocontrol study that were untreated. Presence of parasitoids at untreated sites corresponds with previous observations suggesting that parasitoids in FL were capable of reaching *M. hirsutus* infestations 3-10 miles away from known release sites (Amalin et al. 2003). Sites randomly selected to be treated with biocontrol agents had numerically more nymphs and adult females than the untreated site in May 2007, but these differences were not significant due to high variance. More mealybug nymphs and adults were found at biological control than untreated sites, and this was reflected by a sharp increase in

males captured (>4,000) at four out of five biocontrol treated sites during the period of peak abundance (May – Nov.) in 2007. The number of males captured at both biocontrol and untreated sites in 2008 were relatively consistent, except for one untreated and one treated site. This suggests that the biocontrol program that began in June 2002 in southern FL (Amalin et al. 2003) has kept *M. hirsutus* at consistent levels at most untreated sites and that the release of biocontrol agents can reduce populations and males captured within 1 yr. While males captured at most sites remained constant, there were periods in which high numbers of males were trapped in 2008. During the period of peak abundance (May – Nov.) in 2008, traps at a single biocontrol site where 417 mealybugs were sampled captured ≈5,000 males, and at an untreated site where 1701 mealybugs were sampled captured ≈10,000 males. Traps captured many males at sites where mealybugs populations were high, and few males at sites where mealybug populations were low during periods of peak abundance, suggesting that the number of males captured in pheromone traps can correlate with mealybug populations in the environment, even when *M. hirsutus* are being managed with biocontrol agents. During the peak period of 2008, the mealybugs at the biocontrol treated sites did not increase considerably from May to August, while a single untreated site showed a dramatic increase, suggesting that by the second year after release, natural enemies were able to suppress *M. hirsutus* populations. The absence of mealybugs on sampled terminals in December 2008, at both biocontrol and untreated sites may be due in part to an active hurricane season. Monitoring utilizing sex pheromone traps is ideal for the cyclic nature of *M. hirsutus* populations and would help determine risk as periods of peak abundance draw near.

Sex pheromone lures were attractive to vine mealybug males, *Planococcus ficus* (Signoret), with an effective range of at least 50 m. However, the development of a model to

estimate *P. ficus* infestation levels based on males capture was limited, because traps often resulted in positive captures in vineyards where no female *P. ficus* were located during visual searches (Walton et al. 2004). A study in California vineyards by Millar et al. (2002) reported significant correlations between the capture of male *P. ficus* in sex pheromone traps, with visual estimates of population density and economic damage.

Compared with many insect pests, monitoring mealybugs is particularly challenging due to their small size and to a general lack of data for developing sampling programs (Geiger and Daane 2001). Much progress has been made towards the development of optimally efficient and standardized trapping protocols for *M. hirsutus*.

The studies described herein have added further information regarding the effects of trap placement and the effects of management tactics on sex pheromone trapping. The ongoing spread of pink hibiscus mealybug represents a threat to southern states and continued development of pheromone traps as a quantitative and predictive sampling tool based on a standardized protocol is warranted. There are a multitude of factors that influence the response of adult males to sex pheromone traps and their subsequent capture. Understanding the influence of trapping parameters (height and location of placement) as well as management tactics (insecticide and biocontrol) on level of males trapped, aids in interpretation of trap data for more accurate correlation with a given areas infestation, helping mitigate risk from this pest.

Table 5.1. Mean ± SE number of pink hibiscus mealybug adult males captured in pheromone traps placed on hibiscus plants or 3 m upwind at untreated and insecticide treated sites during the 3 wks prior to insecticide treatment, 12 wks after treatment, and total from 1 July to 24 October, 2008.

		Week #						
Trap placement	Treatment	1	2	3*	4	5	6	7
On plant	Untreated	78 ± 28	32 ± 10	10 ± 4	8 ± 4	11 ± 4	21 ± 8	14 ± 4
	Insecticide	83 ± 26	37 ± 12	32 ± 10	33 ± 7	32 ± 7	23 ± 7	13 ± 2
3 m upwind	Untreated	28 ± 10	9 ± 4	3 ± 1	5 ± 2	5 ± 2	5 ± 3	3 ± 2
	Insecticide	39 ± 14	15 ± 6	11 ± 4	9 ± 3	11 ± 3	10 ± 3	5 ± 1

		Week #					
		8	9	10	11	12	13
On plant	Untreated	15 ± 5	14 ± 4	34 ± 12	33 ± 6	14 ± 7	39 ± 24
	Insecticide	16 ± 5	13 ± 5	32 ± 14	27 ± 7	8 ± 2	12 ± 4
3 m upwind	Untreated	6 ± 2	2 ± 1	2 ± 1	3 ± 2	1 ± 0.6	0.5 ± 0.2
	Insecticide	7 ± 4	5 ± 3	7 ± 2	9 ± 3	1 ± 0.25	2 ± 1

		Week #		Total captures	
		14	15	Weeks 1-3	Weeks 4 - 15
On plant	Untreated	14 ± 5	5 ± 2	121 ± 38a	220 ± 57a
	Insecticide	9 ± 4	6 ± 2	151 ± 45a	223 ± 48a
3 m upwind	Untreated	0.4 ± 0.2	0.1 ± 0.1	40 ± 15b	30 ± 13b
	Insecticide	1 ± 0.2	0.3 ± 0.2	64 ± 23b	68 ± 18b

* Weeks 1-3 prior to pesticide application.

Means within columns followed by the same letter are not significantly different ($P<0.05$) using logarithmically transformed data according to Tukey's HSD test.

Table 5.2. Mean ± SE number of nymph and adult female pink hibiscus mealybugs present on sampled plants, encapsulated mealybugs, and their percent parasitism at biocontrol and untreated sites at 3-mo intervals.

Date	Mealybugs	Biocontrol	Untreated	t-statistic (df)	*P*
25 May 2007	No. present	236 ± 112	16 ± 7	1.95 (4.02)	0.123
	No. encapsulated	55 ± 19	9 ± 5	-	-
	% parasitism	10 ± 6	7 ± 3a	0.45 (8)	0.668
31 Aug 2007	No. present	57 ± 19	26 ± 11	1.31 (7)	0.232
	No. encapsulated	17 ± 6	8 ± 3	-	-
	% parasitism	4 ± 2	13 ± 8	-0.79 (7)	0.456
25 Jan 2008	No. present	5 ± 3	0 ± 0	1.34 (4.04)	0.250
	No. encapsulated	4 ± 2	NA	-	-
	% parasitism	5 ± 4	NA	-	-
23 May 2008	No. present	131 ± 74	31 ± 14	1.34 (4.30)	0.248
	No. encapsulated	14 ± 3	6 ± 2	-	-
	% parasitism	33 ± 13	5 ± 4	2.10 (7)	0.074
5 Sept 2008	No. present	184 ± 162	340 ± 380	-0.58 (7)	0.580
	No. encapsulated	14 ± 7	4 ± 3	-	-
	% parasitism	21 ± 10	8 ± 7	1.26 (7)	0.247
28 Nov 2008	No. present	0 ± 0	0 ± 0	-	-
	No. encapsulated	NA	NA	-	-
	% parasitism	NA	NA	-	-

Figure 5.1. The effect of trapping interval on the capture of adult male pink hibiscus mealybugs in pheromone traps in May and September 2008. Means with the same letter in May (lower case) and September (upper case) are not significantly different ($P<0.05$) using logarithmically transformed data according to Tukey's HSD test.

