

BIOLOGY, ECOLOGY AND MANAGEMENT OF KEY PESTS OF  
SATSUMA CITRUS IN ALABAMA

by

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## Abstract

Satsuma mandarin (*Citrus unshiu* Marcovitch) production is an emerging industry in Alabama. Recent surveys identified *Leptoglossus zonatus* (leaffooted bug), *Phyllocnistis citrella* (citrus leafminer), and *Panonychus citri* (citrus red mite), as important pests of satsuma in Alabama. My dissertation comprised of three sections with the focus on the ecology and management of the above three key pests, and included laboratory and field studies. The first section (chapters II and III) focused on *L. zonatus*. Host preference and development of *L. zonatus* was studied in chapter II. The results showed that tomato was the most preferred fruit, but satsuma is also a suitable host. Damage to satsuma by *L. zonatus* was evaluated in chapter III. Feeding by *L. zonatus* on satsuma produced external damage, and resulted in significant fruit weight loss, fruit abortion, and reduced soluble solids content (SSC). Section 2 (chapters IV and V) focused on *P. citrella*. The seasonal phenology and natural enemy fauna of *P. citrella* in Alabama was investigated in chapter IV. The results showed multiple overlapping generations of *P. citrella*, and effective pheromone-baited traps were identified. At least 21 species of beneficial arthropods were recorded. In chapter V, I investigated the relative contributions of key beneficial arthropods to natural mortality of *P. citrella* in Alabama using exclusion techniques. Predation by spiders was the most important natural mortality factor, whereas parasitism was minimal. Section 3 (chapters VI and VII) focused on *P. citri*. Chapter VI presented a laboratory evaluation of three commercially available predacious mite species in the family Phytoseiidae (*Phytoseiulus persimilis*, *Galendromus occidentalis*, and *Neoseiulus californicus*), as potential biological

control agents of *P. citri*. All three species were effective in regulating *P. citri* density, but *P. persimilis* showed the highest predation potential followed by *G. occidentalis*. The results of field evaluations of small-scale releases of *P. persimilis* or *G. occidentalis* against *P. citri* (chapter VII) showed that two timed releases of either species at an appropriate release rate (100 or more per tree) provided effective season-long suppression of *P. citri*. These results support the development of an IPM program for satsuma production in the Gulf Coast region.

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# CHAPTER 1

## INTRODUCTION AND LITERATURE REVIEW

### INTRODUCTION

#### Satsuma Mandarin Production

*Citrus* spp. (Sapindales: Rutaceae) is one of the most widely grown nutrient-rich fruit crops around the world, and also a major fruit crop with an average annual market value of about \$10 billion in the United States. In the United States (U.S.), citrus is produced mainly in Florida, California, and Texas. However, the last two decades have seen an increase in citrus acreage in southern Alabama and other parts of the Gulf Coast region of the U.S. (Campbell et al. 2004, Fadamiro et al. 2007, 2008)

Satsuma mandarin (*Citrus unshiu* Marcovitch), a seedless and easy-peeling fruit, is one of the most appealing citrus species. Native to Asia, satsuma has been grown for over a century in the Gulf Coast of the U.S. (English and Turnipseed 1940). However, growth and expansion of the industry have been hampered by periodic freezes (Winberg 1948, Campbell et al. 2004). Since the early 1990s, satsuma production has significantly increased, particularly in the two coastal counties (Mobile and Baldwin) in southern Alabama. Renewed interest in satsuma production by the region growers is fueled by improved production and tree protection methods, as well as strong industry and state support (Campbell et al. 2004).

Currently, satsuma mandarin is a minor citrus crop in the U.S. produced commercially in the states of Alabama, California, Florida, Louisiana, Mississippi and Texas. California is the top satsuma producing state with approximately 3,000 acres, and Louisiana is second with about 300 acres (Boudreaux and Vaughn, 2006). Alabama is the third leading state for satsuma production in the U.S. with over 100 acres. Based on acreage of mature trees and production studies, Alabama's current annual level of satsuma production is 1.4 to 1.8 million pounds with a market value of approximately \$1 million per year (Fadamiro et al. 2007). The predominant variety of satsuma grown in Alabama is 'Owari'. Other varieties are also planted including 'Armstrong Early', 'Brown's Select', and 'Early St. Ann' (Ebel et al. 2004, Fadamiro et al. 2007). In Alabama, satsuma is marketed entirely for fresh consumption. The public school system in Alabama is also a major market for satsumas; about one-third of the local Satsuma mandarin crop has been sold annually to the Alabama public school system since 2003. Growers also market fruit to produce brokers and to fresh produce market vendors.

### **Major Arthropod Pests of Satsuma Mandarin in Alabama**

As in other citrus growing regions, one of the key factors blocking the expansion of the budding Alabama citrus industry is insect and mite pest damages and management. The first published studies on life history and control of pests of Alabama satsuma mandarin were conducted in the early part of the last century (Dozier 1924, English and Turnipseed 1933, 1940). These studies identified several arthropods as key pests of the crop in Alabama including citrus whitefly, *Dialeurodes citri* (Ashmead) (Hemiptera: Aleyrodidae), purple scale, *Lepidosaphes beckii* (Newman) (Hemiptera: Diaspididae), Glover scale, *L. gloveri* (Packard) (Hemiptera: Diaspididae), citrus red mite, *Panonychus citri* (McGregor) (Acari: Tetranychidae); and citrus

rust mite, *Phyllocoptruta oleivora* (Ashmead) (Acari: Eriophyidae). After these early publications, commercial production of satsuma mandarins in Alabama was largely abandoned due to severe freezes. The recent expansion of commercial satsuma production in the state has called for a renewed attention to pest management.

A recent comprehensive survey of Alabama satsuma orchards identified at least twenty eight arthropod pest species (Fadamiro et al. 2007, 2008). These included 24 insect species from five orders: Hemiptera (18 species), Hymenoptera (1 species), Lepidoptera (2 species), Orthoptera (2 species), and Thysanoptera (1 species). In addition, four species of pest mites (Acari) were identified (Fadamiro et al. 2007, 2008). Seven species were identified as major pests of satsuma in Alabama including citrus whitefly, *Dialeurodes citri* (Ashmead) (Hemiptera: Aleyrodidae), purple scale, *Lepidosaphes beckii* (Newman) (Hemiptera: Diaspididae), Glover scale, *L. gloveri* (Packard) (Hemiptera: Diaspididae), citrus red mite, *Panonychus citri* (McGregor) (Acari: Tetranychidae); and citrus rust mite, *Phyllocoptruta oleivora* (Ashmead) (Acari: Eriophyidae); citrus leafminer, *Phyllocnistis citrella*, Stainton (Lepidoptera: Gracillariidae), and leaffooted bug, *Leptoglossus zonatus*. (Hemiptera: Coreidae). The minor (secondary) pests include citrus mealybug, *Planococcus citri* (Risso), black citrus aphid, *Toxoptera auranti* (Fonscolombe), green citrus aphid, *Aphis spiraecola* Patch, cotton aphid, *Aphis gossypii* Glover, flower thrips, *Frankliniella bispinosa* Morgan, Brown stink bug, *Euschistus servus* Say, green stink bug, *Acrosternum hilare* Say, and red imported fire ant, *Solenopsis invicta* Buren, Orangedog, *Papilio cresphontes* (Cramer), Citrus snow scale, *Unaspis citri* (Comstock), Cottony cushion scale, *Icerya purchasi* Maskell (Fadamiro et al. 2007, 2008). Most of the arthropod pests identified by Fadamiro et al. (2007, 2008) had first been identified in Alabama in the early 1900s (English and Turnipseed 1933, 1940). However, a few arthropod

species which have recently become pests of satsuma in Alabama were also reported by Fadamiro et al. (2007, 2008) including leaffooted bugs, *Leptoglossus* spp., and citrus leafminer, *P. citrella*. In general, the arthropod fauna of satsuma mandarin in Alabama is similar to the pest fauna of citrus in Florida, Louisiana, and Texas, although some recently-introduced pests of citrus in Florida such as brown citrus aphid, *Toxoptera citricida* (Kirkaldy), and citrus root weevil, *Diaprepes abbreviatus* (L.) (Aerts and Mossler 2006), were not recorded in the Alabama orchards in the surveys by Fadamiro et al (2008). However, the Asian citrus psyllid, *Diaphorina citri* Kuwayama was first detected in Alabama in August 2008 on citrus at two locations in Baldwin County. *D. citri* was first found in the U.S. in Florida in 1998 and has also been recently discovered in Louisiana, Texas, Georgia, Mississippi, South Carolina, and California. This insect is the most efficient vector of citrus greening disease bacterium, *Candidatus Liberobacter asiaticum*. Citrus greening (also called Huanglongbing) is one of the most serious diseases of citrus causing reduced production and eventual tree death. Fortunately, so far none of the *D. citri*-infested citrus trees and insect samples tested positive for greening. Nevertheless, Baldwin County has since been placed under quarantine by the State Department of Agriculture. The Alabama Citrus Greening/Vector working group was recently established to formulate and coordinate a plan for managing the pest/disease complex in the state, including quarantine, education, research and management strategies. The following sections provide a detailed review of the three key pests of satsuma, which were the focus of this dissertation.

### **Leaffooted bugs, *Leptoglossus zonatus***

Leaffooted bugs, *Leptoglossus* spp. (Hemiptera: Coreidae), are polyphagous pests of various field, vegetable and fruit crops in the United States (Hussey 1953, Allen 1969, Hall and

Teetes 1982, Johnson and Allain 1998, Schaefer and Panizzi 2000, Henne et al. 2003, Buss et al. 2005, Fadamiro et al. 2008). Recently, two species, *L. zonatus* (Dallas) and *L. phyllopus* (L.), were identified as key direct pests of satsuma in the Gulf Coast region with the potential for major economic losses (Henne et al. 2003, Fadamiro et al. 2008). However, the predominant species in Alabama is *L. zonatus*.

*L. zonatus* presents two large whitish-yellow spots on the anterior portion of the pronotum, and a zigzag white band across the hemelytra that easily distinguish it from related species (Hussey 1953, Allen 1969). *L. zonatus* is a pest of many economically important crops such as maize (Schaefer and Panizzi 2000), sorghum (Matrangolo et al. 1994), cotton (Jackson et al. 1995), citrus (Albrigo and Bullock 1977, Henne et al. 2003, Fadamiro et al. 2007, 2008), pecan (Ebeling 1950), and physic nut (Grimm and Guharay 1998). *Leptoglossus* spp. feed primarily on fruits and developing seeds, occasionally feeding on vegetative tissues for moisture uptake (Schaefer and Mitchell 1983). Several cover-crop plants, e.g., watermelons, citrons, velvet beans mentioned by Griffiths and Thompson (1957), and thistles can also serve as important host plants for nymph development (Hubbard (1858). In Alabama, *L. zonatus* typically move from crop fields (e.g., cotton, tomatoes, watermelons, etc) into adjacent satsuma orchards in the fall (September) when the fruit start to ripen.

*L. zonatus* immatures pass through five nymphal stages, which are found in the same environment as the adults. Several aspects of the biology of *L. zonatus* documented in several crops showed various life history parameters on different hosts (Panizzi 1989, Jackson et al. 1995, Grimm and Somarriba 1999). For example, development time on a meridic diet (Jackson et al. 1995) was different from that on physic nut (Grimm 1999). However, little is known about the development or life history of *L. zonatus* on satsuma mandarin. Fruit feeding by leaffooted

bug immatures and adults can result in premature color break and fruit drop, and render the fruit unmarketable (Ebeling 1950, Griffiths and Thompson 1957, Albrigo and Bullock 1977, Grimm 1999; Henne et al. 2003). In addition, *Leptoglossus* spp, can transmit a yeast disease pathogen (*Nematocera coryli*) to fruit, causing dryness in the affected wedges of the fruit and producing bad fruit flavor (Clarke and Wilde 1970, Henne et al. 2003). The economic damage due to *Leptoglossus* spp. has been estimated in sorghum (Hall and Teetes 1982) and physic nut (Grimm 1999). Leaffooted bugs are primarily controlled using conventional insecticides (Abudulai et al. 2001, Grimm and Guharay 1998), but only a few products (mainly pyrethroids) are presently labeled for leaffooted bug control in the U.S. Despite their importance as pests of many crops, several aspects of the biology and management of leaffooted bugs have not been studied. In particular, little is known about their host preference, and their development on satsuma mandarin has not been evaluated. Knowledge of the biology and ecology of *L. zonatus* is necessary for the development of an effective IPM program for this pest.

### **Citrus Leafminer, *Phyllocnistis citrella***

The citrus leafminer, *Phyllocnistis citrella* (Lepidoptera: Gracillariidae), is of southern Asian origin (Stainton 1856, Clausen 1931), and has now spread to all major citrus-growing areas around the world (Heppner 1993, Hoy and Nguyen 1997, Legaspi et al. 2001, Garcia-Mari 2004, Diez et al. 2006). *P. citrella* is prevalent in most Alabama satsuma orchards (Fadamiro et al. 2007, 2008), and is generally regarded as an important pest in young citrus nurseries and top-grafted trees (Knapp et al. 1995, Diez et al. 2006).

The biology and ecology of *P. citrella* has been reported by a number of researchers in different countries (Clausen 1931, Ba-Angood 1978, Huang et al. 1989, Heppner 1993, Knapp et

al. 1995). *P. citrella* goes through four developmental stages: eggs, larvae, pupae, and adults. Total developmental time from eggs to adult emergence was ~ 13-52 days, and had 6 -13 generations per year depending on weather conditions (Clausen 1931, Lal 1950, Heppner 1993, Knapp et al. 1995). *P. citrella* attacks all citrus cultivars or related species within the family Rutaceae (Badawy 1967, Heppner 1993), including *Citrus* spp., grapefruit (*Citrus paradisi* Macfad.), and kumquat (*Fortunella crassifolia* Swingle) (Badawy 1967, Heppner 1993). Larvae of *P. citrella* feed on the leaf epidermis ingesting sap and causing chlorosis. They typically make serpentine tunnels called mines under the leaf cuticle, resulting in twisted and malformed leaves (Heppner 1993, Legaspi et al. 1999). The serpentine mines may reduce the photosynthetic capacity of leaves and increase their susceptibility to plant pathogens, such as citrus canker bacterium, *Xanthomonas axopodis* pv. *citri* (Sohi and Sandhu 1968, Cook 1988, Gottwald et al. 1997, 2002). Virtually all citrus cultivars are susceptible to *P. citrella* (Heppner 1993, Legaspi and French 1999) and heavy infestations can result in significant yield loss (Peña et al. 2000).

The seasonal dynamics of *P. citrella* and associated natural enemies have been documented in many regions (Chen et al. 1989, Pena et al. 1994, Pena 1998, Urbaneja et al. 2000, Legaspi et al. 2001, Diez et al. 2006, Lapointe and Leal 2007). The species is multivoltine with number of generations per year ranging from six in Japan (Clausen 1931) to 13 in Northcentral India (Lal 1950). In Florida, *P. citrella* is present throughout the season, but peak flight activity occurs from late March through early October, coinciding with flushes of vegetative growth (Lapointe and Leal 2007). The female-produced *P. citrella* sex pheromone was first partially identified by Ando et al. (1985). Recent studies have identified the full composition of this sex pheromone as consisting of three active components: (*Z, Z, E*)-7, 11, 13-hexadecatrienal, (*Z, Z*)-7, 11-hexadecadienal, and (*Z*)-7-hexadecenal (Leal et al. 2006, Moreira et al. 2006). Follow-up

field tests demonstrated strong attraction of *P. citrella* males to traps baited with a binary or tertiary lure of the active sex pheromone components (Mafi et al. 2005, Lapointe et al. 2006, Leal et al. 2006, Moreira et al. 2006, Lapointe and Leal 2007). A recent study also reported on the utility of traps baited with a commercially produced synthetic sex pheromone lure of *P. citrella* for monitoring the pest in Florida (Stelinski and Rogers 2008).