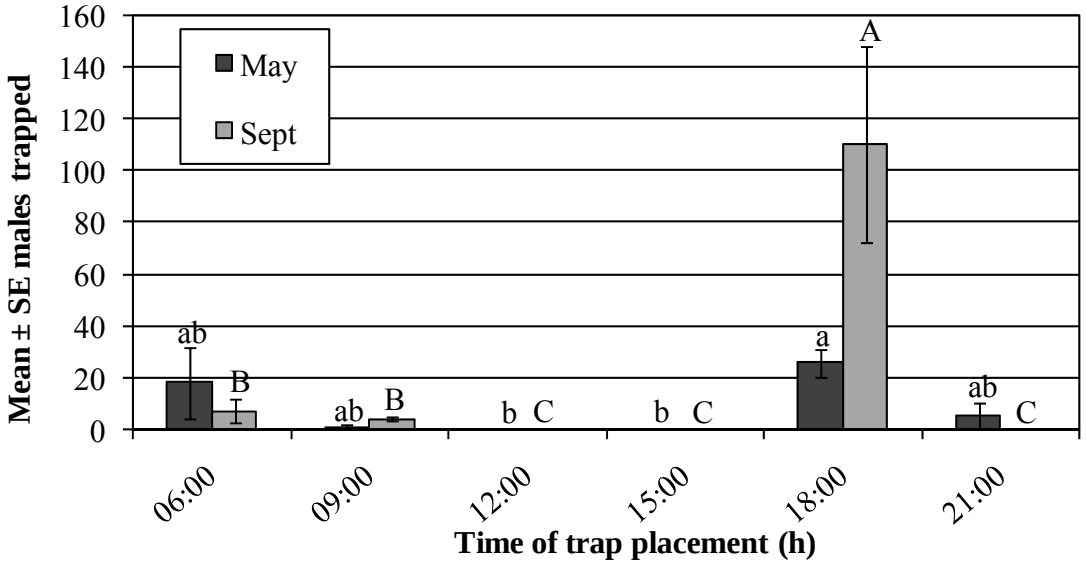
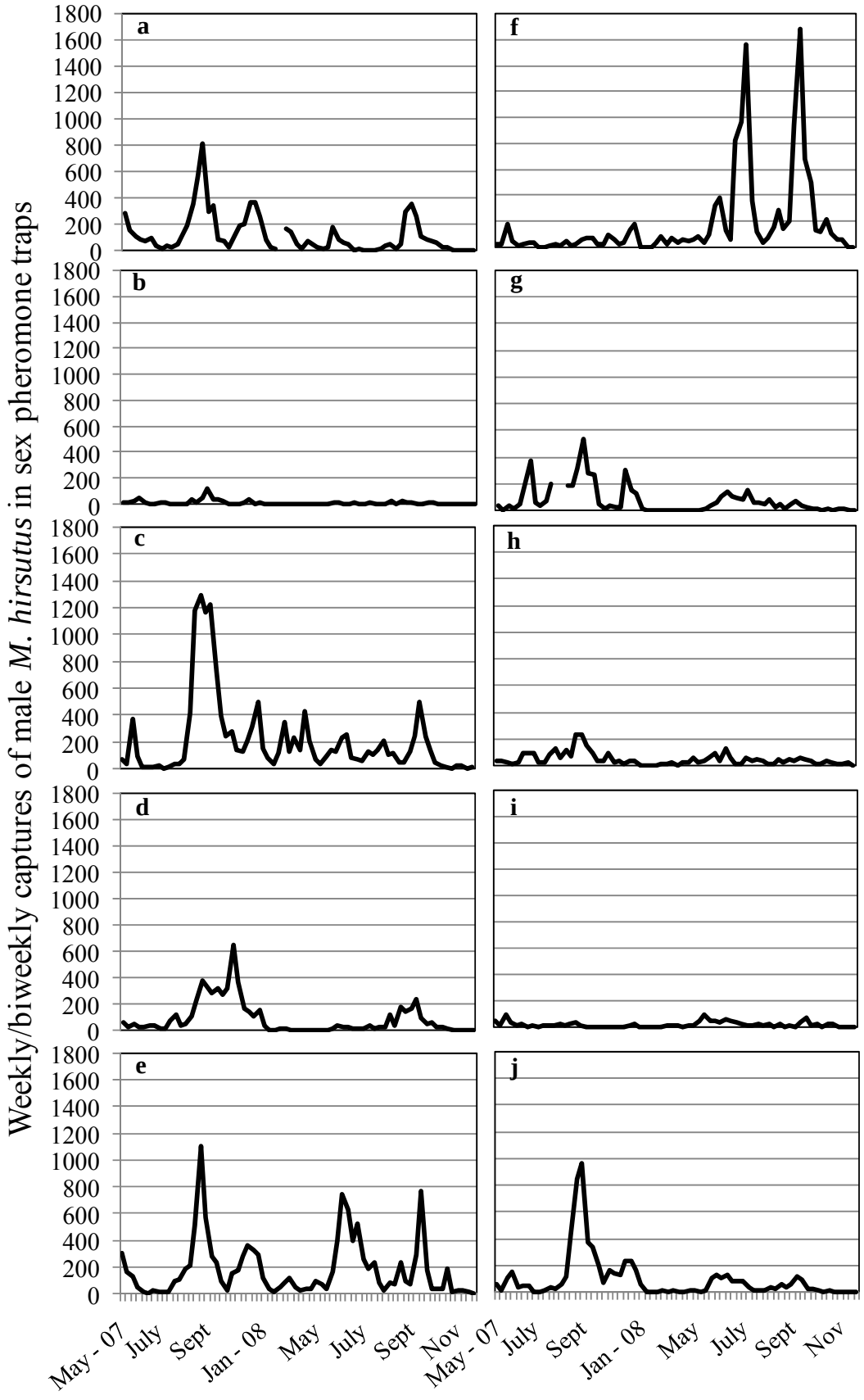


Figure 5.2. Captures of male pink hibiscus mealybugs in pheromone traps deployed from May 2007 through November 2008 at residential sites at which parasitoids were released (a-e) and untreated sites (f-j) in southern Florida. One trap was deployed at each site. The number of parasitoids released at biocontrol sites (*A. kamali*: *G. indica*) on 13 June, 2007 were as follows: site a – 0:800, site b – 0:400, site c – 200:200, site d – 200:600, site e – 800:800. The numbers of parasitoids released at biocontrol sites on 5 July, 2007 were as follows: site a – 400:800, site b – 400:800, site c – 400:800, site d – 400:800 and site e – 800:1600.

(figure on next page)


Weekly/biweekly captures of male M. hirsutus in sex pheromone traps
a
b
c
d
e
f
g
h
i
j
1800
1600
1400
1200
1000
800
600
400
200
0
May - 07
July
Sept
Jan - 08
May
July
Sept
Nov

CONCLUSIONS

The pink hibiscus mealybug (PHM), *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae), is an important pest in the southern US. A USDA (2003) internal document stated that, "there is no question that PHM will become widely distributed throughout the Western Hemisphere in the next 10 years", and this statement is slowly being realized. The status of PHM in the southern US is as follows. Since 2002, PHM has been identified in 37 Florida counties (G. Azore, FDACS, DPI, pers. comm.). Since 2006, PHM has been identified in four Louisiana parishes (Jefferson, Orleans, St. John, and Plaquemines). Pheromone traps deployed in Terrebonne, Lafourche, and St. Bernard parishes in Louisiana trapped no PHM males, and as of May 2009, the Louisiana Department of Agriculture and Forestry (LDAF) is no longer actively conducting formal surveys for PHM, except for regular inspections at nursery grower and nursery stock dealer locations (Tad Hardy, Admin. Coordinator & State Entomologist, Quarantine Programs, Horticulture and Quarantine Division, LDAF, pers. comm.). Since 2007, PHM has been identified in six Texas counties (Nueces, Hidalgo, Galveston, Harris, Brazoria, and Montgomery) located in the southeastern part of the state along the Gulf coast from samples of landscape plants (C. Bográn, Associate Professor and Extension Specialist, Texas AgriLife Extension Service, pers. comm.). Since 2008, PHM has been identified in one Georgia county (Forsyth, NE of Atlanta on ornamentals) (Mike Evans, Division Director, Plant Protection Division, Georgia Department of Agriculture, pers. comm.). Due to the location of initial

infestations in Texas and Louisiana it is possible that tropical storms have carried PHM north from Florida and/or the Caribbean where they then established and dispersed locally. The location of the infestation in Georgia suggests shipment of infested plant material as the means of dispersal. PHM spread is facilitated by a variety of factors and if its current distribution is an indicator of the effectiveness of current prevention methods, its spread will continue in the southern US.

Research presented in this dissertation to meet primary objectives should benefit stakeholders and researchers as they continue to manage pink hibiscus mealybug. Currently the PHM sex pheromone is commercially produced by South Carolina Scientific, Inc. This concluding chapter will address each research objectives in terms of 1) the current situation, 2) what my research has shown and 3) questions that remain to be addressed.

Objective 1 - Compare the expression of PHM feeding symptoms among hibiscus cultivars and among PHM infestation densities.

During my studies in Florida, I only witnessed PHM "bunchy top" feeding symptoms on hibiscus plants. In Homestead, the majority of PHM infested study sites that were used for trapping during my research have been removed by homeowners, and there has been a shift in the composition of landscape plants toward a reduction in hibiscus. PHM feed on numerous plants and my research demonstrated that there is a difference in susceptibility to PHM feeding symptoms among hibiscus cultivars. Over time I observed a reduced abundance of hibiscus in the FL landscape, and consequently less feeding symptoms. This reduction was not due to an absence of PHM, extensive pheromone trapping throughout the Homestead area over four years showed that PHM populations remain common and widely distributed.

When making future planting selections, landscapers should select PHM tolerant plants for locations within infested areas. While biocontrol agents keep PHM at low levels, current levels in Homestead would not be low enough to prevent feeding symptoms on *H. rosa-sinensis* 'President'. Feeding symptoms exhibited by 'President' would be unacceptable to most homeowners, but cultivars such as 'Double Red' and 'Snow Queen' showed almost no symptoms. In addition to the current hibiscus cultivars, future breeding of PHM tolerant cultivars could be warranted. The variability in host expression of feeding symptoms demonstrates the necessity for monitoring techniques that do not rely on presence of feeding symptoms. As homeowners have removed plants highly susceptible to feeding symptoms, their absence in the landscape could allow low levels of PHM to go undetected and potential population surges could occur, negatively impacting plants that are unaffected by low PHM levels. This creates a situation where sex pheromone monitoring would be appropriate. By planting less PHM susceptible plants and utilization of sex pheromone monitoring, future cost savings would include reduced insecticide use in the landscape and at nurseries, effective mapping of populations in the field by a more efficient means than visual inspection, better utilization of biological control agents, and a reduction in labor for management and scouting.

Objective 2 - Investigate short-range dispersal of PHM.

Investigation of the dispersal capabilities of PHM within the Florida landscape has shown that PHM passively dispersed on wind currents as crawlers were captured at distances up to 50 m from an infested source that was 2 m from the ground. Dispersal of crawlers from plants taller than 2 m would disperse further under similar wind conditions. A single dispersing crawler that reaches female adulthood may be able to begin an infestation, if a male can locate her using her sex pheromone and mate. Dispersal of PHM directly influences risk of infestation of new host

plants. The dispersal of nymphs will not be unidirectional as prevailing wind direction changes during the year, especially due to tropical storms, making prediction of spread difficult. The USDA suggested that egg, crawler, and male (adult) stages have the potential to be blown by wind currents in the upper atmosphere for hundreds of miles (Stibick 1997) and Gautam (2002) determined that crawlers can survive without food for 1-2 d.

Predominantly (97%) first instar crawlers dispersed aerially with a diel periodicity that peaked between 14:00 and 18:00 h and was significantly influenced by mean wind speed. The number of dispersing crawlers carried passively on air currents did not differ in number of dispersing individuals captured from plants initially infested with 5, 10, or 20 adult females and equal numbers of crawlers were captured at distances of 5, 10, 25, and 50 m from infested plants.

Long range dispersal will likely have the most impact on biological control release programs, as the fragmented patterning of the US landscape may decouple natural enemies and reduce the odds of parasitism (Roland and Taylor 1997). The large size and varied habitats of the southern US creates a large challenge when attempting to monitor/control PHM. Short range dispersal will have a greater impact on the infestation of commercial nursery operations within infested areas and the possibility of shipping infested plants. Commercial production of ornamental plants in southern Florida occurs in heterogeneous urban landscapes in which nurseries are interspersed with both residential and undeveloped areas containing many potential hosts, creating a situation of ongoing risk of infestation.

Objective 3 - Evaluate the relationship between local PHM infestations, dispersal/colonization of new hosts, and males captured in sex pheromone traps.

Developments in pheromone based monitoring of PHM to correlate the number of males captured in traps with dispersing individuals would be beneficial. Sex pheromone traps are far

superior to visual scouting. However, males were captured at many sites where no signs or feeding symptoms were present. The relationship between dispersing local PHM populations and the capture of males in pheromone traps may be as follows. Crawlers disperse and likely settle at a feeding site within 1-2 days, supported by the fact that predominantly (97%) first instar crawlers dispersed aerially. Due to the period during which crawlers are capable of surviving without food and extensive host range, crawlers are likely to settle on a susceptible hosts within the vicinity of their birth. These crawlers develop into adult females and males in ≈23-35 d and live 3 to 5 d. The poor flight ability of males combined with the fact that males only live from 3-5 d suggests the majority of males trapped in a given area are from local infestations. Given a 1:1 sex ratio, males trapped directly correspond with the number of adult females present in the area. The relationship between males and the next generation of nymphs is secondary and depends on many factors. If a pheromone trap is placed at a given location, the crawlers disperse downwind and develop, which is the same location from where males would fly upwind to locate a pheromone trap and correlate with adult females. The ability for this correlation to occur will be contingent on many factors, especially abiotic factors and the physical makeup of the local environment, as adult males are poor fliers. The trap placement data suggest their poor flight ability, which is further supported by attempted studies in which heavily infested plants placed in open fields resulted in few to no males subsequently captured in traps, which can likely be attributed to high winds. If trapping is attempted in a setting in which there are hosts acting as wind breaks, it is likely males trapped will correlate better to populations, than in an open environment with high wind speeds.

Investigation to determine the relationship between population density and males captured in sex pheromone traps is of central importance. The experiment examining the

relationship between dispersal and population density (Chapter 3) had a sex pheromone trap component, where one sex pheromone trap was placed above each plant infested with different initial PHM densities. However, no males were captured, which I believe was due to the inability of males to locate traps in the open field, high wind environment. Additionally I attempted a large scale field trial in the same open field, infesting plants with more PHM than in Chapter 3. The study was cut short by the hurricane season, but after 3 wk no males were trapped. I would recommend small scale trials within a greenhouse or exclusion cage to determine the initial relationship between males trapped and population density, then conduct field trials to evaluate the added influence of environmental factors and dispersing crawlers.

As PHM crawlers disperse and distribute themselves in the environment, determining the active space of pheromone traps is also important. Over what distance do males respond to sex pheromones, and how well does the number of males captured correlate with surrounding populations? In the environment there are multiple generations of PHM and males are captured continuously. Developing a model or determining a sample interval at which the number of males indicates population density is difficult. Males captured during the entire exclusion cage study and those captured during periods of peak yearly abundance in the biological control field trial suggest that males trapped provided a good indicator of populations, high and low levels of mealybugs corresponded with high and low levels of males captured. However, the ability of trapping over a short period to act as a "snap shot" and provide information on surrounding populations has yet to be determined.

The study utilizing a two week survey in May as a predictor of future captures (Chapter 3) did not show a strong relationship between the number of males captured during the survey, and subsequent captures over the course of the summer. This result would be expected in a

region where biological control programs have been implement and corresponds with data from my study (Chapter 5) in which parasitoids were released. Variability in males trapped in May occurs as parasitoid populations begin to build, while subsequent captures should all be relatively equal as parasitoids reduce PHM populations, as was seen in 2007. However, the inability of surveys in May to predict future captures, has no bearing on the ability of traps to correlate the number of males captured with surrounding PHM populations.