*P. citrella* is usually controlled using insecticides but chemical control is rarely effective because the larvae are protected from insecticide by leaf cuticles (Legaspi et al. 2001). Thus, biological control is generally regarded as a sustainable management solution for *P. citrella* (Knapp et al. 1995, Hoy and Nguyen 1997, Legaspi et al. 2001, Garcia-Mari et al. 2004). Several predatory arthropods are known to feed on *P. citrella*, including lacewing larvae, ants, and hunting spiders (Argov et al. 1996, Pomerinke 1999, Amalin et al. 2001, Hoy et al. 2007, Xiao et al. 2007), and many studies have identified predation as the most important natural mortality factor acting on *P. citrella* in many parts of the world (Chen et al. 1989, Amalin et al. 1996, 2001, 2002, Hoy et al. 2007, Xiao et al. 2007). In addition, many species of parasitoids have been reared from *P. citrella* worldwide (Hoy and Nguyen 1997, Schauff 1998, Legaspi et al. 1999), but indigenous parasitoids were found to provide only minimal levels of parasitism in Florida (Pena et al. 1996). Consequently, *Agonaspis citricola* Logvinovskaya (Hymenoptera: Encyrtidae), an exotic specialist endo-parasitoid of *P. citrella* larvae, was recently introduced for classical biological control of the pest in Florida and Texas (Hoy and Nguyen 1997, Pomerinke and Stansly 1998).

*P. citrella* is a key pest of satsuma mandarin in Alabama (Fadamiro et al. 2007, 2008) but little is known about its seasonal phenology, associated natural enemies, and key mortality factors in the Gulf Coast region. Because pest phenology and response to pheromone traps may

vary from region to region due to several factors including variations in climate and crop varieties (Fadamiro 2004a, b), data obtained from other citrus growing regions may not provide accurate information on the abundance and seasonal activity of *P. citrella* in southern Alabama. Several aspects of the biology and ecology of *P. citrella* in Alabama were studied in this dissertation.

### **Citrus red mite, *Panonychus citri***

Citrus red mite, *Panonychus citri* (McGregor) (Acari: Tetranychidae), was first identified on citrus in Florida in 1885 (English and Turnipseed 1940) and is now an important pest of citrus in many parts of the world (Gotoh and Kubota 1997, Jamieson et al. 2005, Childers et al. 2007). Like most spider mites, *P. citri* goes through five stages: egg, larvae, protonymph, deutonymph and adult (Krantz 1978). The oval eggs are laid on the leaf surface by females and the immatures resemble the adults (Krantz 1978). The female is larger than the male (Childers et al. 2007). The developmental time from egg to adult is ~12 days and adults live for ~10-23 days depending on environmental conditions (24 - 26 °F and RH 50 -70 %) (Childers and Fasulo 2009). Like other spider mites, *P. citri* adults deposit fine webbing to maintain beneficial environmental conditions on the leaf surface, as well as for protection (Krantz 1978). Adults and immatures feed primarily on leaves and fruits, but leaf damage is usually more common (Childers et al. 2007), producing stippling damage, and severe infestations can result in premature leaf drop, decreased plant vigor (Krantz 1977).

*P. citri* is a major pest of satsuma mandarin in Alabama (English and Turnipseed 1940, Fadamiro et al. 2007, 2008). The results of recent field surveys identified *P. citri* as one of the most numerically abundant pests in Alabama satsuma orchards (Fadamiro et al. 2007, 2008).

Two to three generations per year were recorded in southern Alabama and the pest was most abundant in the spring (Fadamiro et al. 2008), when population densities were typically greater than the economic threshold of five motiles per leaf, proposed by Childers et al. (2007). The results also showed that *P. citri* was more abundant in the exterior canopy than in the interior canopy, and infestations were more severe in conventionally sprayed orchards than in unsprayed orchards (Fadamiro et al. 2008).

Traditionally, control of *P. citri* on citrus has been accomplished through the use of conventional acaricides (Childers 1994). However, there are some reports of documented or suspected cases of resistance of some phytophagous mites to acaricides (Omoto et al. 1995, Bergh et al. 1999). Furthermore, indiscriminate use of broad spectrum pesticides may induce or exacerbate populations of phytophagous mites by disrupting the activity of predatory mites and other natural enemies (Welty 1995, Antonelli et al. 1997, Jamieson et al. 2005).

Many authors have reported on the biological control of phytophagous mites of citrus and other fruit crops with predacious mites, in particular those belonging to the families Phytoseiidae and Stigmaeidae (Childers et al. 1975, McMurtry 1983, Childers 1994, Wood et al. 1994, Jamieson et al. 2005). Like their spider mite prey, predacious mites also have five stages: egg, larva, protonymph, deutonymph and adult (Krantz 1978). Most phytoseiids are ovoid and often move much faster than spider mites. Phytoseiid mites have been reported to use kairomones from host plants to locate their prey (Sabelis 1985). Predacious mites often overexploit spider mites patches and are therefore forced to move to a new patch or die of starvation (Croft and Jung 2001).

Biological control with predacious mites may present an alternative to chemical control of *P. citri* in Alabama. A recent survey of the predacious mite fauna of satsuma mandarin in

Alabama identified a total of 29 species from nine families, including 18 species in the family Phytoseiidae and one species in the family Stigmaeidae (Fadamiro et al. 2009). The dominant predacious mite species recorded were *Typhlodromalus peregrinus* Muma and *Proprioseiopsis mexicanus* (Garman) (Phytoseiidae), and *Agistemus floridanus* Gonzalez (Stigmaeidae) (Fadamiro et al. 2009). However, these predacious mites were recorded in the orchards at densities too low for effective suppression of *P. citri* (Fadamiro et al. 2009). Furthermore, recent attempts to mass rear *T. peregrinus* and other indigenous predacious mites for augmentative releases against *P. citri* have not been successful (unpublished data). This prompted our interest in the evaluation of some commercially available phytoseiids, such as, *Phytoseiulus persimilis* Athias Henriot, *Galendromus occidentalis* (Nesbitt), and *Neoseiulus californicus* (McGregor) as potential biological control agents of *P. citri*. Predacious mites in the family Phytoseiidae can be classified into four categories based on their feeding habits and related biological and morphological traits (McMurtry and Croft 1997). Type I phytoseiids (e.g., *Phytoseiulus* spp.) are specialized predators of spider mites, *Tetranychus* spp. Type II phytoseiids are selective predators of *Tetranychus* species. Examples include *Galendromus* spp. and some *Neoseiulus* spp. Type III phytoseiids (e.g., *Amblyseius* spp.) are generalist predators, while Type IV phytoseiids (e.g., *Euseius* spp) are specialized pollen feeders/generalist predators.

In this dissertation, the above commercially available predacious mite species were evaluated in the laboratory and field to determine their potential as biological control agents of *P. citri*.

## JUSTIFICATION

Historically, pest control in citrus and other fruit crops has largely been accomplished by multiple applications of conventional, broad-spectrum pesticides. In many cases, the pesticides have been applied on a calendar basis. This intensive chemical use has resulted in many drawbacks, including increasing concern over food safety, environmental pollution, pest resistances, and reducing activities of beneficial insects (Thistlewood 1991) The passage of the Federal Food Quality Protection Act (FQPA) of 1996 called for a significant reduction in pesticide use on food crops, and has resulted in the restriction or loss of many insecticides and miticides popularly used to control citrus pests. For instance, use of Azinphos-methyl (Guthion) Carbaryl (Sevin), and AgriMek (Abamectin), is now restricted on many fruit crops

Integrated pest management (IPM) programs based on pest sampling, biological control, and use of reduced-risk and selective pesticides have been developed in major citrus producing states or regions (Childers et al. 2007), but not in Alabama. In the absence of local scientific data on efficacy and suitability of alternative strategies, citrus growers in Alabama have been reluctant to adopt IPM. In southern Alabama, leaffooted bug, *L. zonatus*, citrus leafminer, *P. citrella*, and citrus red mite, *P. citri* are three pests with great concern to satsuma growers (Fadamiro et al. 2007, 2008).. However, little is known about several aspects of their biology and ecology. Knowledge of the biology and ecology of the pests is central to the development of an effective satsuma IPM program in Alabama.

## DISSERTATION OUTLINE, GOAL AND OBJECTIVES

The goal of this dissertation is to support the development of effective and ecologically friendly pest management strategies for managing key pests of satsuma in Alabama through a

knowledge of their biology, ecology and natural mortality factors. The dissertation is arranged under three sections with the basic aim of investigating the biology, ecology and management of three of the key pests of satsuma in Alabama, namely *L. zonatus* (leaffooted bug), *P. citrella* (citrus leafminer), and *P. citri* (citrus red mite). Section 1 (Chapters II and III) focused on the host preference, development, and feeding damage of *L. zonatus*. The seasonal phenology and natural mortality factors of *P. citrella* were investigated in section 2 (Chapters IV and V). The focus of section 3 (Chapters VI and VII) was on biological control of *P. citri*.

In Chapter II, I investigated host preference and suitability of satsuma fruit as host for *L. zonatus* under laboratory conditions. Tomato was the most preferred fruit by both the nymphs and adults, with satsuma a distant second. Development experiments showed that satsuma is a suitable host which can maintain modest to high populations of the pest.

The main objective of Chapter III was to assess the injury and amount of damage to satsuma fruit caused by *L. zonatus* nymphs and adults at various densities (0-3 individuals per fruit) and feeding durations (0-14 days after infestation) in laboratory and field experiments. Feeding by *L. zonatus* on satsuma produced typical damage symptoms including the presence of green spots and dark spots on the outer rind and the collapsing and drying out of the juice vesicles in the inner rind. Insect density and feeding duration had significant effects on most damage parameters. Fruit weight loss, percentage of damaged fruit sections, fruit abortion and premature fruit ripening increased with insect density and feeding duration.

The objective of Chapter IV was to evaluate the seasonal population dynamics of *P. citrella* and associated natural enemies in Alabama satsuma orchards. Multiple overlapping generations with at least three distinct peaks were recorded for *P. citrella* immatures in Alabama, and population densities progressively increased from spring to summer and declined during fall.

At least 21 species of beneficial arthropods were also recorded, the most prevalent being spiders (13 species. e.g., *Hibana* sp.), ants (e.g., *Solenopsis invicta* Buren), *Chrysoperla* sp., *Harmonia axyridis* Pallas, and two parasitoid species (*Ageniaspis citricola* Logvinovskaya and *Cirrospilus* sp.). Additional studies were conducted to evaluate two popular trap types (Pherocon VI “Delta” trap versus Pherocon 1C “Wing” trap) baited with a commercially available sex pheromone lure for monitoring *P. citrella*. No significant differences were recorded in captures of male moths in both types of traps. The highest trap captures were recorded from August to October in 2006 and from June to October in 2007. The phenology of *P. citrella* moths generally followed the same pattern as that of the immatures: highest densities were recorded in summer.

In Chapter V, I investigated the relative contributions of key beneficial arthropods identified in Chapter IV to natural mortality of *P. citrella* in Alabama using cage or sticky barrier exclusion methods. Overall mortality of *P. citrella* on unprotected (control) satsuma tree branches ranged from 39% to 52 % depending upon location and year. Predation was the dominant natural mortality factor acting on *P. citrella*. In unprotected (control) satsuma tree branches, predation accounted for ~87-96 % of all deaths. In particular, predation by spiders was the single most important mortality element, which accounted for ~50-70 % of all deaths. Predation by ants was second, accounting for ~10-19 % of all deaths. Predation by predatory insect larvae accounted for ~ 3-27 % of all mortalities, while parasitism contributed the least (0-10 %) to *P. citrella* mortality. Predation by spiders was excluded by a cage barrier, whereas a sticky barrier was more effective in excluding predation by ants.

The objective of Chapter VI was a laboratory evaluation of the potential of three commercially available predacious mite species (Phytoseiidae), *Phytoseiulus persimilis*, *Galendromus occidentalis*, and *Neoseiulus californicus*, as biological control agents for *P. citri*.

All three phytoseiids species were effective in regulating *P. citri* density, but very few eggs were laid by each. Regression analysis showed a functional type II (convex) response for all three species: the number of prey consumed increased with prey availability up to a maximum point after which it slowly began to decrease. Results from experiments on prey-stage preference showed that all three phytoseiids preferred nymphs to eggs of *P. citri*. Among the three species, *P. persimilis* was a slightly more effective predator, particularly at high prey densities.

The objective of Chapter VII was to further investigate the potential of biological control of citrus red mite, *P. citri*, by conducting field trials to further evaluate the effectiveness of small-scale releases of *G. occidentalis* or *P. persimilis*, for suppression of *P. citri* in a commercial satsuma orchard in southern Alabama. Both predacious mite species were selected based on the results of Chapter VI in which they were identified as promising biological control agents for *P. citri*. In four separate experiments, releases of each single species at different release rates (0-200 per tree) and frequencies (1 versus 2 releases per season) were compared on satsuma trees with either high initial density (i.e. > 5 motiles per leaf) or moderate initial density (i.e. 3-4 motiles per leaf) of *P. citri*. The results showed that release rate and frequency were two key factors determining the ability of the predacious mites to maintain effective long-term suppression of the prey. In general, both predacious mite species were more effective at high release rates than at lower rates: the best treatment was two timed releases of either species at a rate of 200 per tree. Furthermore, predacious mite releases were more successful at moderate than at high prey densities. Both species showed similar efficacy in suppressing *P. citri*, although *P. persimilis* was slightly more effective in at least one trial. However, the two predacious mite species were detected at very low population densities throughout the trial, suggesting that they were unable to establish and proliferate in the orchard.

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## CHAPTER 2

### HOST PREFERENCE AND DEVELOPMENT OF THE LEAFGOOTED BUG, *LEPTOGLOSSUS ZONATUS* (HEMIPTERA: COREIDAE) ON SATSUMA MANDARIN (*CITRUS UNSHIU*)

#### INTRODUCTION

Leafhopper bugs, *Leptoglossus* spp. (Hemiptera: Coreidae), are polyphagous pests of various field, vegetable and fruit crops in the United States (U.S.) (Hussey 1953, Allen 1969, Hall et al. 1982). *L. zonatus* (Dallas) is an important emerging pest of a wide range of crops in the southern U.S., including cotton, tomato, eggplant, peaches, citrus, watermelons, corn, pecans, and citrus (Albrigo et al. 1977, Johnson and Allain 1998, Schaefer and Panizzi 2000, Buss et al. 2005). This species was also recently identified as a major pest of satsuma mandarin (*Citrus unshiu* Marcovitch) in the Gulf Coast region (Henne et al. 2003, Fadamiro et al. 2007, 2008). Feeding on satsuma fruit by nymphs and adults of *L. zonatus* or the closely related *L. phyllopus* (L.) can result in premature color break and fruit drop, and render the fruit unmarketable (Henne et al. 2003). Damage can also reduce the soluble solids content of satsuma fruit (Y. F. X., unpublished data). In addition, *Leptoglossus* spp. can transmit yeast disease pathogen (*Nematocera coryli*) to fruit, which causes dryness in the affected wedges of the fruit and produces bad fruit flavor (Henne et al. 2003).