Objective 4 - Evaluate and compare conventional and experimental management tactics for PHM under semi-field and field conditions.

A considerable amount of investigation has been carried out on the effectiveness of management tactics for pink hibiscus mealybug. Chemical control programs are not pragmatic for expansive areas, although there are effective chemicals for nursery and landscape settings. Insecticides recommended for PHM control in nurseries by the Florida Cooperative Extension Service (Osborne 2005) include foliar applied organophosphates (chlorpyrifos and acephate), pyrethroids (bifenthrin), insect growth regulators (buprofezin and pyrproxyfen), or insecticidal soaps and pesticide oils, as well as soil applied neonicotinoids (imidacloprid, dinotefuran, and thiamethoxam). Homeowners can find many of the recommended active ingredients for PHM in over the counter products at their local home and garden store. For long-term, self-sustaining management of PHM, biological control programs have shown some success.

Additionally, biological control programs utilizing the release of encyrtid parasitoids, *Anagyrus kamali* (Moursi) and *Gyranusoidea indica* (Shafee, Alam and Agarwal) in combination with the predator *Cryptolaemus montrouzieri* (Mulsant) continue to be implemented in Florida and have been implemented in Louisiana. It has been seven years since the first release of biocontrol agents in FL for PHM and the FDACS continues to mass-rear and release parasitoids

(G. Azore, pers. comm.). The FDACS report from 4 May, 2004 to 2 June 2006, 118,800 *A. kamali* and 172,200 *G. indica* parasitoids were released at approximately 142 sites in the city of Homestead, FL. FDACS positively confirmed 70 samples as infested with PHM at 48 nursery and garden centers in FL from 5 January 2007 to 9 March 2009. Of the 70 samples, 15 occurred where biological control agents had been previously released, and of the 48 sites, seven were forced to destroy their infested stock, and biological control agents were released at 31 of the sites (G. Azore, pers. comm.). In Louisiana a biocontrol program was established with USDA-APHIS-PPQ on 11 October 2006. From the program inception through 18 April 2007, *A. kamali* and *G. indica* (250,000 individuals in total) were released at more than 400 residences. The program resumed in July 2007 and continued until December 2007 when releases were suspended indefinitely. In 2007, LDAF released 309,500 total parasites at 778 sites in Orleans, Jefferson, St. John and Plaquemines parishes. Dr. Amy Roda, USDA-APHIS-PPQ entomologist has been monitoring LA for successful wasp parasitism, and based on her data, the parasitoids appear to be providing "good" control (Tad Hardy, LDAF, pers. comm.). Moffitt (1999) calculated that a biocontrol program involving an annual expenditure of approximately $500,000 for 3 to 5 years would bring PHM infestation on the US mainland under control. While Moffitt (1999) may have underestimated the cost and duration necessary to control pink hibiscus mealybug in the southern US utilizing a biological control program, this underestimation is of little consequence since losses to agriculture in the continental US and the US Virgin Islands have been estimated at $750 million per year in the absence of control.

The semi-field trial exclusion cage studies demonstrated that dinotefuran and predators reduced nymphs captured in band traps. Upon completion of the study, the number of nymphs recovered from hibiscus terminals was significantly reduced relative to the controls, by the

dinotefuran, predator and parasitoid treatments, while only the predator treatment significantly reduced the number of adult females and egg sacs recovered. Depending upon the management goal, soil applied dinotefuran, *C. montrouzieri* and parasitoids are all viable management options. For the quickest control, based on tested treatments, dinotefuran and predators would be the best choice. Although parasitoids take more time to reduce PHM, they offer the best option for long-term self-sustaining control.

At this point biocontrol agents can be considered to provide "good" management of PHM in the United Sates due to the fact that they have prevented a situation similar to that of Grenada where PHM was affecting forests, killing individual trees and even whole groves. Due to this success, the present attention in FL seems to have shifted away from PHM to newer pests. However, the point at which mass-rearing and release of parasitoids can be ceased has yet to be determined and PHM remains a quarantine pest. In spite of the success of biocontrol programs, the information contained within this dissertation towards managing PHM and optimization of sex pheromone monitoring of PHM would greatly benefit newly and previously infested regions around the globe, nursery operations at risk of quarantine in FL, and biocontrol programs that wish to assess effectiveness and persistence of biocontrol agents. Sex pheromone trapping data indicate widespread prevalence of PHM in southern Florida, and weekly captures have numbered in the 1000's in 2008 as PHM continues to be found in the landscape at unacceptable levels that have very negative implications for nurseries in the area.

The attempt at mating disruption for *Planococcus ficus* by Walton et al. (2006) was only successful at reducing mealybug density compared with controls when mating disruption was combined with buprofezin. Mating disruption and the suppression of pink hibiscus mealybug populations at the concentrations tested was not shown in exclusion cages, similar to the results

of *P. ficus* trials in vineyards. This may have been due to the exclusion cage design or high populations which may have allowed other cues to facilitate mate location. Investigation under field conditions where males rely on sex pheromones for mate location may be warranted. However, if the cost and dose of sex pheromone necessary to cause mating disruption is high, this management tactic may not be financially feasible for pest managers.

Objective 5 - Evaluate factors that influence the capture of male PHM in sex pheromone traps.

Influence of management tactics within exclusion cages resulted in captured males accurately indicating nymphs captured in band traps, exhibiting a linear relationship for all treatments except insecticide. For the insecticide treatment, while there was no linear relationship, there was a significant decline in males captured compared to the untreated corresponding with nymphs captured. The ratio of mean adult males captured over the course of the study and either mean nymphs captured in band traps or those found upon completion of the study was very similar for all treatments except pheromone. The effect of treatments on the number of nymphs present was reflected by subsequent number of males captured, even though it is not a direct relationship. However, in field experiments, hibiscus plants with equivalent initial numbers of trapped males treated with soil applied dinotefuran had similar total numbers of trapped males at the conclusion of the study. Plants treated in the landscape were all larger than those used in field cages, some landscape plants had a canopy 8x larger. When examined at 4 wk intervals, the mean number of males trapped in plants after treatment with insecticide captured 101 (1-4 wk), 87 (5-8 wk), and 34 (9-12 wk) males, compared with traps in untreated plants that captured 55 (1-4 wk), 95 (5-8 wk), and 70 (9-12 wk) males. While these divisions are arbitrary, I can’t ignore the trend of decreased capture of males over time at insecticide treated

plants and the consistent level of males captured at untreated plants. It would have been interesting to see the number of males captured from week 13 to 16 after treatment.

Similarly, the release of parasitoids in the field did not have a significant effect on the total number of males captured in sex pheromone traps after 18 mo. However, parasitoids did reduce the number of males trapped during May to November from 3,184 ± 1,028 in 2007 to 2,478 ± 957 in 2008. The mean ± SE number of mealybugs on sampled terminals in May and September 2008 was 131 ± 74 and 184 ± 162 at treated sites and 31 ± 14 and 340 ± 380 at untreated sites, respectively. By the second year after release, parasitoids kept mealybugs at a consistent level through the peak abundance period, while those at untreated showed an increase. Spikes in the number of males captured in Figure 5.2 were consistent with increased mealybugs sampled from plants in table 5.2, just as periods of low males trapped and low PHM populations sampled corresponded with each other. Mealybugs found at both treated and untreated sites were highly variable and correspond with males captured in sex pheromone traps. The precision of the relationship between trapped males and populations still needs further investigation, but the relationship.

Biocontrol program value and future effectiveness can be enhanced though the research reported in this dissertation. The expense of producing biocontrol agents, low number of plant protection technicians relative to number of plants and labor intensity to scout for insects, creates a need that pheromone based monitoring can fill to effectively target infested areas. Previous studies have suggested that parasitoids in FL were capable of reaching PHM infestations 3-10 miles away from known release sites. As advances continue in the understanding of PHM dispersal, monitoring, and interaction with biocontrol programs, we will become more adept at mitigating the risk from this pest.