Certain aspects of the life history of *L. zonatus* have been documented in some host crops (Panizzi 1989, Matrangolo and Waquil 1994, Jackson et al. 1995, Grimm and Somarriba 1999), showing different developmental and survival rates for *L. zonatus* on different crops. For instance, total developmental time at ~ 30 °C from second instars to final molt on maize was ~ 42 days (Panizzi 1989) compared to ~ 21 days on physic nut (*Jatropha curcas* L.) (Grimm and Somarriba 1999). In southern Alabama, *L. zonatus* adults typically move from crop fields (e.g., cotton, tomatoes, watermelons, etc) into adjacent/nearby fruit orchards (e.g., satsuma, peach, etc) in the fall when the fruits start to ripen. However, little is known about its host preference. Furthermore, it is not clear whether or not *L. zonatus* can complete its life cycle on satsuma. The development of an effective program for managing *L. zonatus* on satsuma requires knowledge of several aspects of its ecology and biology, including host preference and developmental biology. This study was conducted to determine: i) host preferences in *L. zonatus* by testing relative attraction of adults and nymphs to various fruit species in multiple choice tests, and ii) life history parameters of *L. zonatus* on satsuma fruit including developmental times, survival rates, female fecundity, and adult longevity.

## MATERIALS AND METHODS

### **Rearing of *L. zonatus***

Adults of *L. zonatus* collected from satsuma orchards in southern Alabama in the fall of 2007 were used to start laboratory colonies, which were supplemented by adults collected from the field in 2008. Adults were reared in wooden sleeve cages (60 × 40 × 30 cm; 5 pairs per cage) with screened walls, on lima bean seedlings (3 potted plants per cage) supplemented with fresh ripened satsuma fruit (3 per cage), and water. The cages were checked daily to collect freshly

laid eggs and to replace diet, as necessary. Lima bean seedlings were replaced monthly, whereas satsuma fruit were replaced biweekly. The rearing conditions were  $25 \pm 2$  °C,  $50 \pm 10$  % RH, and a photoperiod of 14:10 (L: D) h.

### **Host Preference in *L. zonatus***

Three separate multiple choice experiments were conducted to evaluate attraction of *L. zonatus* nymphs and adults to the fruit of the following crops: tomato (*Solanum lycopersicum* L. syn. *Lycopersicon lycopersicum*), satsuma mandarin (*Citrus unshiu* Marcovitch), peach (*Prunus persica* (L.) Batsch), kumquat (*Fortunella* spp.), and lemon (*Citrus limon* (L.) Burm. F.). In experiment 1, attraction of *L. zonatus* to the following five fruit treatments was compared: tomato (ripened, deep red color), satsuma (unripened, green), satsuma (ripened, orange), peach (ripened, yellow), and kumquat (ripened, yellow). In experiment 2, attraction of *L. zonatus* to the following four fruit treatments was compared: tomato (ripened, deep red color), satsuma (ripened, orange), peach (ripened, yellow), and lemon (ripened, yellow). Experiment 3 was conducted to test whether the attraction of *L. zonatus* to tomato (as observed in experiments 1 and 2) is related to color (deep red). For this experiment, green, unripened tomato was compared with the other three treatments tested in the second experiment. The tests were conducted in a wooden cage (60 x 40 x 30 cm) with screened (nylon screen net) walls at the above conditions used for insect rearing. In experiment 1, the cage was divided into five sections (four corners and one middle section). One single fruit of each species/type (treatments) was placed in the bottom of one of the five sections. The experiment was replicated three times and the position of each treatment in the cage was determined randomly and rotated during each replication. *L. zonatus* of the following stages were tested: early instars (2<sup>nd</sup> - 3<sup>rd</sup>), late instars (4<sup>th</sup> - 5<sup>th</sup>), adult females, and adult males.

For each stage, 10 individuals, previously starved for 8 h, were introduced into the center of the cage. The number of individuals on each fruit treatment 24 h later was counted.

In experiments 2 and 3, the cage was divided into four corner sections. One fruit of each species/type (treatments) was placed in the bottom of one of the four corners of the cage (one corner for each treatment). Ten individuals of late stage (4<sup>th</sup> - 5<sup>th</sup>) nymphs or adult females, previously starved for 8 h, were introduced into the center of the cage. Each experiment was replicated eight times. The replication and treatment rotation schemes ensured that each treatment was located in each of the four corners of the cage twice. The number of individuals on each treatment was counted at 1, 6, 12, and 24 h after the insects were released into the cage.

Data obtained from the three experiments were first normalized by using the square-root transformation ( $\sqrt{x + 0.5}$ ). Significant differences in the number of individuals recorded on each treatment were established using one-way analysis of variance (ANOVA) followed by Tukey-Kramer honestly significant difference (HSD) comparison test ( $P < 0.05$ , JMP Version 7.01, SAS Institute 2007).

### **Development of *L. zonatus* on Satsuma Fruit**

The life history parameters of *L. zonatus* on satsuma fruit were determined under laboratory conditions [ $25 \pm 2$  °C,  $50 \pm 10$  % RH and a photoperiod of 14:10 (L: D) h], to evaluate the suitability of the fruit as host for the pest. Since it is difficult to obtain large number of eggs of *L. zonatus* on satsuma fruit in the laboratory, egg masses freshly laid on leaves of lima beans seedlings were collected daily and placed (with the leaves) into a glass jar (18 long  $\times$  7.5 cm diameter), where they were allowed to hatch and molt into second instars. Each observation consisted of 15 - 24 eggs with five replications for a total of 94 eggs. The durations of the egg

stage and the first instars (both on lima bean) were recorded. Newly molted second instars were reared on satsuma fruit in a glass jar (dimensions above) until they emerged as adults. The number and stage of the nymphs were recorded daily until adult emergence. From these data the following life cycle parameters were computed: development time for each stage, total development time from egg to adult, stage-specific survivorship (survival of the different stages), and cumulative survivorship (calculated by dividing the total number of individuals observed at each stage (survivors) by the total number of eggs observed at the beginning of the experiment = 94 eggs). Newly emerged adults were sexed and the data used to calculate sex ratio, and then placed in pairs (male and female) in a glass jar (dimensions above) and reared on satsuma fruit at the above stated conditions. Twelve pairs of adults were observed and the following data were recorded: pre-oviposition time, oviposition period, longevity, female fecundity (mean number of eggs oviposited), and egg hatch rate (%). Data were checked for normality and then subjected to one-way ANOVA followed by Tukey-Kramer HSD test ( $P < 0.05$ , JMP Version 7.01, SAS Institute 2007), to determine significant differences in developmental time among the different stages, and stage-specific survivorship. Significant sexual differences in adult longevity were established using Student's  $t$  test ( $P < 0.05$ , JMP Version 7.01, SAS Institute 2007).

## RESULTS

### **Host Preference of *L. zonatus***

When simultaneously presented with the different fruit species (tomato, satsuma, peach, and lemon) in the three multiple choice experiments, nymphs and adults of *L. zonatus* were always found in greater numbers on tomato than on the other fruit species. In experiment 1, significantly higher numbers of *L. zonatus* of all stages were recorded on tomato compared to the

other four treatments, 24 h after insects were released in the cage (early nymphs:  $F = 18.14$ ;  $df = 4,10$ ;  $P < 0.0001$ ; late nymphs:  $F = 6.55$ ;  $df = 4,10$ ;  $P = 0.007$ ; females:  $F = 11.88$ ;  $df = 4,10$ ;  $P = 0.0008$ ; males:  $F = 6.10$ ;  $df = 4,10$ ;  $P = 0.009$ ; Table 1). In general, no significant differences were recorded in the numbers of *L. zonatus* of all stages found on unripened versus ripened satsuma (Table 1).

In experiment 2, significantly more late stage nymphs were found on tomato (ripened, red) than on the remaining three fruit treatments (all ripened) at 6 h ( $F = 16.55$ ;  $df = 3, 28$ ;  $P = 0.0001$ ), 12 h ( $F = 46.4$ ;  $df = 3, 28$ ;  $P = 0.0001$ ), and 24 h ( $F = 51.8$ ;  $df = 3, 28$ ;  $P = 0.0001$ ) after the insects were released in the cage (Fig. 1A). Similarly, greater number of females were recorded on tomato (ripened, red) than on the remaining treatments at 6 h ( $F = 7.59$ ;  $df = 3, 28$ ;  $P = 0.0007$ ), 12 h ( $F = 46.01$ ;  $df = 3, 28$ ;  $P = 0.0001$ ), and 24 h ( $F = 84.15$ ;  $df = 3, 28$ ;  $P = 0.0001$ ) after insect release (Fig. 1B). The next most attractive fruit species was satsuma, on which was recorded greater numbers of nymphs and adult females compared to peach and lemon, at 24 h after insect release (Figs.1A and B). In experiment 3, unripened (green) tomato was tested to determine whether the attraction of *L. zonatus* to tomato documented in experiments 1 and 2 is related to its deep red color. The results were generally similar for both the nymphs and adult females. The numbers of *L. zonatus* found on unripened tomato were greater than the numbers found on each of the remaining three fruit treatments (all ripened) at 6 h ( $F = 5.5$ ;  $df = 3, 28$ ;  $P = 0.0042$ ), 12 h ( $F = 89.72$ ;  $df = 3, 28$ ;  $P = 0.0001$ ), and 24 h ( $F = 25.9$ ;  $df = 3, 28$ ;  $P = 0.0001$ ) after insect release (late stage nymphs, Fig. 2A), and at 6 h ( $F = 7.7$ ;  $df = 3, 28$ ;  $P = 0.0001$ ), 12 h ( $F = 12.03$ ;  $df = 3, 28$ ;  $P = 0.0001$ ), and 24 h ( $F = 34.4$ ;  $df = 3, 28$ ;  $P = 0.0001$ ) after insect release (adult females, Fig. 2B). No significant differences were recorded at 1 h after insect release. As recorded in the second experiment, the numbers of nymphs and adult females recorded on

satsuma, 24 h after insect release, were significantly greater than the numbers recorded on peach or lemon.

### **Development of *L. zonatus* on Satsuma Fruit**

***Basic Description of the Stages.*** The egg masses of *L. zonatus* are golden brown color, cylindrical and flattened, and laid in a straight line on the hosts. An egg mass contained 19.4 eggs on average (range: 15-32 eggs). Five nymphal stages (instars) were recorded. First stage nymphs typically congregate under leaves (lima bean seedlings) or on the fruit (fruit crops), and feeding was initiated by the second stage nymphs. The nymphs were bright red and do not have wings.

***Immature Development and Survival.*** Under our rearing conditions an egg took 14.7 days to hatch. The mean developmental times for the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, and 5<sup>th</sup> instars were 3.5, 5.1, 5.9, 9.1, and 11.4 days, respectively (Table 2). Total developmental time from eggs through the fifth instars was ~ 50 days (Table 1). Development time of early instars was significantly shorter compared to the eggs and late instars ( $F = 490.11$ ;  $df = 5, 465$ ;  $P = 0.0001$ ). High survivorship was recorded for all the stages but the fourth instars had 100% survivorship. Cumulative survivorship from eggs through the fifth instars was 75.6 % (Table 2).

***Adult Survival and Reproduction.*** Mean pre-oviposition period was 13 days, and eggs were laid over ~39 days (Table 3). Mean female longevity (72.6 days) was significantly greater than mean male longevity (57 days) ( $t = 2.45$ ;  $df = 1, 11$ ;  $P = 0.039$ ). Percentage female emergence was 49.3%, which resulted in a female: male sex ratio of 1:1.03. Mean fecundity was ~39 eggs per female, with a hatch rate of ~ 98 % (Table 3).

## DISCUSSION

The results from the three multiple choice experiments indicated that tomato was the most preferred by *L. zonatus* of all tested fruit species, with satsuma a distant second. *L. zonatus* and related species are polyphagous insects with a wide host range. Schaefer and Mitchell (1983) listed host plants in 28 families for *L. phyllopus*, and three families for *L. zonatus*. Leaffooted bugs are economic pests of important crops including maize (Schaefer et al. 2000, Panizzi 2004), sorghum (Hall et al. 1982, Matrangolo et al. 1994), cotton (Essig 1926), and have recently emerged as key pests of satsuma mandarin (Henne et al. 2003, Fadamiro et al. 2007, 2008) and other fruit and vegetable crops in the U.S. Some species of plant bugs in the family Coreidae are known to feed preferentially on vegetative tissues like shoots, petioles and foliages, whereas others preferentially attack reproductive organs (Schaefer and Mitchell 1983). *L. zonatus* feed primarily on fruits and developing seeds, but also feed occasionally on vegetative tissues for moisture uptake (Schaefer and Mitchell 1983). In contrast, the principal host of *L. phyllopus* is thistles, *Cirsium* spp (Hubbard 1885). We are not aware of any previous studies on host preference in leaffooted bugs, and in particular studies which compared the fruit species tested in our study.

Our results are in agreement with observations on the field ecology and migration pattern of leaffooted bugs in the Gulf Coast region. Adults typically migrate from adjacent/nearby crop fields (e.g., cotton, tomatoes, watermelons, etc) and miscellaneous non crop hosts into citrus groves and other tree fruit crops at the time of blooming to feed on opening buds or tender shoots, and also later in the season when the fruits start to ripen (Ziegler and Wolfe 1961, personal observation). However, the movement pattern and seasonal abundance of leaffooted bugs in different host crops remain largely unquantified in the region.

The results also showed that the relatively greater attraction of *L. zonatus* to tomato is not due mainly to color but may be mediated by chemical cues, possibly via olfaction or taste. Our observations also showed that tomato was almost always the first fruit species selected by both nymphs and adult females, further suggesting that olfaction is likely the main mechanism behind their preference for tomato. Host chemicals play a key role in mediating host preference in herbivorous insect species, most of which use host secondary chemicals (semiochemicals) as kairomones for host location (Duffey 1980, Brown 1984, Pasteels et al. 1988, Bowers 1990; Rank et al. 1998). Host biochemistry is dominant in the coevolution of plants and herbivorous insects, and thus in determination of diet breadth (Ehrlich and Murphy 1988). Our results suggest that *L. zonatus* may initially select a host based on chemical characteristics rather than morphological characteristics. The similarities in the data obtained for unripened versus ripened fruit of tomato and satsuma suggest that the chemical cues mediating attraction of *L. zonatus* to both fruit species also occur in unripened fruits, and that fruit maturity did not have a major effect on our results. Furthermore, results of an ongoing field study have demonstrated significant attraction and aggregation of *L. zonatus* on some noncrop plants in the family Solanaceae, a family which also includes tomato, suggesting that the chemical cues mediating this attraction are shared by members of this plant family. If confirmed, highly attractive noncrop Solanaceous plants could potentially be used as trap plants or sentinel monitoring plants for leaf-footed bugs in fruit orchards and other economically important crops. Further studies including field tests are necessary to confirm our results and to identify the semiochemicals (kairomones) in tomato and satsuma that mediate the observed attraction of *L. zonatus* to both fruit crops.

This study demonstrated that *L. zonatus* can survive, develop and reproduce on a diet consisting solely of satsuma fruit with no access to free water or supplementary food resources. Under laboratory conditions, Total developmental time from egg through the fifth stage was ~50 days. High survivorship was recorded for all the immature stages ranging from 100% for fourth instars to 89.1% for first instars. A few studies have examined the development of *L. zonatus* on other crops or artificial diets (Panizzi 1989, Matrangolo and Waquil 1994, Jackson et al. 1995, Grimm and Somarriba 1999). Many of our data are similar to those reported in the above studies but with some key differences. For instance, the total developmental time for the nymphal stages recorded on satsuma in this study (36.4 days) is much longer than the 28.7 days on maize and 31.6 days on sorghum reported by Matrangolo and Waquil (1994), or the 25.6 days recorded on physic nut (Grimm and Somarriba 1999). On the other hand, the 23.8 % mortality rate which we recorded for the nymphal stage on satsuma was much lower than the 53.8% and 55.1% recorded on maize and sorghum, respectively (Matrangolo and Waquil 1994), or the 59.7% mortality recorded on physic nut (Grimm and Somarriba 1999). The differences between our data and those previously reported may be related to differences in experimental conditions, and may not truly reflect the suitability of the different crops as hosts for *L. zonatus*.

Female and male *L. zonatus* fed on satsuma fruit lived an average of 73 and 57 days, respectively, which are similar to the ~ 71 and ~ 54 days recorded on sorghum (Matrangolo and Waquil 1994), but shorter than the ~ 87 and 84 days recorded on physic nut, respectively (Grimm and Somarriba 1999). However, the ~39 eggs deposited per female on satsuma was less than the 96 eggs laid on sorghum (Matrangolo and Waquil 1994), the 229 eggs on physic nut (Grimm and Somarriba 1999), and the 348 eggs deposited on a meridic diet (Jackson et al. 1995).

Altogether, these results suggest that satsuma fruit is an excellent host for development of *L. zonatus*, but not an optimal host for oviposition. The reduced oviposition on satsuma relative to those reported on other crops may be related to the waxy texture of the fruit, or to other yet unknown factors. Further studies are necessary to investigate the basis for the reduced oviposition by *L. zonatus* on satsuma and to compare oviposition and development on other fruit crops, such as tomato and peaches. Despite the lower number of eggs deposited on satsuma fruit in this study, our data, including the near 1:1 sex ratio of emerged adults recorded in this study, suggest that high populations of biologically fit *L. zonatus* can be maintained on this fruit crop.

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**Table 1. Mean ( $\pm$  SE) number of *L. zonatus* of different stages recorded on various fruit species after they were released in multiple choice tests**

Stages	Tomato (ripened)	Satsuma (unripened)	Satsuma (ripened)	Kumquat (ripened)	Peach (ripened)
Early nymphs 2 <sup>nd</sup> -3 <sup>rd</sup>	6.3 $\pm$ 0.3a	0.0 $\pm$ 0.0b	1.0 $\pm$ 0.6b	0.3 $\pm$ 0.3b	0.0 $\pm$ 0.0b
Late nymphs, 4 <sup>th</sup> - 5 <sup>th</sup>	2.7 $\pm$ 0.6a	1.3 $\pm$ 0.0ab	0.6 $\pm$ 0.3ab	1.3 $\pm$ 0.0ab	0.0 $\pm$ 0.0b
Females	2.7 $\pm$ 0.0a	0.0 $\pm$ 0.0b	0.7 $\pm$ 0.3b	0.3 $\pm$ 0.3b	0.0 $\pm$ 0.0b
Males	1.7 $\pm$ 0.0a	0.0 $\pm$ 0.0b	1.0 $\pm$ 0.0ab	0.3 $\pm$ 0.3ab	0.3 $\pm$ 0.3b

Means ( $\pm$  SE) within the same row followed by different letters are significantly different ( $P < 0.05$ , Tukey-Kramer HSD test). For each stage, ten individuals were released per test and replicated three times.

**Table 2. Life history parameters of leaf footed bug, *L. zonatus*, (eggs and nymphs) on satsuma mandarin fruits under laboratory conditions**

Stage	Duration (days)	Total number observed (n)	Stage-specific survivorship (%)	Cumulative survivorship (%)
Eggs	14.7 ± 0.2a	92	97.8	97.8
1st	3.5 ± 0.1e	82	89.1	87.2
2nd	5.1 ± 0.2d	78	95.1	82.9
3rd	5.9 ± 0.2d	74	94.8	78.7
4th	9.1 ± 0.2c	74	100.0	78.7
5th	11.4 ± 0.7b	71	95.9	75.6

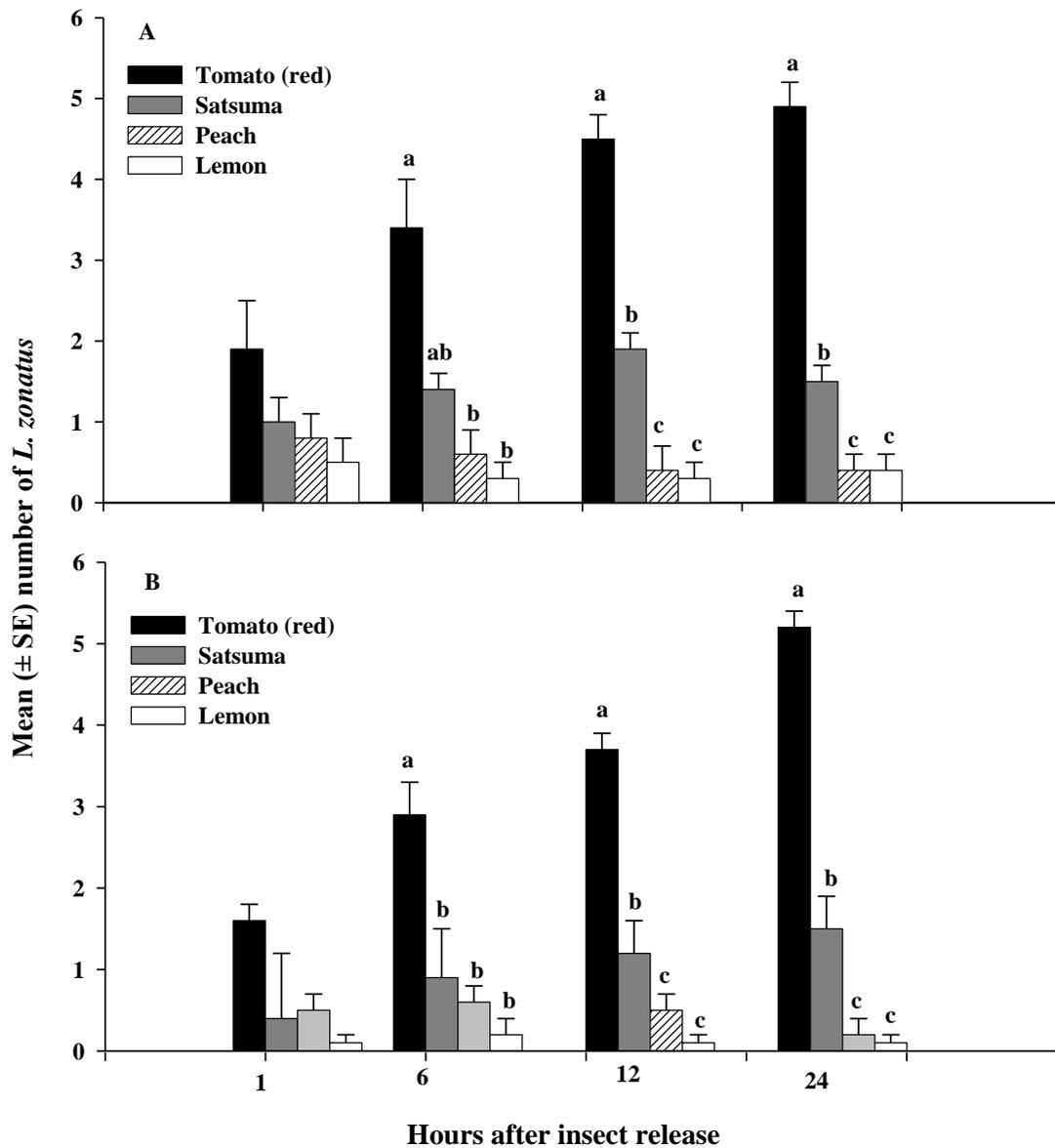
Means (± SE) within the same column followed by different letters are significantly different ( $P < 0.05$ , Tukey-Kramer HSD test). Cumulative survivorship was calculated by dividing the total number of individuals observed at each stage (survivors) by the total number of eggs observed at the beginning of the experiment (94 eggs).

**Table 3. Life history parameters of *L. zonatus* on satsuma mandarin fruit under laboratory conditions**

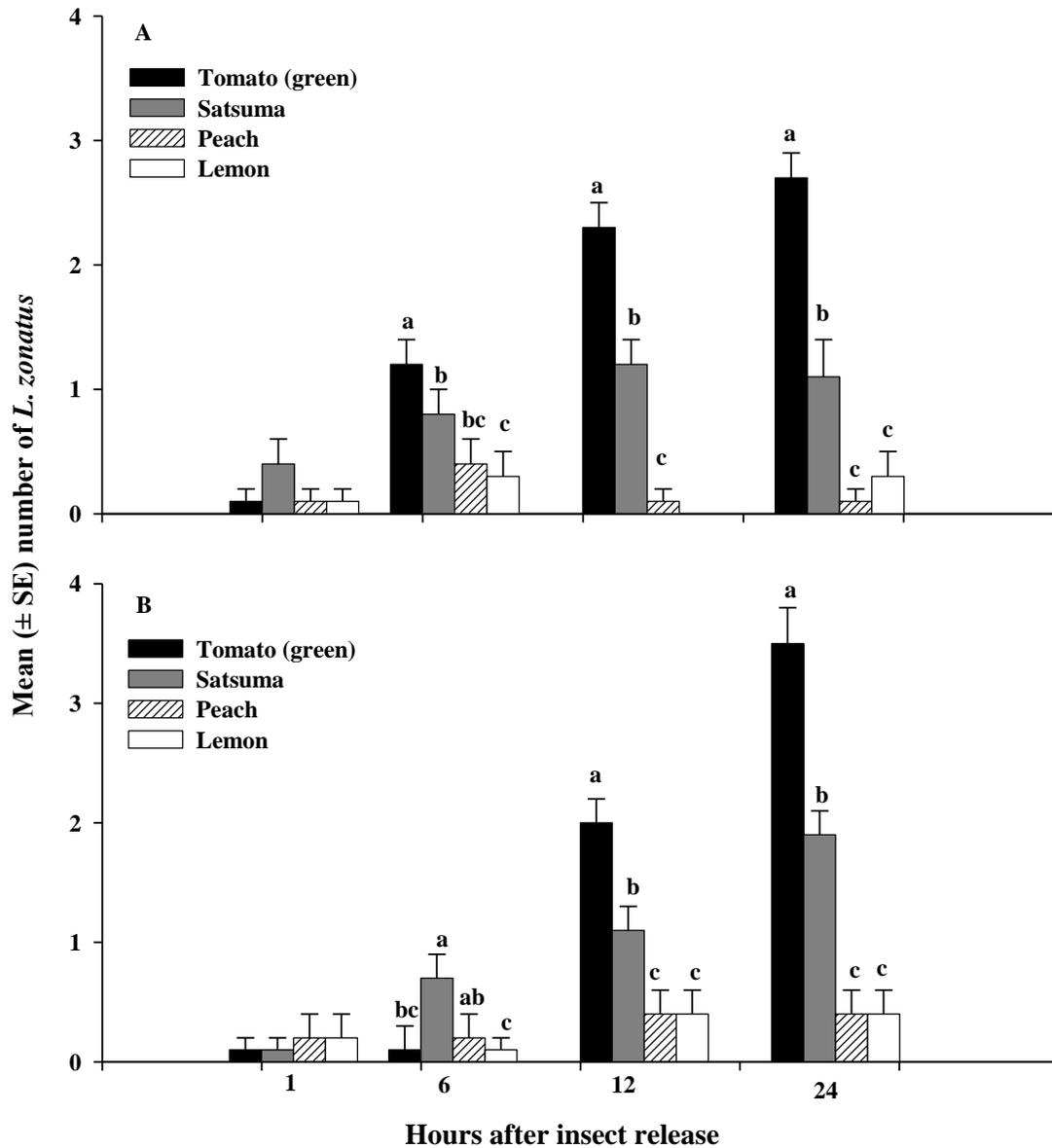
		Mean $\pm$ SE	Range
Female	Pre-oviposition (days)	13.0 $\pm$ 1.1	11.0 - 15.0
	Oviposition (days)	38.5 $\pm$ 4.5	12.0 - 64.0
	Fecundity (eggs/female)	38.8 $\pm$ 3.9	0.0 - 44.0
	Egg hatch rate (%)	98.3 $\pm$ 1.7	91.7 - 100.0
	Female emergence (%)	49.3 $\pm$ 2.0	41.0 - 53.0
Female	Longevity (days)	72.6 $\pm$ 8.0a	19.0 - 125.0
Male	Longevity (days)	57.0 $\pm$ 6.5b	21.0 - 93.0

Significant sexual difference in longevity established using *t* - test ( $P < 0.05$ )

**Figure 1.** Mean ( $\pm$  SE) of *L. zonatus* nymphs (A) and adult females (B) recorded on different fruit species (all ripened) at different periods after they were released in a multiple choice bioassay. Ten individuals were released per test and replicated eight times. Means for the same period having different letter in common are significantly different ( $P < 0.05$ , Tukey-Kramer HSD test).



**Figure 2.** Mean ( $\pm$  SE) of *L. zonatus* nymphs (A) and adult females (B) recorded on unripened tomato versus ripened satsuma, peach and lemon at different periods after they were released in a multiple choice bioassay. Ten individuals were released per test and replicated eight times. Means for the same period having different letter in common are significantly different ( $P < 0.05$ , Tukey-Kramer HSD test).



## CHAPTER 3

### EVALUATION OF DAMAGE TO SATSUMA MANDARIN, *CITRUS UNSHIU*, BY THE LEAFFOOTED BUG, *LEPTOGLOSSUS ZONATUS*, (HEMIPTERA: COREIDAE)

#### INTRODUCTION

Satsuma mandarin, *Citrus unshiu* Marcovitch, has been grown for over a century in the Gulf Coast region of the United States (English and Turnipseed 1940). However, the last decade has recorded a major increase in the production of this crop in southern Alabama, fueled by improved production and tree protection methods, and strong industry and state support (Campbell et al. 2004). Recent studies have identified several pest species capable of limiting the expansion of satsuma mandarin production in the region (Henne et al. 2003, Fadamiro et al. 2007, 2008). In particular, two species of leaffooted bugs (Hemiptera: Coreidae), *Leptoglossus zonatus* (Dallas) and *L. phyllopus* (L.), were identified as key direct pests of satsuma with the potential for major economic losses (Henne et al. 2003, Fadamiro et al. 2008). In southern Alabama, the predominant *Leptoglossus* spp. on satsuma is *L. zonatus* (Dallas), whereas *L. phyllopus* (L.) is considered a minor pest (Fadamiro et al. 2008).

Like many other *Leptoglossus* species, *L. zonatus* has recently risen in status as a pest of a wide range of crops in the southern U.S., including cotton, tomato, eggplant, sorghum, watermelons, corn, peaches, citrus, pecans (Albrigo and Bullock 1977, Matrangolo and Waquil

1994, Johnson and Allain 1998, Schaefer and Panizzi 2000, Buss et al. 2005). In southern Alabama, *L. zonatus* adults typically move from crop fields (e.g., cotton, tomatoes, watermelons, etc) into adjacent or nearby satsuma orchards in the fall when the fruits start to ripen (personal observation). A recent study by our group suggests that satsuma fruit is a suitable host for *L. zonatus*, which can maintain modest to high populations of the pest, but tomato is preferred over satsuma (Xiao and Fadamiro in press). Feeding on citrus and other fruit by leaf-footed bug nymphs and adults can result in reduction in quality and quantity of fruits, including premature color break, fruit drop, and unmarketable fruit (Ebeling 1950, Griffiths and Thompson 1957, Albrigo and Bullock 1977, Grimm 1999, Henne et al. 2003). The insects can also transmit yeast disease pathogen, *Nematocera coryli* Peglion, to fruit, which causes dryness in the affected wedges of the fruit and produces bad fruit flavor (Henne et al. 2003). However, much of the background information necessary for effective management of *L. zonatus* on satsuma is missing, including the absence of a systematic assessment of injury and economic thresholds for *L. zonatus* on satsuma. The objectives of this study were to 1) characterize interior and exterior damage symptoms on satsuma fruit caused by *L. zonatus*; 2) determine relationship between insect density, feeding duration and fruit damage; and 3) propose an economic injury level for *L. zonatus* on satsuma based on the data from the above laboratory and field studies. It is hoped that the results will support the development of an effective integrated pest management program for managing *L. zonatus* on satsuma mandarin.