I have shown that trap placement influences the number of males captured. More males were captured in traps placed within a known host and in traps placed at low versus high elevations in non-host trees. Anecdotal observations in which more males were captured in traps highest from the ground in host trees would likely be due to the biology of PHM. As PHM colonizes a plant, the terminal ends of shoots are where feeding symptoms occur, adult females hide to oviposit, and nymphs disperse from terminals. The presence of females at the terminal ends of host plants would likely result in more males at these locations. When males are flying to a non-host plant with a trap in it from somewhere else in the environment, more male were captured closest to the ground. Determining the time of day males are captured in sex pheromone traps in a controlled environment is also important. While males are captured at dawn and dusk under field conditions, this may be caused by the relatively high wind speed during the day and inability of males to reach traps. If males are flying at midday, it is likely they are caught by the wind and carried long distances, and this would have major implications on relationships between trap captures and surrounding infestations.

Monitoring the pink hibiscus mealybug in southern Florida is a very complex endeavor. It is less straightforward than the successful monitoring of the vine mealybug in vineyards or San Jose scale in orchards, which are essentially monocultures. There is also the added influence of varied management tactics from nurseries and biocontrol programs. Sex pheromone monitoring of mealybugs, while not a new concept, is not as far along in its development as that of lepidopteran or even armored scale sex pheromone monitoring. Future research towards optimization of PHM pheromone traps will continue to elucidate the factors influencing resultant trap capture.

Future investigation towards improving the practical significance of pheromone based monitoring should involve nurseries within infested areas. The block shape of nurseries creates a situation where crawlers are likely to enter from the windward side and males will seek pheromones coming from that direction as well to investigate the level of PHM males captured in sex pheromone traps at the upwind perimeter (where nymphs would enter), middle (where nymphs would establish), and downwind perimeter (where nymphs are leaving), possibly utilizing trap transects. In addition to trapping within nurseries, the use of aerial dispersal traps and visual inspections of areas surrounding each trap would determine the number of crawlers entering the area and the establishment of active colonies. This would aid in furthering the understanding of trapping relationships, determining trapping parameters, and more accurately evaluating the risk of plant material infestation relative to trapped males.

REFERENCES

Abdel-Moniem, A. S. H., N. A. Farag, and M. H. Abbass. 2005. Vertical distribution of some piercing sucking insects on some roselle varieties in Egypt and the role of amino acids concentration in infestation. Archives of Phytopathology and Plant Protection 38: 245-255.

Agnello, A. M., S. M. Spangler, W. H. Reissig, D. S. Lawson, and R. W. Weires. 1992. Seasonal development and management strategies for Comstock mealybug (Homoptera: Pseudococcidae) in New York pear orchards. J. Econ. Entomol. 85: 212-225.

Amalin, D. M., K. A. Bloem, D. Meyerdirk , and R. Nguyen. 2003. Biological control of pink hibiscus mealybug in south Florida: a one-year assessment. USDA-APHIS manuscript.

Anonymous. 1999. New mealybug found in Imperial county. California Grower 23: 25.

Anonymous. 2005. *Maconellicoccus hirsutus*. Bulletin OEPP/EPPO Bulletin 35: 413-415.

Babu, A. M., K. Vineet, K. Sathyaprasad, and B. K. Kariappa. 2004. Anatomical alterations associated with tukra symptoms in the leaf and stem of mulberry. Indian Journal of Sericulture 43: 46-49.

Babu, T. R., and K. M. Azam. 1987a. Toxicity of different fungicides to adult *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae). Crop Prot. 6: 161-162.

Babu, T. R., and K. M. Azam. 1987b. Residual toxicity of different insecticides to the adult *Cryptolaemus montrouzieri* Mulsant (Coccinellidae: Coleoptera). Trop. Pest Manage. 33: 180-181.

Babu, T. R., and K. M. Azam. 1987c. Studies on biology, host spectrum, and seasonal population fluctuation of the mealybug, *Maconellicoccus hirsutus* on grapevine. Indian J. Hortic. 44: 284-288.

Babu, T. R., and K. M. Azam. 1988. Effect of low holding temperature during pupal instar on adult emergence, pre-oviposition and fecundity of *Cryptolaemus montrouzieri* Mulsant (Coccinellidae: Coleoptera). Insect Sci. App. 9: 175-177.

Barrass, I. C., P. Jerie, and S. A. Ward. 1994. Aerial dispersal of first- and second-instar longtailed mealybug, *Pseudococcus longispinus* (Targioni Tozzetti) (Pseudococcidae: Hemiptera). Aus. J. Exp. Agr. 34: 1205-1208.

Beardsley, J. W. 1985. Notes and exhibitions. *Maconellicoccus hirsutus* (Green). Proceedings of the Hawaiian Entomological Society 25: 27.

Berg, G. H. 1996. Analisis de riesgo por una via respecto a *Maconellicoccus hirsutus* (Green) (cochinilla rosada), a nivel global. Informe tecnico, OIRSA, El Salvador: 32.

Bierl-Leonhardt, B. A., D. S. Moreno, M. Schwarz, J. Farger-Lund, J. R. Plimmer, and E. D. Devilbiss. 1981. Isolation, identification and synthesis of the sex pheromone of the citrus mealybug *Planococcus citri* (Risso). Tetrahedron Lett. 22: 389-392.

Bierl-Leonhardt, B. A., D. S. Moreno, M. Schwarz, H. S. Forster, J. R. Plimmer, and E. D. Devilbiss. 1982. Isolation, identification, synthesis and bioassay of the pheromone of the comstock mealybug and some analogs. J. Chem. Ecol 8: 689-699.

Bogran, C. E., and S. Ludwig. 2007. Pink hibiscus mealybug, a new pest in Texas. Texas Cooperative Extension, The Texas A&M University System E-454.

Brown, C. E. 1958. Dispersal of the pine needle scale, *Phenacaspis pinifoliae* (Fitch), (Diaspididae: Homoptera). Can. Entomol. 90: 685-690.

Byrne, D. N., R. Isaacs, and K. H. Veenstra. 1996. Local dispersal and migration by insect pests and their importance in IPM strategies. *In* E. B. Radcliffe and W. D. Hutchison [eds.], Radcliffe's IPM World. University of Minnesota, St. Paul, MN.

CABI/EPPO. 2004. *Maconellicoccus hirsutus,* distribution map of plant pests, pp. Map 100. CAB International, Wallingford (GB).

Chang, L. W. H., and C. E. Miller. 1996. Pathway risk assessment: pink mealybug from the Caribbean. Planning and Risk Analysis Systems, Policy and Program Development, APHIS, USDA.

Chong, J., A. Roda, and C. M. Mannion. 2008. Life history of the mealybug, *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae), at constant temperatures. Environ. Entomol. 37: 323-332.

Cloyd, R. A., and A. Dickinson. 2006. Effect of insecticides on mealybug destroyer (Coleoptera: Coccinellidae) and parasitoid *Leptomastix dactylopii* (Hymenoptera: Encyrtidae), natural enemies of citrus mealybug (Homoptera: Pseudococcidae). J. Econ. Entomol. 99: 1596-1604.

Cornwell, P. B. 1960. Movement of the vectors of virus diseases of cacao in Ghana, wind movements and aerial dispersal. Bull. Entomol. Res. 51: 175-201.

Daane, K. M., W. J. Bentley, V. M. Walton, R. Malakar-Kuenen, J. G. Millar, C. A. Ingels, E. A. Weber, and C. Gispert. 2006. New controls investigated for vine mealybug. Calif. Agr. 60: 31-38.

Das, L. K., and B. Singh. 1986. Economic control of *Maconellicoccus hirsutus* Green infesting mesta. Indian J. Agric. Sci. 56: 373-375.

Debach, P. 1949. Population studies of the long-tailed mealybug and its natural enemies on citrus trees in southern California, 1946. Ecology 30: 14-25.