## MATERIALS AND METHODS

### Rearing of *L. zonatus*

Adults of *L. zonatus* collected from satsuma orchards in south Alabama in the fall of

2007 were used to start laboratory colonies. Adults were reared in wooden sleeve cages ( $60 \times 40 \times 30$  cm; 5 pairs per cage) with screened walls, and provided lima bean seedlings (3 potted plants per cage), fresh mature satsuma fruits (3 per cage), fresh tomato (3 per cage) and water. The cages were checked daily to collect freshly laid eggs and to replace diet, as necessary. Lima bean seedlings were replaced monthly, whereas satsuma and tomato fruits were replaced biweekly and weekly, respectively. Satsuma fruits used were harvested from the field and stored in the refrigerator until use (fruits stored for up to 2 months). The rearing conditions were  $25 \pm 2$  °C,  $50 \pm 10\%$  RH, and a photoperiods of 14:10 (L: D) h.

### **Laboratory Tests**

Damage to satsuma fruit by *L. zonatus* was evaluated under laboratory conditions [ $25 \pm 2$  °C,  $50 \pm 10\%$  RH, and a photoperiod of 14:10 (L: D) h] during fall 2008. Fresh, mature satsuma fruits were weighed and placed individually into glass jars (18 cm long  $\times$  7.5 cm diameter, 1 fruit per jar). Newly-emerged *L. zonatus* females (2-5 days old) were introduced into each jar at one of four densities: 0 (control), 1, 2, or 3 per fruit. The jar was covered with screening nets. The set up consisted of a total of 48 jars distributed into four groups of 12 jars for each insect density. The fruit was weighed at 1 (12 replications), 3 (12 replications), 7 (9 replications), 10 (6 replications), and 14 (3 replications) days after infestation. At 3, 7, 10, and 14 days after infestation, three fruits were selected from each group and dissected to determine interior damage. Fruit weight loss was calculated by comparing weight loss before and after infestation. The percentage of damaged sections (PDS) was calculated for each fruit by dividing the number of damaged sections by the total number of sections (usually 10 sections). The soluble solids content (SSC) of the fruit was then measured with a refractometer (Westover model RHB-

32ATC). Similar experiments were performed with *L. zonatus* males and late (4<sup>th</sup> - 5<sup>th</sup>) instars. Early instars (2<sup>nd</sup> - 3<sup>rd</sup>) were not tested based on a preliminary experiment which showed that they produce very little measurable damage to satsuma.

## **Field Tests**

Field experiments were performed in two satsuma orchards located in Baldwin county, southern Alabama during 2007 (preliminary trial) and 2008: Gulf Coast Research and Extension Center (GCREC) orchard and Coker orchard. Both orchards were comprised mainly of satsuma mandarin (Owari variety) and were not conventionally sprayed during the trials. For the 2007 trial, three premature satsuma fruits on a tree branch were randomly selected and covered with a cage (35 x 15 cm diameter) made from white color screening net. *L. zonatus* females were placed into the cages at an approximate density of 0 (control), 0.3, 0.7, 1.4, or 2 individuals per fruit for a period of 14 days. Each insect density treatment was replicated four times (4 cages) per orchard. On day 15, the fruits were harvested (by hand) and carefully transported (in plastic bag kept in ice) to the laboratory where they were weighed and inspected for external and interior damage. A similar methodology was used for the 2008 trial but with some modifications. Two premature fruits were caged (instead of 3 in 2007) and *L. zonatus* were placed in the cages at a density of 0, 0.5, 1, 2 or 3 individuals per fruit. Also both females and males were tested separately in 2008. Each insect density treatment was also replicated four times (4 cages) per orchard. In both years, fruits were assessed for damage by collecting the following data: fruit color, premature fruit abortion (PFA), fresh fruit weight (FFW), percentage of damaged sections (PDS), and soluble solids contents (SSC).

## **Statistical Analysis**

For the laboratory test, fruit weight loss was calculated as the difference in fruit weight before and after infestation. Fruit weight loss (FWL) data were converted to percentages and used in the statistical analyses. Data on damage fruit sections (PDS) and soluble solids contents (SSC) were also expressed as percentages. All data were first normalized by using the arcsine square-root transformation ( $\sqrt{x + 0.5}$ ). Laboratory data (FWL, PDS, and SSC) were first analyzed with two-way analysis of variance (ANOVA) with insect density and feeding duration (days after infestation) as the main factors. Data collected on day 14 after infestation were further analyzed with one-way ANOVA to test for effect of insect density on FWL, PDS and SSC. Field data (premature fruit abortion or PFA, fresh fruit weight or FFW, PDS, and SSC) collected from each orchard on day 15 after infestation were analyzed with one-way ANOVA using insect density as the independent variable. In all tests, data for the different stages (females, males or nymphs) were analyzed separately. Significant differences between means were established by using Tukey-Kramer honestly significant difference (HSD) comparison test ( $P < 0.05$ , JMP Version 7.01, SAS Institute Inc. 2007).

## RESULTS

### Description of Fruit Damage

*L. zonatus* pierced satsuma fruit skin with its mouthparts and sucked fluids directly from the underlying vesicles. A “green island” on outer rind (Fig. 1A) and necrosis on inner rind (Fig. 1B) often appeared on injured satsuma fruits. Dark spots were also observed on some injured fruits. Feeding may also result in interior fruit damage including collapsing and drying out of the juice vesicles (Fig. 1C). Premature ripening (yellowing) of infested fruit was also observed in the

field tests. The symptoms expressed by fruit infested by *L. zonatus* females, males or nymphs were identical in appearance.

### **Laboratory Tests**

For *L. zonatus* females, two-way ANOVA revealed significant effects of insect density and feeding duration (days after infestation) on all three parameters evaluated: fruit weight loss (FWL), percentage of damaged sections (PDS), and soluble solids content (SSC), as well as a significant density  $\times$  duration interaction on PDS and SSC (Table 1). Insect density and feeding duration each exerted a significant positive effect on FWL and PDS (increase fruit damage), but a negative effect on SSC (decline Soluble solids content). In other words, FWL and PDS increased with increasing insect density and feeding duration (Fig.2), while SSC decreased with both factors. Similar results were obtained for *L. zonatus* males and nymphs. The statistics for both sexes and the nymphs are shown in Table 1. For brevity, data obtained on day 14 of infestation (feeding duration of 14 days) were analyzed by one-way ANOVA and presented in Table 2. In general, FWL and PDS increased with increasing insect density and feeding duration, while SSC decreased with both factors. For instance, FWL increased from 19.6 to 24.3% and PDS from 3 to 71%, whereas SSC decreased from 8.4 to 6.3% at densities of 1 female/fruit and 3 females/fruit, respectively (Table 2). The data also indicated that slightly higher fruit damage (FWL and PDS) were caused by *L. zonatus* females or males than by the nymphs (Fig. 3), although no statistical comparisons were made since the data for the different stages were not collected at the same time.

## Field Tests

Feeding by *L. zonatus* females or males caused notable damage to satsuma fruits as determined by fruit color, premature fruit abortion (PFA), fresh fruit weight (FFW), percentage of damaged sections (PDS), and soluble solids contents (SSC). In the 2007 trial, only females were evaluated at both orchards. Analysis of the data collected at Coker orchard showed that female density had a significant positive effect on PFA (increased fruit abortion) ( $F = 23.3$ ;  $df = 4, 55$ ;  $P < 0.0001$ ) and PDS (increased percentage of damage) ( $F = 118.2$ ;  $df = 4, 55$ ;  $P < 0.0001$ ), but a negative effect on FFW ( $F = 19.6$ ;  $df = 4, 55$ ;  $P < 0.0001$ ) and SSC ( $F = 21.8$ ;  $df = 4, 55$ ;  $P < 0.0001$ ). Similar results were obtained at GCREC orchard: insect density had a significant positive effect on PFA ( $F = 33.4$ ;  $df = 4, 55$ ;  $P < 0.0001$ ) and PDS ( $F = 96.4$ ;  $df = 4, 55$ ;  $P = 0.0001$ ) and a negative effect on FFW ( $F = 3.33$ ;  $df = 4, 55$ ;  $P = 0.01$ ) and SSC ( $F = 96.3$ ;  $df = 4, 55$ ;  $P = 0.001$ ). Premature fruit yellowing was recorded at densities of 0.7 or more insects per fruit. In 2008, both females and males were evaluated at the two orchards and the results were generally similar to those obtained in 2007. At both orchards, female density had a significant positive effect on PFA and PDS, but a negative effect SSC (Table 3, Fig.4). Similar results were recorded also for males: increasing male density resulted in increased PFA and PDS and reduced SSC (Table 4). An effect of insect density on fruit color was also recorded at both locations and for both sexes. Premature fruit yellowing was recorded at densities of 1 or more insects per fruit, whereas fruits caged with  $\leq 1$  insects per fruit were still mainly green 14 days after infestation. However, no significant effect of insect density on fresh fruit weight (FFW) was recorded in 2008 (Tables 3 and 4). In general, no significant differences were recorded between the densities of 0 (control) and 0.5 insect per fruit for most damage parameters.

## DISCUSSION

The symptoms of *L. zonatus* feeding damage on satsuma fruit recorded in this study have been previously reported on satsuma or other citrus species (Ebeling 1950, Griffiths and Thompson 1957, Albrigo and Bullock 1977, Kubo and Filho 1992, Henne et al. 2003, Buss et al. 2005). Henne et al. (2003) reported external damage to the rind of satsuma fruit infested by *L. zonatus*, in the form of green spots that remain for a time after the rind has turned orange. *Leptoglossus* spp., have also been reported to cause similar damage to other crops (Wheeler and Miller 1990, Hall and Teetes 1982, Grimm 1999). The premature abortion of *L. zonatus*-infested fruit recorded in our field tests has also been documented in other citrus species (Griffiths and Thompson 1957; Albrigo and Bullock 1977), as well as in other crops (Ebeling 1950, Wiseman and McMillian 1971, DeBarr and Kormanik 1975, Bolkan et al. 1984, Grimm 1999). DeBarr and Kormanik (1975) reported that *L. corculus* (Say) nymphs fed on the cytoplasm of cells of the nucellar tissue of pine conelets resulting in total conelet abortion after only four days of feeding. Albrigo and Bullock (1977) suggested that citrus fruit drop from leaftooted bug feeding may be due to secondary infections by microorganisms. However, this hypothesis was not tested in the present study.

In addition to direct feeding injury, *L. zonatus* and other related species have also been reported to transmit the yeast, *Nematospora coryli*, which causes further fruit damage including drying rot (Clarke and Wilde 1970, Henne et al. 2003). Henne et al. (2003) attributed the collapsing and drying out of the juice sacs inside the rind of satsuma fruit infested by *L. zonatus* to this yeast. Although, we did not attempt to isolate this pathogen and other microorganisms, it is possible that some of the symptoms of internal fruit damage observed in this study were due to yeast or similar pathogens.

As expected, our results showed major effects of *L. zonatus* density and feeding duration on most fruit damage parameters. In general, higher insect density and longer feeding duration resulted in increased fruit weight loss and percentage of damaged fruit sections (PDS), but reduced soluble solids content (SSC). The results were fairly consistent between laboratory and field experiments although minor differences were recorded. This is the first systematic evaluation of damage by *L. zonatus* or related species to satsuma or other citrus species. Similar results have been reported for some non-citrus crops damaged by *Leptoglossus* spp. (Hall and Teetes 1982, Grimm 1999). Hall and Teetes (1982) attributed the significant reduction in the yield of sorghum infested by *L. phyllopus* to direct seed feeding. Similarly, physic nut damaged by *L. zonatus* also showed a significant reduction in fruit weight and a slight reduction in oil contents (Grimm 1999).

We were not surprised to find a negative correlation between the percentage of damaged sections (PDS) and soluble solids content (SSC) of satsuma fruit infested by *L. zonatus*. The SSC is commonly used as a measure of fruit juice quality (Widodo et al. 1996). Our data showed that a significant decline in SSC was not always recorded at densities lower than 2 individuals per fruit, suggesting that this parameter may not be a very reliable measure of *L. zonatus* feeding damage on satsuma. Also, SSC declined faster in the field trials than in the laboratory tests. For instance, the decrease in SSC was significant at the density of 1-2 individuals per fruit in the field trials, but a significant decrease in SSC was not recorded in the laboratory tests below the density of 3 individuals per fruit. These differences may be related to the hotter and unstable field conditions. In general, *L. zonatus* females and males produced similar amounts of damage, which were slightly more than the damage produced by the nymphs. Grimm (1999) reported a

similar finding on physic nut: *L. zonatus* adults of both sexes produced more damage than the nymphs.

One of the objectives of this study was to extrapolate the fruit damage data to possibly propose an economic threshold for *L. zonatus* on satsuma. *L. zonatus* feeding affected fruit weight, yield, and quality, but the data showed that not all feeding resulted in significant measurable damage. For example, compared to uninfested (control) fruits, a significant fruit weight loss was not recorded below the density of 1 individual per fruit or at feeding durations of less than 10 days. Hall and Teetes (1982) reported a similar result on sorghum and concluded that two adults of *L. phyllopus* were generally not enough to cause significant reduction in the yield of green sorghum. Data from the laboratory tests showed that fruit weight loss (FWL) and PDS values of uninfested control fruits were not significantly different from values for fruits infested by *L. zonatus* at a density of one individual per fruit, but significantly different from values for fruits infested at a density of two or more individuals per fruit. In contrast, PDS and premature fruit abortion were significantly greater at the density of one individual per fruit compared to the control. We also observed premature ripening of infested fruit at densities of one or more individuals per fruit. Similar results on premature fruit abortion were reported for pistachio fruits infested by *L. clypealis* L., which resulted in an increase in premature fruit abortion from 3.8% in the control to 32.9 % in fruit clusters fed upon for 48 hours by a single *L. clypealis* (Bolkan et al. 1984). However, Grimm (1999) cautioned against reliance on premature fruit abortion to diagnose economic damage by bugs, since fruit abortion may be caused by other factors which are unrelated to insect feeding. For instance, Umaña and Carballo (1995) reported that only 33-49 % of aborted macadamia nuts showed symptoms of bug feeding damage. Thus, our data on premature fruit abortion may not be used as a reliable indicator of economic damage

by *L. zonatus*. In general, our field results corroborated our laboratory data. The minor differences between our laboratory and field results may be related to differences in environmental conditions, as well as the impact of other field factors. Although our data are a little conflicting, they suggest an economic injury level (lowest insect density that will result in economic damage) of 1-2 *L. zonatus* per satsuma fruit. At present, it is difficult to accurately propose an economic or action threshold (insect density that will trigger control measures to prevent the density from reaching economic injury level) for *L. zonatus* on satsuma, due to several factors including lack of effective monitoring and sampling techniques and the inability to accurately predict its movement from adjacent crop fields into satsuma orchards. Knowledge of these factors and the economics of managing the pest are requisite to the development of a reliable and practical economic threshold for *L. zonatus* on satsuma mandarin.

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**Table 1. Two-way ANOVA testing for effects of insect density, feeding duration, and interactions of both factors on fruit weight loss (FWL), percent damaged sections (PDS), and soluble solid contents (SSC) of satsuma fruits infested by *Leptoglossus zonatus* in laboratory tests.**

Effect		Df	Females		Males		Nymphs	
			<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
FWL	Density	3	74.8	0.0001	37.1	0.0001	16.4	0.0001
	Duration	4	1424.8	0.0001	738	0.0001	599	0.0001
	Density × Duration	12	2.8	0.0016	1.55	0.112	0.59	0.84
PDS	Density	3	166.7	0.0001	62.9	0.0001	59.7	0.0001
	Duration	3	70.9	0.0001	39.0	0.0001	42.6	0.0001
	Density × Duration	9	35.1	0.0001	12.9	0.0001	14.2	0.0001
SSC	Density	3	7.4	0.0007	18.2	0.0001	25.7	0.0001
	Duration	3	4.9	0.006	9.1	0.0002	10.8	0.0001
	Density × Duration	9	1.4	0.217	1.2	0.34	4.1	0.001

Four levels of insect density (0, 1, 2 or 3 per fruit) were evaluated at feeding durations of 1, 3, 7, 10, and 14 days after infestation for FWL and at 3, 7, 10 and 14 days after infestation for PDS and SSC.

**Table 2. Effect of insect density on fruit weight loss (FWL), percent damaged section (PDS), and soluble solid contents (SSC) of satsuma fruits infested by *Leptoglossus zonatus* for 14 days in laboratory tests.**

Stage	Density (insect no. per fruit)	Mean ( $\pm$ SE) per fruit		
		FWL (%)	PDS (%)	SSC (%)
Females	0.0	15.9 $\pm$ 0.3c	0c	8.7 $\pm$ 0.2a
	1.0	19.6 $\pm$ 1.2bc	3.0 $\pm$ 3.0c	8.4 $\pm$ 0.2a
	2.0	21.6 $\pm$ 0.5ab	17.2 $\pm$ 0.5b	8.1 $\pm$ 0.2a
	3.0	24.3 $\pm$ 1.3a	71.1 $\pm$ 4.4a	6.3 $\pm$ 0.7b
		$F = 13.84$ $P = 0.0015$	$F = 98.32$ $P = 0.0001$	$F = 2.86$ $P = 0.104$
Males	0.0	15.9 $\pm$ 1.5c	0c	8.5 $\pm$ 0.1a
	1.0	18.4 $\pm$ 0.4bc	5.8 $\pm$ 2.9bc	8.1 $\pm$ 0.1ab
	2.0	20.9 $\pm$ 0.9ab	23.3 $\pm$ 6.7b	7.8 $\pm$ 0.1ab
	3.0	23.3 $\pm$ 1.1a	63.1 $\pm$ 6.8a	6.7 $\pm$ 0.6b
		$F = 9.4$ $P = 0.005$	$F = 31.04$ $P = 0.0001$	$F = 4.8$ $P = 0.032$
Nymphs	0.0	14.4 $\pm$ 1.1b	0c	8.8 $\pm$ 0.2a
	1.0	17.3 $\pm$ 1.4ab	3.0 $\pm$ 1.5bc	8.7 $\pm$ 0.2a
	2.0	19.6 $\pm$ 0.6ab	14.4 $\pm$ 3.1b	8.3 $\pm$ 0.2a
	3.0	20.7 $\pm$ 1.3a	42.1 $\pm$ 4.1a	6.8 $\pm$ 0.4b
		$F = 5.71$ $P = 0.021$	$F = 39.11$ $P = 0.001$	$F = 12.36$ $P = 0.002$

Means ( $\pm$  SE) within the same column for each stage followed by different letters are significantly different ( $P < 0.05$ , Tukey-Kramer HSD test; df = 3, 8).

**Table 3. Evaluation of damage to satsuma fruit infested by *Leptoglossus zonatus* females in two south Alabama satsuma orchards in 2008**

Location	Density (insect no. per fruit)	Fruit Color	Mean ( $\pm$ SE) per fruit			
			PFA (%)	FFW (g)	PDS (%)	SSC (%)
Coker	0	G	0b	103.9 $\pm$ 3.5	0d	9.1 $\pm$ 0.2a
	0.5	G	0b	85.7 $\pm$ 3.1	3.6 $\pm$ 1.8d	8.7 $\pm$ 0.2a
	1	Y	87.5 $\pm$ 12.5a	97.8 $\pm$ 8.3	15.2 $\pm$ 2.3c	8.2 $\pm$ 0.2a
	2	Y	75.0 $\pm$ 16.2a	88.3 $\pm$ 7.7	31.9 $\pm$ 3.9b	6.0 $\pm$ 0.4b
	3	Y	100 $\pm$ 0a	80.8 $\pm$ 7.5	56.8 $\pm$ 6.8a	5.0 $\pm$ 0.2bc
			$F = 28.0$ $P < 0.0001$	$F = 2.14$ $P = 0.09$	$F = 41.34$ $P < 0.0001$	$F = 45.99$ $P < 0.0001$
GCREC	0	G	0c	113.2 $\pm$ 6.7	0d	9.4 $\pm$ 0.3a
	0.5	G	12.5 $\pm$ 6.5bc	105.7 $\pm$ 3.9	3.0 $\pm$ 1.7cd	8.6 $\pm$ 0.3a
	1	Y	75.0 $\pm$ 16ab	102.3 $\pm$ 1.3	11.0 $\pm$ 2.7c	7.1 $\pm$ 0.2b
	2	Y	62.5 $\pm$ 18.2a	106.7 $\pm$ 6.8	37.0 $\pm$ 2.4b	5.8 $\pm$ 0.3c
	3	Y	100 $\pm$ 0.0a	108.3 $\pm$ 6.0	71.0 $\pm$ 3.2a	5.1 $\pm$ 0.3c
			$F = 11.8$ $P < 0.0001$	$F = 0.512$ $P = 0.691$	$F = 122.38$ $P < 0.0001$	$F = 39.99$ $P < 0.0001$

Means ( $\pm$  SE) within the same column for each location followed by different letters are significantly different ( $P < 0.05$ , Tukey-Kramer HSD test;  $df = 4, 35$ ). GCREC = Gulf Coast Research and Extension Center, Fairhope, AL; G = green color, Y = yellow color; PFA = premature fruit abortion (%); FFW = fresh fruit weight (in grams); SSC = soluble solids content (%); PDS = damaged fruit sections (%).

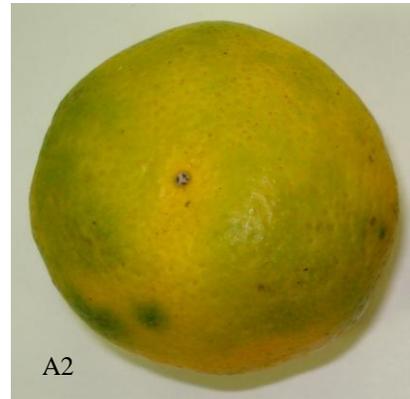
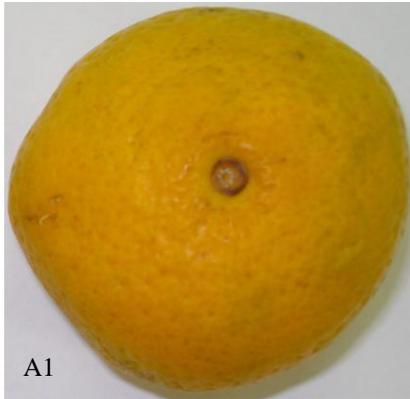
**Table 4. Evaluation of damage to satsuma fruit infested by *Leptoglossus zonatus* males in two south Alabama satsuma orchards in 2008**

Location	Density (insect no. per fruit)	Fruit Color	Mean ( $\pm$ SE) per fruit			
			PFA (%)	FFW (g)	PDS (%)	SSC (%)
Coker	0	G	0c	100.7 $\pm$ 4.3	0d	9.5 $\pm$ 0.3a
	0.5	G	0c	90.8 $\pm$ 2.3	2.1 $\pm$ 1.4d	8.6 $\pm$ 0.1a
	1	Y	37.5 $\pm$ 18.2bc	90.8 $\pm$ 4.5	17.2 $\pm$ 4.5c	6.8 $\pm$ 0.5b
	2	Y	50.0 $\pm$ 18.9b	94.9 $\pm$ 4.8	34.3 $\pm$ 2.3b	6.3 $\pm$ 0.4b
	3	Y	100 $\pm$ 0.0a	104.5 $\pm$ 3.9	64.8 $\pm$ 2.7a	5.3 $\pm$ 0.5b
			<i>F</i> = 12.4 <i>P</i> < 0.0001	<i>F</i> = 2.36 <i>P</i> = 0.07	<i>F</i> = 99.37 <i>P</i> < 0.0001	<i>F</i> = 18.37 <i>P</i> < 0.0001
GCREC	0	G	0c	101.0 $\pm$ 2.1	0c	8.9 $\pm$ 0.3a
	0.5	G	0c	99.8 $\pm$ 1.9	1.4 $\pm$ 0.7c	8.4 $\pm$ 0.2a
	1	Y	50.0 $\pm$ 18.8b	100.7 $\pm$ 2.2	8.1 $\pm$ 2.6c	6.9 $\pm$ 0.3b
	2	Y	87.5 $\pm$ 12.5a	100.9 $\pm$ 3.6	34.5 $\pm$ 4.1b	6.9 $\pm$ 0.5b
	3	Y	100 $\pm$ 0.0a	91.6 $\pm$ 3.1	65.9 $\pm$ 4.1a	5.0 $\pm$ 0.3c
			<i>F</i> = 21.6 <i>P</i> < 0.0001	<i>F</i> = 2.34 <i>P</i> = 0.09	<i>F</i> = 89.13 <i>P</i> < 0.0001	<i>F</i> = 27.12 <i>P</i> < 0.0001

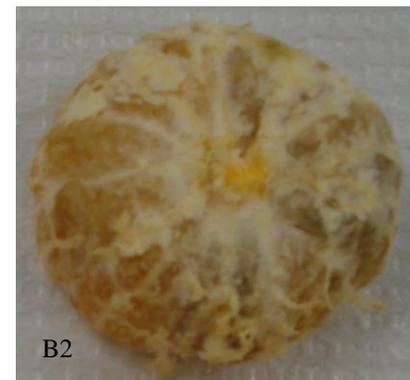
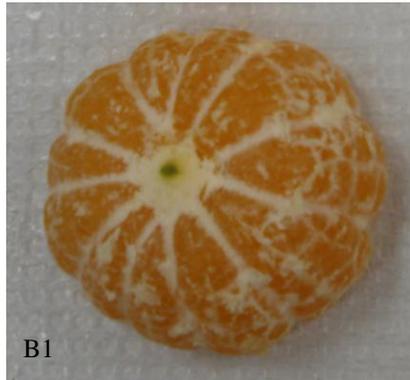
Means ( $\pm$  SE) within the same column for each location followed by different letters are significantly different ( $P < 0.05$ , Tukey-Kramer HSD test;  $df = 4, 35$ ). GCREC = Gulf Coast Research and Extension Center, Fairhope, AL; G = green color, Y = yellow color; PFA = premature fruit abortion (%); FFW = fresh fruit weight (in grams); SSC = soluble solids content (%); PDS = % damaged fruit sections.

**Figure 1.** Damage symptoms on satsuma fruits infested by *L. zonatus*. **A** (external damage): control fruit showing no damage (A1), “green islands” on outer rind of infested fruit (A2); **B** (necrosis): control fruit showing no necrosis (B1), necrosis on inner rind of infested fruit (B2); **C** (internal damage): control fruit showing no internal damage (C1), infested fruit showing dried-out juice sacs (C2).

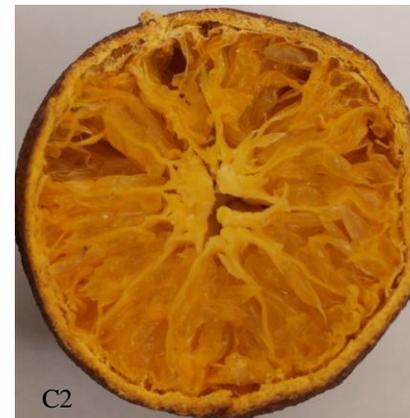
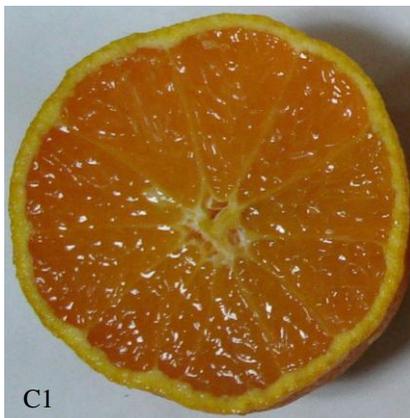
A