Dunn, R., R. Henley, R. Mizell, J. Norcini, D. Norman, L. Osborne, J. Price, G. Simone, and R. Stamps. 1999. Crop profile for ornamentals in Florida. (http://www.ipmcenters.org/cropprofiles/docs/FLornamentals.html)

FDACS. 2004. Bureau of methods development & biological control. (http://www.doacs.state.fl.us/pi/methods/phm.html)

FDACS. 2005. Control strategies to limit the spread of the pink hibiscus mealybug from Florida nurseries. (http://www.doacs.state.fl.us/pi/plantinsp/docs/phm_control_strategies.pdf)

Figadère, B. A., J. S. McElfresh, D. Borchardt, K. M. Daane, W. Bentley, and J. G. Millar. 2007. Trans-alpha-necrodyl isobutyrate, the sex pheromone of the grape mealybug, *Pseudococcus maritimus*. Tetrahedron Lett. 48: 8434-8437.

Fletcher, T. B. 1919. Report of the imperial entomologist. Sci. Rep. Agric. Res. Inst. Pusa, 1918-1919: 86-103.

Follet, P. A. 2004. Generic vapor heat treatments to control *Maconellicoccus hirsutus* (Homoptera: Pseudococcidae). J. Econ. Entomol. 97: 1263-1268.

Francis, A., K. A. Bloem, A. L. Roda, S. L. Lapointe, A. Zhang, and O. Onokpise. 2007. Development of trapping methods with synthetic sex pheromone of the pink hibiscus mealybug, *Maconellicoccus hirsutus* (Hemiptera: Pseudococcidae). Fla. Entomol. 90: 440-446.

Francois, B. 1996. An analysis of the economic, environmental and social impacts of pink mealybug infestation in Grenada, pp. 61. Inter-American Institute for Cooperation on Agriculture.

Frost, W. E. 1997. Polyphenic wax production in *Abacarus hystrix* (Acari: Eriophyidae), and implications for migratory fitness. Physiol. Entomol. 22: 37-46.

Furness, G. O. 1976. The dispersal, age-structure and natural enemies of the long-tailed mealybug, *Pseudococcus longispinus* (Targioni-Tozzetti), in relation to sampling and control. Aust. J. Zool. 24: 237-247.

Gaskalla, R. 2006. Pink hibiscus mealybug (PHM) advisory. *In* Florida-Department-Agriculture-Consumer-Services [ed.]. (http://www.doacs.state.fl.us/pi/plantinsp/docs/phm_advisory_2-14-06.pdf)

Gautam, R. D. 2002. An exciting experience with regard to sustainable management of pink mealybug, pp. 87-99. *In* D. Prasad and S. N. Puri [eds.], Crop Pest and Disease Management: Challenges for the Millennium. Jyoti Publishers, Inderpuri, New Delhi.

Geiger, C. A., and K. M. Daane. 2001. Seasonal movement and distribution of the grape mealybug (Homoptera: Pseudococcidae): developing a sampling program for San Joaquin Valley vineyards. J. Econ. Entomol. 94: 291-301.

Ghose, S. K. 1971a. Predators, parasites and attending ants of the mealybug, *Maconellicoccus hirsutus* (Green) (Pseudococcidae: Hemiptera). Plant Prot. Bull. 22: 22-30.

Ghose, S. K. 1971b. Morphology of various instars of both sexes of the mealybug *Maconellicoccus hirsutus* (Green) (Pseudococcidae: Hemiptera). Indian J. Agric. Sci. 41: 602-611.

Ghose, S. K. 1972. Biology of the mealybug *Maconellicoccus hirsutus* (Green) (Pseudococcidae: Hemiptera). Indian Agric. 16: 323-332.

Gieselmann M. J., and R. E., Rice. 1990. Use of Pheromone Traps, pp. 349-352 *In* D. Rosen [ed.], World Crop Pests, Armored Scale Insects - Their Biology, Natural Enemies and Control. Elsevier Sciences B.V., New York, NY.

Goolsby, J. A., A. A. Kirk, and D. E. Meyerdirk. 2002. Seasonal phenology and natural enemies of *Maconellicoccus hirsutus* (Hemiptera: Pseudococcidae) in Australia. Fla. Entomol. 85: 494-498.

Grafton-Cardwell, E. E., J. G. Millar, N. V. O'Connell, and L. M. Hanks. 2000. Sex pheromone of yellow scale, *Aonidiella citrina* (Homoptera: Diaspididae): evaluation as an IPM tactic. J. Agric. Urban Entomol. 17: 75-88.

Greathead, D. J. 1972. Dispersal of the sugar-cane scale *Aulacaspis tegalensis* (Zhnt.) (Hemiptera: Diaspididae) by air currents. Bull. Entomol. Res. 61: 547-558.

Greathead, D. J. 1990. Crawler behavior and dispersal, pp. 305-305. *In* D. Rosen [ed.], World Crop Pests, Armored Scale Insects - Their Biology, Natural Enemies and Control. Elsevier Science B.V., New York, NY.

Greathead, D. J. 1997. Crawler behavior and dispersal, pp. 339-342. *In* Y. a. C. J. H. Ben-Dov [ed.], World Crop Pests, Soft Scale Insects - Their Biology, Natural Enemies and Control. Elsevier Science B.V., New York, NY.

Gross, S., E. Dunkelblum, F. Assael, M. Harel, A. Zada, and Z. Mendel. 2001. Characterization of the performance of pheromone traps as a basis for the management of the citrus mealybug *Planococcus citri* (Risso) in citrus groves. Boll. Zool. agr. Bachic. 33: 283-295.

Gullan, J. P. 2000. Identification of the immature instars of mealybugs (Hemiptera: Pseudococcidae) found on citrus in Australia Aust. J. Agric. 39: 160-166.

Hall, D. G., and S. L. Lapointe. 2005. Seasonal activity of pink hibiscus mealybug in east central Florida based on pheromone trapping of males., Proc. 88th Ann. FL Entomol. Soc., Ft. Myers, FL.

Hall, D. G., A. Roda, S. L. Lapointe, and K. Hibbard. 2008. Phenology of *Maconellicoccus Hirsutus* (Hemiptera: Pseudococcidae) in Florida based on attraction of adult males to pheromone traps. Fla. Entomol. 91: 305-310.

Hall, W. J. 1921. The hibiscus mealybug, *Phenacoccus hirsutus* (Green), pp. 17, Tech. and Sci. Ser. Bull. . Egypt Minist. Agric., Cairo.

Hall, W. J. 1926. The hibiscus mealybug in Egypt in 1925 with notes on the introduction of *Cryptolaemus montrouzieri*. Ministry of Agric., Egypt, Tech. & Sci. Service 70: 1-15.

Hanski, I. 1998. Metapopulation Ecology. Oxford Univ. Press.

Hara, A. H., and C. M. Jacobsen. 2005. Hot water immersion for surface disinfestations of *Maconellicoccus hirsutus* (Homoptera: Pseudococcidae). J. Econ. Entomol. 98: 284-288.

Hill, W. B., and E. C. Burts. 1982. Grape mealybug (Homoptera: Pseudococcidae) on pear in north central Washington. J. Econ. Entomol. 75: 501-503.

Ho, H. Y., C. C. Hung, T. H. Chuang, and W. L. Wang. 2007. Identification and synthesis of the sex pheromone of the passionvine mealybug, *Planococcus minor* (Maskell). J. Chem. Ecol 33: 1986-1996.

Hodges, G. S., and A. C. Hodges. 2005. Pink Hibiscus Mealybug Training Manual, Pink Hibiscus Mealybug Workshop, Gainesville, FL.

Hoelscher, C. E. 1967. Wind dispersal of brown soft scale crawlers, *Coccus hespiridum* (Homoptera: Coccidae), and Texas citrus mites, *Eutetranychus banksi* (Acarina: Tetranychidae), from Texas citrus. Ann. Entomol. Soc. Am. 60: 673-678.