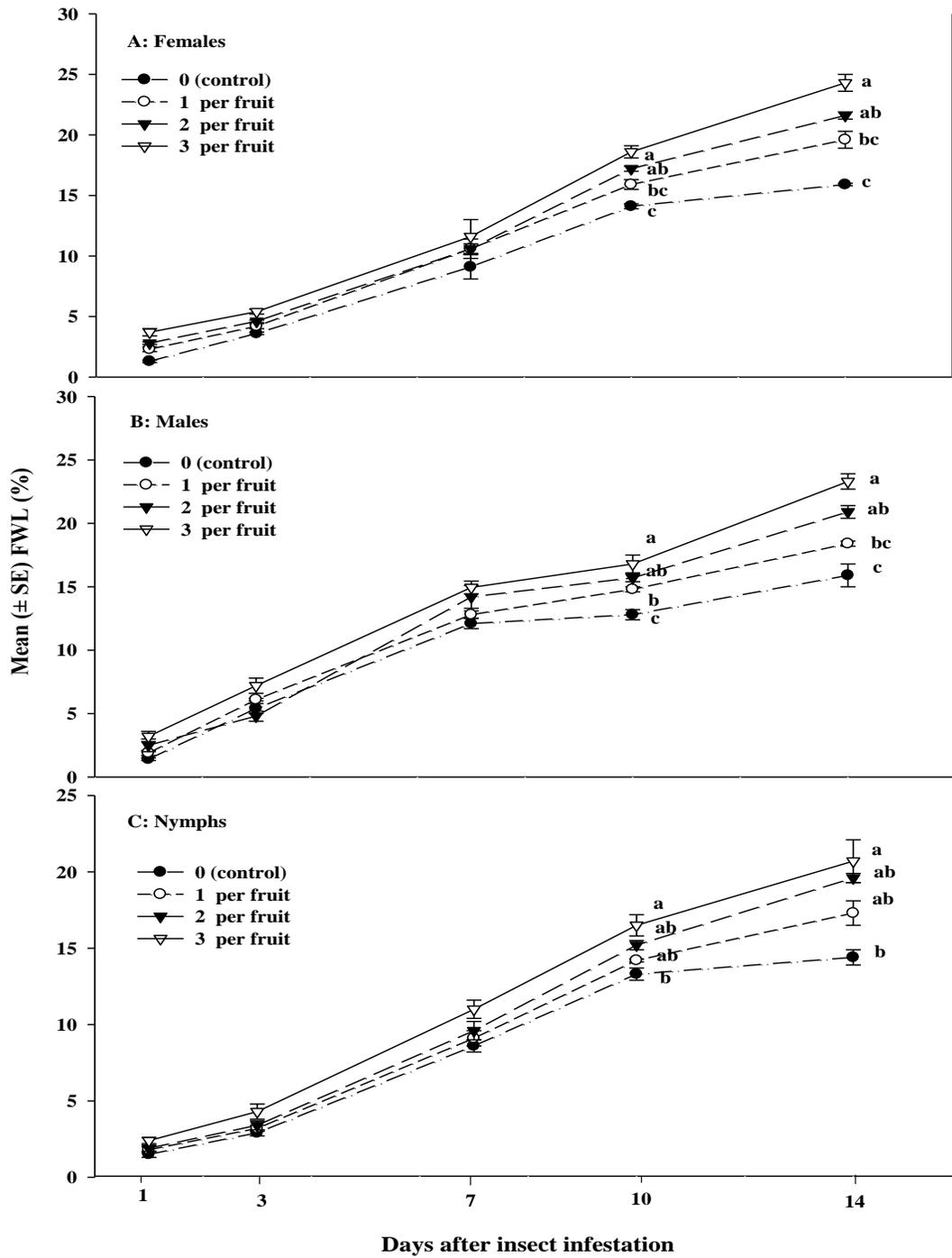
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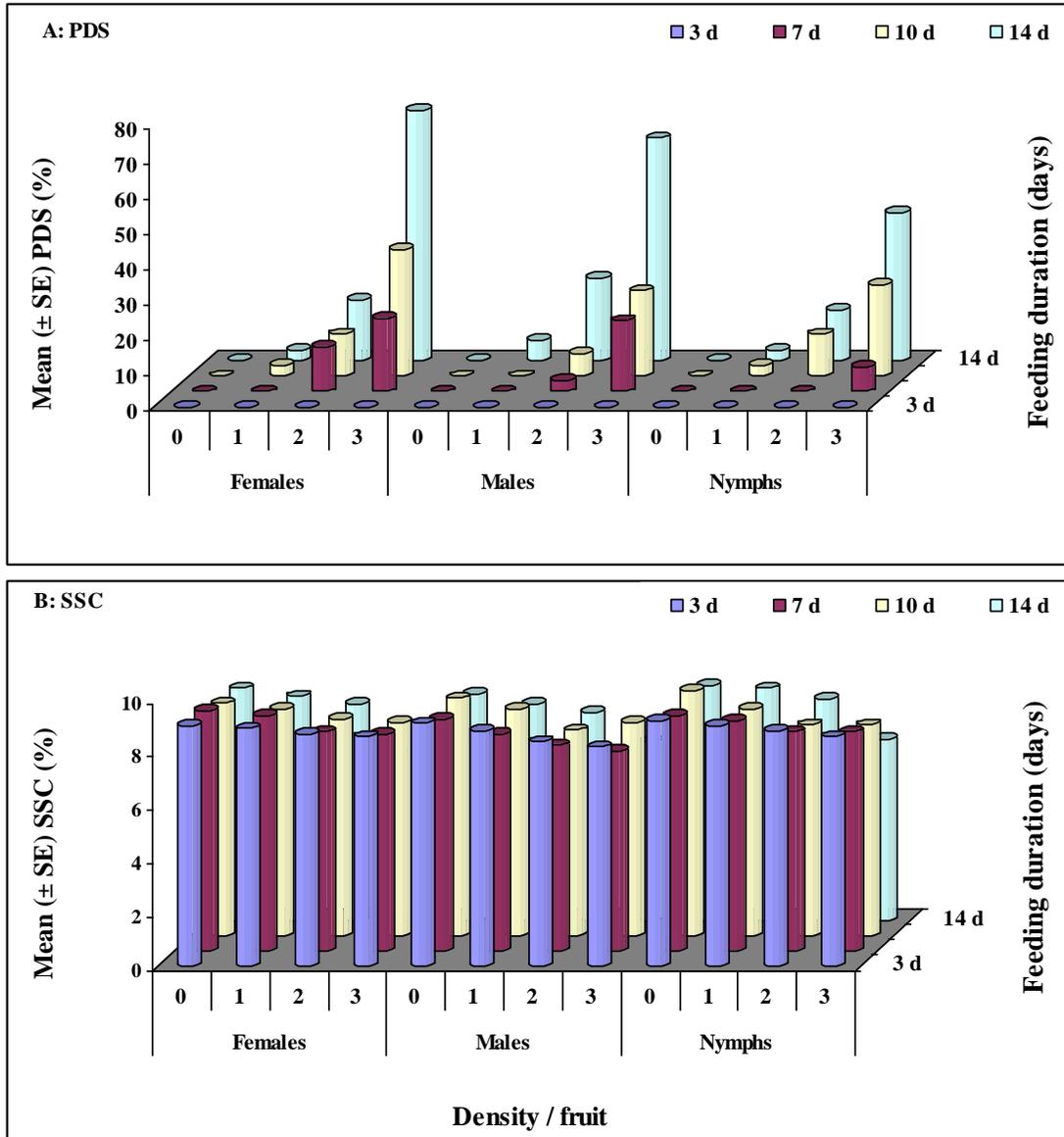
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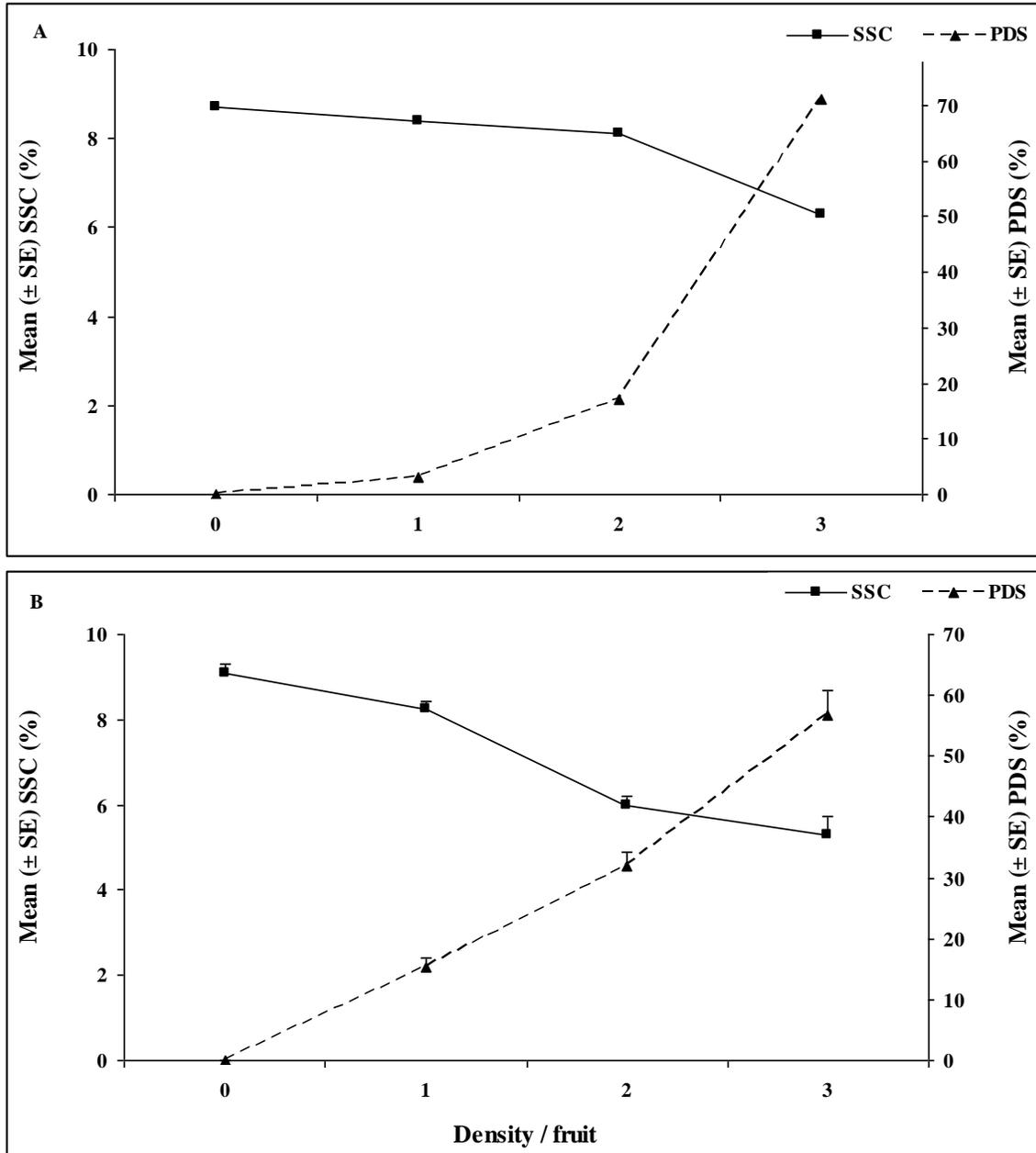
**Figure 2.** Effects of insect density and feeding duration on percent fruit weight loss (% FWL) of satsuma fruits infested by *Leptoglossus zonatus* in laboratory tests in 2008. **A:** Females, **B:** Males; **C:** Nymphs. Figure shows mean ( $\pm$  SE) number of FWL (%) over different feeding periods.



**Figure 3.** Effects of insect density and feeding duration on (A) percent damaged sections (PDS) and (B) soluble solids content (SSC) of satsuma fruits infested by *L. zonatus* females in laboratory tests in 2008.



**Figure 4.** Negative correlation between percent damaged sections (PDS) and soluble solids content (SSC) of satsuma fruits infested by *L. zonatus* females at different densities. **A:** Data from the laboratory test in 2008, **B:** Data from the field trial in Coker orchard in 2008.



## CHAPTER 4

### SEASONAL PHENOLOGY AND NATURAL ENEMY FAUNA OF CITRUS LEAFMINER, *PHYLLOCNISTIS CITRELLA* (LEPIDOPTERA: GRACILLARIIDAE), AND EVALUATION OF PHEROMONE-BAITED FOR MONITORING THE PEST IN ALABAMA SATSUMA ORCHARD

#### INTRODUCTION

The citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) is a major pest of citrus in many parts of the world including Africa, Australia, the Middle East, the Caribbean, Central, South, and North America, (Heppner 1993, Knapp et al. 1995, Heppner and Dixon 1995, Peña et al. 1996, Perales-Gutierrez et al. 1996, Hoy and Nguyen 1997, Legaspi et al. 2001, Diez et al. 2006). Native to southeast Asia, *P. citrella* was first recorded in the United States (U.S.) in 1993 in citrus nurseries in Dade County, Florida and quickly spread rapidly throughout the state (Heppner 1995). The pest is now found in several citrus producing states in the U.S. including Alabama, Louisiana, Texas and California (Gill 1999, Legaspi et al. 1999). Satsuma mandarin production is an emerging fruit industry in the Gulf Coast region of the U.S. (Campbell et al. 2004). Recent surveys have identified *P. citrella* as a major pest of satsuma mandarin (*Citrus unshiu* Marcovitch) in Alabama (Fadamiro et al. 2007, 2008).

Larvae of *P. citrella* feed on the leaf epidermis ingesting sap and causing chlorosis. They typically make serpentine tunnels called mines under the leaf cuticle, resulting in twisted and

malformed leaves (Heppner 1993, Legaspi et al. 1999). The serpentine mines may reduce the photosynthetic capacity of leaves and increase their susceptibility to plant pathogens, such as the citrus canker bacterium, *Xanthomonas axopodis* pv. *citri* (Sohi and Sandhu 1968, Cook 1988, Schubert and Sun 1996, Gottwald et al. 1997, 2002). Virtually all citrus cultivars are susceptible to *P. citrella* (Heppner 1993, Legaspi et al. 1999) and heavy infestations can result in significant yield loss (Peña et al. 2000). The pest is potentially damaging to young nurseries and top-grafted trees since damage can prevent young leaves from expanding and sometimes cause leaves to fall (Diez et al. 2006).

Several aspects of the biology and ecology of *P. citrella* have been studied in many citrus growing regions of the world (Ba-Angood 1978, Huang et al. 1989, Heppner 1993, Peña et al. 1996, Peña 1998, Legaspi et al. 2001, Diez et al. 2006, Xiao et al. 2007, Stelinski and Rogers 2008). Many of the above studies reported on the seasonal population dynamics of the pest and associated natural enemies in different regions. The species is multivoltine with number of generations per year ranging from six in Japan (Clausen 1931) to 13 in Northcentral India (Lal 1950). In Florida, *P. citrella* is present season long but peak flight activity occurs from late March through early October, during the availability of new leaf flushes (Lapointe and Leal 2007). The female produced sex pheromone of *P. citrella* was first partially identified by Ando et al. (1985). Recent studies have identified the full composition of this sex pheromone as consisting of three active components: (Z, Z, E)-7, 11, 13-hexadecatrienal, (Z, Z)-7, 11-hexadecadienal, and (Z)-7-hexadecenal (Leal et al. 2006, Moreira et al. 2006). Follow-up field tests demonstrated strong attraction of *P. citrella* males to traps baited with a binary or tertiary lure of the active sex pheromone components (Mafi et al. 2005, Lapointe et al. 2006, Leal et al. 2006, Moreira et al. 2006, Lapointe and Leal 2007). A recent study also reported on the utility of

traps baited with a commercially produced synthetic sex pheromone lure of *P. citrella* for monitoring the pest in Florida (Stelinski and Rogers 2008).

*Phyllocnistis citrella* is a perennial pest of satsuma mandarin in southern Alabama (Fadamiro et al. 2007, 2008) but little is known about its seasonal abundance and natural mortality factors in the region. Furthermore, the utility of pheromone-baited traps for monitoring *P. citrella* in southern Alabama and other parts of the Gulf Coast has not been demonstrated. Because pest phenology and response to pheromone traps may vary from region to region due to several factors including variations in climate and crop varieties (Fadamiro 2004a, b), data obtained from other citrus growing regions may not provide accurate information on the abundance and seasonal activity of *P. citrella* in southern Alabama. The objectives of this study were to 1) determine seasonal abundance of *P. citrella* immatures and associated natural enemies in Alabama satsuma orchards, and 2) evaluate the efficacy of two popular types of commercially available traps baited with synthetic sex pheromone lures for monitoring adult populations of the pest in Alabama. The results are expected to support the development of monitoring guidelines and management tools for *P. citrella* in southern Alabama and other parts of the Gulf Coast region.

## **MATERIALS AND METHODS**

### **Seasonal Abundance of *P. citrella* Immatures**

The seasonal abundance of immature stages of *P. citrella* was studied during 2006 and 2007 at several locations in southern Alabama. Six orchards (Brantley, Buck, Coker, Ladnier, Gulf Coast Research and Extension Center or GGREC, and McDaniel) were sampled in 2006 and four (Brantley, Coker, GCREC, and McDaniel) in 2007. The survey orchards were located in

Baldwin and Mobile counties, the two main citrus-growing regions in southern Alabama, and were comprised mainly of satsuma mandarin, with a few sweet orange (*Citrus sinensis*), grapefruit (*C. paradise*), and kumquat (*Fortunella* spp.). The predominant cultivar of satsuma mandarin was ‘Owari’, with few trees each of ‘Armstrong Early’ and ‘Brown’s Select’. Five of the orchards (Brantley, Buck, Coker, GCREC and Ladnier) were commercial farms typically managed using conventional practices including routine applications of pesticides, while McDaniel orchard was unsprayed prior to and during the surveys.

Each orchard was repeatedly sampled biweekly (at 2-week intervals) by collecting 5 flushes (each with ~ 10 leaves) from five randomly selected trees for a total of 25 flushes (or ~ 250 leaves) per sampling date per orchard. The flushes were stored in properly-labeled paper bags, held in a cooler and transported to the laboratory, where they were examined under the microscope (10 × magnification) to determine the presence of all stages of *P. citrella*. The number of *P. citrella* immatures (sum of larvae, prepupae and pupae) per flush was recorded. Mean numbers per flush per sampling date were then calculated using the 25 flushes as replicates, and then mean number were used to generate seasonal abundance charts. Observations on natural enemies of *P. citrella* were also periodically made in the six satsuma orchards surveyed in 2006. On three of the sampling dates (May, July, October), predators observed on the surveyed trees were captured by hand or sweep net, collected in glass vials containing ethyl alcohol (75 %) and transported to the laboratory for identification. In addition, the *P. citrella* infested leaves collected on the above three sampling dates were kept in properly labeled Petri dishes (9 cm diameter) in the laboratory [ $25 \pm 2$  °C,  $50 \pm 10\%$  RH, and a photoperiod of 14:10 (L: D) h] to determine parasitism and rear out the parasitoids. Final species determinations of predators and

parasitoids were made by Dr. Charles Ray (Auburn University Plant Diagnostic Laboratory), and voucher specimens were deposited in the Auburn University Entomology museum.