Horton, D. 2008. Pink hibiscus mealybug found in north Georgia. (http://www.plantmanagementnetwork.org/pub/php/news/2008/PHMealybug/)

Ingram, D. L., and L. Rabinowitz. 1991. Hibiscus in Florida. University of Florida, IFAS Extension (http://edis.ifas.ufl.edu/MG020)

Jacobson, C. M., and A. H. Hara. 2003. Irradiation of *Maconellicoccus hirsutus* (Homoptera: Pseudococcidae) for phytosanitation of agricultural commodities. J. Econ. Entomol. 96: 1334-1339.

Kairo, M. T. K. 1998. Biology of the hibiscus (pink) mealybug, *Maconellicoccus hirsutus* (Green), Report for the Pink Hibiscus Mealybug Technology Transfer Workshop, USVI.

Kairo, M. T. K., G. V. Pollard, D. D. Peterkin, and V. F. Lopez. 2000. Biological control of the hibiscus mealybug, *Maconellicoccus hirsutus* Green (Hemiptera: Pseudococcidae) in the Caribbean. Integrated Pest Management Reviews 5: 241-254.

Kamal, M. 1951. Biological control projects in Egypt, with a list of introduced parasites and predators. Bull. Soc. Foud. Ier Entomol. 35: 205-220.

LDAF. 2006. Pink hibiscus mealybugs discovered in Jefferson parish. (http://www.ldaf.state.la.us/portal/News/PressReleaseCurrent/tabid/92/ItemId/1072/Default.aspx)

Loxdale, H. D., J. Hardie, S. Halbert, R. Foottit, N. A. C. Kidd, and C. I. Carter. 1993. The relative importance of short- and long-range movement of flying aphids. Biol. Rev. 68: 291-311.

Mani, M. 1989. A review of the pink mealybug – *Maconellicoccus hirsutus* Green. Insect Sci. App. 10: 157-167.

Mani, M., and A. Krishnamoorthy. 2008. Field efficacy of Australian ladybird beetle *Cryptolaemus montrouzieri* Mulsant in the suppression of *Maconellicoccus hirsutus* (Green) on sapota. J. Biol. Control 22: 471-473.

Mani, M., and T. S. Thontadarya. 1987. Development and feeding potential of coccinellid predator, *Cryptolaemus montrouzieri* Muls. on the grape mealybug *Maconellicoccus hirsutus* (Green). J. Biol. Control 1: 19-22.

Mani, M., and T. S. Thontadarya. 1988. Population dynamics of the mealybug, *Maconellicoccus hirsutus* (Green) and its natural enemies in grapevine ecosystem. J. Biol. Control 2: 93-97.

Manjunath, D., R. Kishore, K. Sathyaprasad, V. Kumar, P. Kumar, and R. K. Datta. 1996. Biology of the mealybug, *Maconellicoccus hirsutus*, causing tukra in mulberry. Sericologia 36: 487-491.

Manjunath, T. M. 1985. Outbreaks and new records. Plant Prot. Bull. 33: 71-76.

McKenzie, H. L. 1967. Mealybugs of California, pp. 32-33. Univ. of Calif. Press, Berkeley & L.A.

Meyerdirk, D. 1997. Field releases of nonindigenous species of *Anagyrus* and *Gyranusoidea* (Hymenoptera: Encyrtidae) for biological control of pink hibiscus mealybug, *Maconellicoccus hirsutus* (Homoptera: Pseudococcidae). CPHST, PPQ, USDA.

Meyerdirk, D. E. 1999. Pre-emptive thrust against the pink hibiscus mealybug: a model for meeting invasive pest threats in the Caribbean, T-Star Workshop X, Homestead, Florida.

Meyerdirk, D. E., and I. M. Newell. 1979. Seasonal development and flight activity of *Pseudococcus comstocki* in California. Ann. Entomol. Soc. Am. 72: 492-494.

Meyerdirk, D. E., I. M. Newell, and R. W. Warkentin. 1981. Biological control of Comstock mealybug. J. Econ. Entomol. 74: 79-84.

Meyerdirk, D. E., R. W. Warkentin, B. Attavian, E. Gersabeck, A. Francis, M. Adams, and G. Francis. 2001. Biological control of pink hibiscus mealybug project manual, pp. 194.

Michaud, J. P., and G. A. Evans. 2000. Current status of pink hibiscus mealybug in Puerto Rico including a key to parasitoid species. Fla. Entomol. 83: 97-101.

Millar, J. G., K. M. Daane, J. C. McElfresii, J. A. Moreira, R. Malakar-Kuenen, M. Guillen, and W. J. Bentley. 2002. Development and optimization of methods for using sex pheromone for monitoring the mealybug *Planococcus ficus* (Homoptera: Pseudococcidae) in California vineyards. J. Econ. Entomol. 95: 706-714.

Millar, J. G., S. L. Midland, J. S. McElfresh, and K. M. Daane. 2005. (2,3,4,4-Tetramethylcyclopentyl) Methyl acetate, a sex pheromone from the obscure mealybug: first example of a new structural class of monoterpenes. J. Chem. Ecol 31: 2999-3005.

Misra, C. S. 1920. "Tukra" disease of mulberry, pp. 610-618, Rep. Proc. 3rd Entomol. Meeting, Pusa.

Moffitt, L. J. 1999. Economic risk to United States agriculture of pink hibiscus mealybug invasion, A report to the APHIS, USDA under Cooperative Agreement No. 98-8000-0104-CA University of Massachusetts, Amherst.

Molles, M. C. 2002. Ecology, concepts and applications. McGraw Hill, New York, NY.

Mossler, M. A., and O. N. Nesheim. 2003. Tropical Fruit Pest Management Strategic Plan (PMSP).

National Agricultural Statistics Service. 2007. Floriculture: top 5 states by value of sales, US. (http://www.nass.usda.gov/Charts_and_Maps/Floriculture_Crops/fleadst0.asp)

Ortu, S., and G. Delrio. 1982. Observations on the field use of the synthetic sex pheromone of *Planococcus citri* (Risso) (Homoptera, Coccoidea). Redia 65: 341-353.

Osborne, L. 2005. Pink hibiscus mealybug management. (http://www.aphis.usda.gov/plant_health/plant_pest_info/phmb/downloads/commercialphm1-05.pdf)

Osborne, L., C. M. Mannion, K. Griffiths, P. Hornby, A. Roda, and D. E. Meyerdirk. 2003. Pink hibiscus mealybug (PHM), *Maconellicoccus hirsutus* (Green). (http://www.aphis.usda.gov/ppq/ep/emerging_pests/phmb/information.pdf)

Ott, R. L., and M. Longnecker. 2001. An Introduction to Statistical Methods and Data Analysis. Duxbury, Pacific Grove, CA.

Parrella, M. P., T. Strehl, and T. P. Costamagna. 2006. Acetamiprid, dinotefuran, thiamethoxam, chenopodium, buprofezin, and flonicamid efficacy and phytotoxicity trial: citrus mealybug (*Planoccocus citri*). (http://ir4.rutgers.edu/Ornamental/OrnData/20061212y.pdf)

Pedgley, D. E. 1993. Managing migratory insect pests - a review. Int. J. Pest Manage. 39: 3-12.

Persad, C. 1995. Preliminary list of host plants of *Maconellicoccus hirsutus* (Green) – hibiscus or pink mealybug in Grenada. Caribbean Agricultural Research and Development Institute, Grenada.

Persad, A. B., and A. Khan. 2006. Attractiveness of hibiscus mealybug to different plant species. Insect Environ. 11: 175-176.

Persad, A., and A. Khan. 2007. Effects of four host plants on biological parameters of *Maconellicoccus hirsutus* Green (*Homoptera*: *Pseudococcidae*) and efficacy of *Anagyrus kamali* Moursi (*Hymenoptera*: *Encyrtidae*). J. Plant Protect. Res. 47: 35-42.

Pollard, G. V. 1998. Update on new pest introductions, Circular Lett. No. 3/98 – FAO.

Quayle, H. J. 1916. Dispersion of scale insects by the wind. J. Econ. Entomol. 9: 486-493.

Rabkin, F. B., and R. R. Lejeune. 1954. Some Aspects of the Biology and Dispersal of the Pine Tortoise Scale, *Toumeyella numismaticum* (Pettit and McDaniel) (Homoptera: Coccidae). Can. Entomol. 86: 570-575.

Ranjan, R. 2004. Economic impacts of pink hibiscus mealybug in Florida and the United States, A Report Presented at the Annual International Agricultural Trade and Policy Center Conference.

Roland, J., and P. D. Taylor. 1997. Insect parasitoid species respond to different spatial scales. Nature 386: 710-713.

Roltsch, W. J., D. E. Meyerdirk, and R. W. Warkentin. 2000. Pink hibiscus mealybug biological control in Imperial Valley, CA. California Department of Food and Agriculture.

Roltsch, W. J., Meyerdirk, D.E., Warkentin, R., Andress, E.R., and K. Carrera. 2006. Classical biological control of the pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green), in southern California. Biol. Control 37: 155-166.

Sagarra, L. A., and D. D. Peterkin. 1999. Invasion of the Caribbean by the hibiscus mealybug, *Maconellicoccus hirsutus* Green (Homoptera: Pseudococcidae). Phytoprotection 80: 3-113.

Sagarra, L. A., D. D. Peterkin, C. Vincent, and R. K. Stewart. 2000a. Immune response of the hibiscus mealybug, *Maconellicoccus hirsutus* Green (Homoptera: Pseudococcidae), to oviposition of the parasitoid *Anagyrus kamali* Moursi (Hymenoptera: Encyrtidae). J. Insect Physiol. 46: 647-653.

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

Sagarra, L. A., C. Vincent, N. F. Peters, and R. K. Stewart. 2000b. Effect of host density, temperature, and photoperiod on the fitness of *Anagyrus kamali*, a parasitoid of the hibiscus mealybug *Maconellicoccus hirsutus*. Entomol. exp. appl. 96: 141-147.

Sagarra, L. A., C. Vincent, and R. K. Stewart. 2000c. Mutual interference among female *Anagyrus kamali* Moursi (Hymenoptera: Encyrtidae) and its impact on fecundity, progeny production and sex ratio. Biocontrol Science and Technology 10: 239-244.

Sagarra, L. A., C. Vincent, and R. K. Stewart. 2001a. Suitability of nine mealybug species (Homoptera: Pseudococcidae) as hosts for the parasitoid *Anagyrus Kamali* (Hymenoptera: Encyrtidae). Fla. Entomol. 84: 112-116.

Sagarra, L. A., C. Vincent, and R. K. Stewart. 2001b. Body size as an indicator of parasitoid quality in male and female *Anagyrus Kamali* (Hymenoptera: Encyrtidae). Bull. Entomol. Res. 91: 363-367.

Sagarra, L. A., C. Vincent, and R. K. Stewart. 2002. Impact of mating on *Anagyrus kamali* Moursi (Hym., Encyrtidae) lifetime fecundity, reproductive longevity, progeny emergence and sex ratio. J. Appl. Entomol. 126: 400-404.

SAS Institute 2001. SAS for Windows. Release 8.12 computer program, version By SAS-Institute, Cary, North Carolina.

Serrano, M. S., and S. L. Lapointe. 2002. Evaluation of host plants and a meridic diet for rearing *Maconellicoccus hirsutus* (Hemiptera: Pseudococcidae) and its parasitoid *Anagyrus kamali* (Hymenoptera: Encyrtidae). Fla. Entomol. 85: 417-425.

Serrano, M. S., S. L. Lapointe, and D. E. Meyerdirk. 2001. Attraction of males by virgin females of the mealybug *Maconellicoccus hirsutus* (Hemiptera: Pseudococcidae). Environ. Entomol. 30: 339-345.

Stibick, J. N. L. 1997. New pest response guidelines, pink hibiscus mealybug *Maconellicoccus hirsutus*. *In* USDA-APHIS-PPQ [ed.].

Strickland, A. H. 1950. The dispersal of *Pseudococcidae* (Hemiptera-Homoptera) by air currents in the gold coast. Proc. R. Ent. Soc. Lond. 25: 1-9.

USDA. 1997. Look out for the pink hibiscus mealybug, pp. 11, Program Aid No. 1606.

USDA. 1998. *M. Hirsutus* (Green): simulation of potential geographical distribution using CLIMEX simulation model, Internal Document.

USDA. 1999. The pink hibiscus mealybug, pp. 2, Pest Alert 81-35-005.

USDA. 2001. Biological Control of Pink Hibiscus Mealybug Project Manual, pp. 194.

USDA. 2003. Status of pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) and related species in North America, pp. 17, Executive Summary.

Vitullo, J., S. Wang, A. Zhang, C. M. Mannion, and J. C. Bergh. 2007. Comparison of sex pheromone traps for monitoring pink hibiscus mealybug, (Hemiptera: Pseudococcidae). J. Econ. Entomol. 100: 405-410.

Vitullo, J., A. Zhang, C. Mannion, and J. C. Bergh. 2009. Expression of feeding symptoms from pink hibiscus mealybug (Hemiptera: Pseudococcidae) by commercially important cultivars of hibiscus. Fla. Entomol., 92: 248-254.

Walton, V. M., K. M. Daane, W. J. Bentley, J. G. Millar, T. E. Larsen, and R. Malakar-Kuenen. 2006. Pheromone-based mating disruption of *Planococcus ficus* (Hemiptera: Pseudococcidae in California vineyards. J. Econ. Entomol. 99: 1280-1290.

Walton, V. M., K. M. Daane, and K. L. Pringle. 2004. Monitoring *Planococcus ficus* in South African vineyards with sex pheromone-baited traps. Crop Prot. 23: 1089-1096.

Williams, D. J. 1986. The Identity and Distribution of the Genus *Maconellicoccus* in Africa. Bull. Entomol. Res. 76: 351-357.

Williams, D. J. 1996. A brief account of the hibiscus mealybug *Maconellicoccus hirsutus* (Hemiptera: Pseudococcidae), a pest of agriculture and horticulture, with descriptions of two related species from southern Asia. Bull. Entomol. Res. 86: 617-628.

Zar, J. H. 1999. Biostatistical Analysis. Prentice-Hall, Upper Saddle River, NJ.

Zettler, J. L., P. A. Follett, and R. F. Gill. 2002. Susceptibility of *Maconellicoccus Hirsutus* (Homoptera: Pseudococcidae) to methyl bromide. J. Econ. Entomol. 95: 1169-1173.

Zhang, A., and D. M. Amalin. 2005. Sex pheromone of the female pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) (Homoptera: Pseudococcidae): biological activity evaluation. Environ. Entomol. 34: 264-270.

Zhang, A., D. M. Amalin, S. Shirali, M. S. Serrano, R. A. Franqui, J. E. Oliver, J. A. Klun, J. R. Aldrich, D. E. Meyerdirk, and S. L. Lapointe. 2004a. Sex pheromone of the pink hibiscus mealybug, *Maconellicoccus hirsutus*, contains an unusual cyclobutanoid monoterpene. Proc. Natl. Acad. Sci. U.S.A. 101: 9601-9606.

Zhang, A., and J. Nie. 2005. Enantioselective synthesis of the female sex pheromone of the pink hibiscus mealybug, *Maconellicoccus hirsutus*. J. Agric. Food Chem. 53: 2451-2455.

Zhang, A., J. Nie, and A. Khrimian. 2004b. Chiral synthesis of maconelliol: a novel cyclobutanoid terpene alcohol from pink hibiscus mealybug, *Maconellicoccus hirsutus*. Tetrahedron Lett. 45.

Zhang, A., S. Wang, J. Vitullo, A. Roda, C. M. Mannion, and J. C. Bergh. 2006. Olfactory discrimination among sex pheromone stereoisomers: chirality recognition by pink hibiscus mealybug males. Chem. Senses 31: 621-626.