### **Evaluation of Pheromone-Baited Traps and Adult Seasonal Phenology**

A second study was conducted at two locations (GCREC and Coker orchards) in southern Alabama during 2006 and 2007 to evaluate two types of sticky traps, Pherocon VI “Delta” trap and Pherocon 1C “Wing” trap (Trécé Inc., Salinas, CA) for monitoring adult *P. citrella*. Both traps types are popular among growers and are commonly used for monitoring adult Lepidoptera in North America (Riedl et al. 1976, Delisle, J. 1992, Fadamiro 2004a, b, Stelinski and Rogers 2008). The two traps have similar liner catch surface area: 379 cm<sup>2</sup> and 394 cm<sup>2</sup> for Pherocon VI Delta trap (evaluated with its opening flap closed) and Pherocon 1C Wing trap, respectively. Each orchard was divided into four blocks where the trap treatments were replicated using a randomized complete block design. The following four treatments were evaluated in each block: i) Pheromone-baited Delta trap; ii) Pheromone-baited Wing trap; iii) Unbaited Delta trap, and iv) Unbaited Wing trap. Pheromone-baited traps were baited with a rubber septum (gray) lure loaded with 0.1 mg of Z7Z11E13-16: Ald and 0.033 mg of Z7Z11-16:Ald (Advanced Pheromone Technologies or APTIV, Portland, OR), which are the active sex pheromone components of *P. citrella* (Leal et al. 2006, Moreira et al. 2006). The unbaited traps of each type (i.e. treatments iii and iv) served as non-lure controls. In each block, traps were randomly placed along a single row of satsuma trees at ~ 1.5 m above the ground and spaced apart by at least 20 m within a block and 40 m between blocks. Satsuma tree canopy heights at the two test orchards ranged from 2.5 m to 3.5 m. Traps were deployed in the last week of April in 2006 and early March in 2007 and removed in mid December of each year, and were checked weekly (2006) or biweekly (2007),

counting and recording the number of *P. citrella* males per trap. The dates of first and peak moth captures were also noted for each trap. Captures of nontarget insects were very minimal in both trap types and thus were not analyzed or presented. The position of each trap was re-randomized every four weeks to minimize potential effect of trap position on moth captures. Pheromone lures were replaced every four weeks in accordance with manufacturer's recommendation, and trap liners were replaced after each weekly or biweekly data collection. Data for each orchard and year were analyzed and presented separately. Captures in unbaited traps were too low for analysis (near zero) and thus were not included in the comparison. Actual captures in pheromone-baited trap were calculated by subtracting captures in unbaited control traps of each type from captures in pheromone-baited traps. Weekly (2006) or biweekly (2007) data were used to general adult seasonal phenology charts. Seasonal mean capture per sampling date was calculated for each block (replicate) and then used to calculate the seasonal mean capture for each pheromone-baited trap treatment (means of four replicates per trap type). Seasonal mean trap captures were first transformed by using the square-root method ( $\sqrt{x + 0.5}$ ), and then analyzed using the student's *t* test ( $P < 0.05$ , JMP Version 7.01, SAS Institute 2007) to determine significant differences between the two trap types.

## **RESULTS AND DISSCUSION**

### **Seasonal Abundance of *P. citrella* Immatures**

The seasonal mean number of *P. citrella* immatures per flush per sampling period (2 weeks) in the surveyed orchards ranged from 3.1 to 5.1 in 2006 and from 4.3 to 6.4 in 2007 (Table 1), indicating moderate to high infestation in all the orchards. Among the orchards, the highest population densities were recorded at Buck, Ladnier and McDaniel in 2006 and at

Brantley, Coker and GCREC in 2007. Data for these top three locations for each year were used to generate seasonal phenology charts (Fig. 1). *P. citrella* immatures were recorded throughout the sampling period from April to November each year, indicative of continuous overlapping generations. However, the highest densities were recorded during summer: densities progressively increased from May to July and remained at high levels throughout the summer (Fig. 1). Population densities of *P. citrella* began to decline in October and eventually reached very low levels in November. At least three distinct peaks of *P. citrella* were recorded per year in each orchard (Fig. 1). The percentage of infested leaves per flush per sampling date was calculated for the top location for each year (i.e. Ladnier in 2006 and Brantley in 2007) to determine possible association between the number of leaves per flush (used as an estimate of flush abundance) and the severity of *P. citrella* infestation (Fig. 2). The number of leaves per flush increased slightly from ~ 8 - 10 in spring (April-May) to 10 - 12 in summer (June-September) and then decreased in fall (October-November). The lowest numbers of leaves per flush (~ 5 - 7) were recorded in November. The percentage of infested leaves per flush was very low in spring and sharply increased to very high levels in summer and decreasing again in fall (Fig. 2).

In general, our results on the seasonal phenology of *P. citrella* in Alabama are similar to those reported in Florida (Peña et al. 1996), Mexico (Bautista-Martínez et al. 1998), and southern Texas (Legaspi et al. 1999): population density of *P. citrella* typically increased from spring to summer and declined during winter. A similar seasonal phenology was also reported in Argentina where the highest densities were recorded in spring and summer (Diez et al. 2006). The relatively greater abundance of *P. citrella* during spring and summer has been attributed to greater availability of leaf flushes and new shoots, as well as higher temperatures (Peña et al.

1996, Legaspi et al. 1999, Diez et al. 2006). However, our data which showed only a slight increase in the availability of leaf flushes in summer compared to the other periods suggest that the higher incidence of *P. citrella* recorded in summer cannot be fully explained by the higher abundance of new leaf flushes during this period. It is likely that lower temperatures starting from fall through early spring (October-May) contributed immensely to the lower incidence of *P. citrella* during this period. Peña et al. (1996) also attributed the decline in *P. citrella* populations during winter in Florida to low temperatures since they observed citrus flushes during this period. Similarly, the increase in the population of this pest during spring and summer in Argentina was ascribed more to increase in temperatures than to the presence of citrus flushes (Diez et al. 2006). Our data, which showed that the percentage of infested leaves per flush were much higher in summer (> 75%) than in spring and fall, further lend support to the proposition that changes in availability of leaf flushes played only a minor role in the observed seasonal population dynamics of *P. citrella* in Alabama. Although not statistically analyzed due to the low number of unsprayed orchards ( $n = 1$ ), no consistent differences were recorded in the population densities of *P. citrella* in sprayed versus unsprayed orchards, as also reported by Diez et al. (2006).

### **Natural Enemy Fauna**

At least 21 species of beneficial arthropods (19 predacious and 2 parasitoid species) were observed in the surveyed orchards (Table 3). However, abundance of the different species and types was not compared due to their occurrence at generally low densities (Table 4). Spiders were the most common predatory arthropods observed in all surveyed orchards (Table 4). Spider communities consisted of 13 species from nine families, including Araneidae (1 species), Anyphaenidae (1 species), Filistatidae (1 species), Lycosidae (1 species), Mitrugidae (1 species),

Oxyopidae (1 species), Salticidae (4 species), Tetragnathidae (2 species), and Theridiidae (1 species) (Table 3). The dominant predacious spider species were the yellow ghost spider, *Hibana* sp. (Anyphaenidae), the long-legged sac spider, *Cheiracanthium* sp. (Miturgidae), and *Hentzia* sp. (Salticidae), which were collected from at least three of the six orchards. Several studies have also identified spiders as important predators of *P. citrella* and other citrus pests (Muma 1965, Dondale et al. 1979, Mansour et al. 1982, Breene et al. 1993, Amalin et al. 2001, Ghavami and Amooz 2008). In south Florida citrus groves, the spider community consisted of 15 species from nine families (Amalin et al. 2001), while 34 species from 13 families were collected in southern Texas (Breene et al. 1993). Our results which showed high diversity and abundance of spiders relative to other beneficial arthropods (Table 4) suggest their importance as natural enemies of *P. citrella* and other pests in Alabama satsuma orchards. In addition, eight predacious insect species in the families Coccinellidae (1 species), Formicidae (2 species), Anthocoridae (1 species), Chrysopidae (1 species), and Phlaeothripidae (1 species) were observed. The key predacious insect species included ants (Hymenoptera: Formicidae), green lacewing, *Chrysoperla* spp. (Neuroptera: Chrysopidae), and multicolored Asian lady beetle, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), which were observed feeding on *P. citrella* larvae in at least five of six surveyed orchards. These predators have also been recorded to feed on *P. citrella* (Chen et al. 1989, Amalin et al. 2001, Xiao et al. 2007, Hoy et al. 2007).

Two parasitoid species were reared out from *P. citrella*: *Ageniaspis citricola* Logvinovskaya (Hymenoptera: Encyrtidae) and *Cirrospilus* sp. (Hymenoptera: Eulophidae). *Ageniaspis citricola*, an exotic specialist endo-parasitoid parasitoid of *P. citrella* which has been introduced in Florida and Texas (Hoy and Nguyen 1997, Pomerinke and Stansly 1998), was reared out in very low numbers from infested leaves collected from one of the six orchards. We

are not aware of any documented purposeful introduction of *A. citricola* in Alabama citrus. Thus, its occurrence is likely due to accidental introductions through movement of rootstock materials from Florida or Texas, or range expansion by natural dispersal. One species in the genus *Cirrospilus* was also reared out in low numbers from two of the six locations (Table 4). *Cirrospilus* spp., are indigenous generalist ecto-parasitoids of *P. citrella* in the U.S. (Pena 1996, Schauff et al. 1998).

### **Evaluation of Pheromone-Baited Traps**

No significant differences were recorded in captures of *P. citrella* in both types of traps (Delta versus Wing traps) at the two locations in both years of the study (Table 2). However, the data showed a slightly higher male captures in Delta traps than in Wing traps. The dates of first moth capture were also similar for both trap types (Table 2), indicating that both traps are equally capable of detecting early flights of *P. citrella* males. In contrast to our results, Stelinski and Rogers (2008) reported that the Pherocon VI Delta trap (with opening flap closed) baited with a sex pheromone lure captured significantly more *P. citrella* males than identically baited Pherocon IC Wing trap. Other studies have also reported on the superiority of Pherocon VI Delta trap over Pherocon IC Wing trap for monitoring adult Lepidoptera of various species (e.g., Knodel and Agnello 1990, Fadamiro 2004a, b).

Several factors may account for the different results obtained in the present study and those of Stelinski and Rogers (2008), in particular differences in population densities in the test orchards. The trap captures reported by Stelinski and Rogers (2008) were very low (averaged 19 - 40 males per trap per week) compared to the present study in which much higher seasonal trap captures averaging about 70 - 200 or more males per trap per week were recorded (Table 2). This

suggests that population densities of *P. citrella* were much higher in our test orchards than in the citrus grove used by Stelinski and Rogers (2008). Assuming that the Delta-style trap is indeed superior to the Wing-style trap, the absence of a significant trap effect in our study may be related to the higher population densities of the pest in our test orchards. This may have resulted in the quick saturation of the trap liners thereby impacting trap efficiency, given that our traps were checked and replaced on a biweekly basis. Future studies are needed to confirm the potential impact of insect population density on efficacy of pheromone-baited traps. Nevertheless, based on the report which showed that the Delta-style trap is more efficient than the Wing-style trap for monitoring *P. citrella* at low to moderate population densities (Stelinski and Rogers 2008) and a consideration of other factors including trap durability and ease of deployment, the Delta-style trap baited with the sex pheromone lure is recommended for monitoring *P. citrella* in Alabama citrus. However, citrus growers who prefer the Wing-style trap may still achieve effective monitoring of the pest with it.

### **Adult Seasonal Phenology**

The weekly or biweekly trap captures of *P. citrella* in pheromone-baited delta trap at both orchards are presented in Figure 3 to illustrate the seasonal phenology of the moths. In both years, *P. citrella* males were captured continuously in both orchards, indicating overlapping generations. However, the highest trap captures were recorded from August to October in 2006 and from June to October in 2007. The data suggest at least two major flight peaks and possibly one or more minor peaks per year. In 2006 at the GCREC location, a small first peak was recorded in early June, followed by a second large peak in late August, and a third larger peak in late October. In 2007 at the same location, a large first peak was recorded in late May-early June,

followed by a larger second peak in late September. Similar to our results, Lapointe and Leal (2007) reported about four flight peaks for *P. citrella* in Florida, including a small first peak in April, followed by a second larger peak in May-June, a large peak in July-August, and a small peak in October. The phenology of *P. citrella* moths generally followed the same pattern as that of the immatures, with peak densities of both recorded in summer. However, male moths were captured in the traps a little earlier than the occurrence of the immature stages, which was not unexpected.

Variable numbers of generations per year have been reported for *P. citrella* in different regions, from six in Japan (Clausen 1931) to 13 in Northcentral India (Lal 1950). Although it is difficult to accurately determine the number of generations for this pest in southern Alabama due to the observed generations overlap, one could deduce the approximate number of generations from a combination of our data and published information on the developmental biology of *P. citrella*. Pandey and Pandey (1964) recorded developmental periods of 13 - 52 days for *P. citrella* depending on weather conditions, while Ba-Angood (1978) reported 47.5, 39.7, 28, and 23.1 days at 20, 25, 30, and 35°C, respectively. Using a modest developmental period of about 30 days in the hot, humid southern Alabama conditions, an estimate of six or more generations per year (from May to October) for *P. citrella* in Alabama is plausible.

In general, densities of *P. citrella* males were lower in 2007 than in 2006 (Table 2) and the major flight peaks occurred earlier in 2007 than in the previous year (Fig. 3). It is unlikely that the reduced adult populations in 2007 is related to lower survival of overwintering populations in winter 2006 or reduced larval development or survival in spring 2007, since slightly higher numbers of immatures were recorded in 2007 compared to 2006 (Table 1). The data suggest the possibility of reduced flight activity of moths during 2007 probably due to lower

temperatures or higher rainfall. To determine if temperature and rainfall could account for the lower adult populations recorded in 2007, historic weather data for Fairhope (nearest weather station to the orchards) were analyzed, comparing climate data for 2006 versus 2007. Temperature did not adequately explain the recorded difference between both years. However, key differences were recorded in the rainfall (precipitation) data with 2007 being a very wet year. The average total precipitation per month for the period when flight activity of *P. citrella* was highest (June - October) was ~ 3.98 and 6.40 for 2006 (June to Oct: 1.27, 3.23, 6.71, 5.27, and 3.44) and 2007 (June to October: 5.97, 6.47, 5.12, 6.09, and 8.33), respectively. In contrast, average rainfall per month in spring (April-May) was higher in 2006 (4.82) (3.48-6.18) than 2007 (2.61) (1.86-3.35). Thus, higher rainfall in summer 2007 could account, at least in part, for the lower adult trap capture compared to summer 2006. Similarly, the rainfall data could also explain the differences in the spring activity of the pest in both years: the relatively lower spring activity of adults in spring 2006 compared to spring 2007 (Fig. 3) could be related to higher rainfall in spring 2006.

In summary, our results confirm that *P. citrella* is an annual pest with multiple overlapping generations in Alabama. The low abundance of parasitoids in the surveyed orchards may suggest that the level of control provided by parasitoids is minimal, and this may partly explain the high abundance of *P. citrella* in Alabama orchards relative to Florida and other citrus growing regions. Studies are ongoing to determine the impact of key natural enemies of *P. citrella* in Alabama satsuma orchards.

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**Table 1. Relative abundance of *Phyllocnistis citrella* immature stages in the Alabama satsuma orchards surveyed during 2006 – 2007**

Years	Location	Mean ( $\pm$ SE) number of <i>P. citrella</i>		Date of peak abundance
		immatures per flush <sup>a</sup>		
		Seasonal abundance	Peak abundance	
2006	Brantley	3.1 $\pm$ 0.4	6.9 $\pm$ 0.6	Sep. 6
	Buck	3.8 $\pm$ 0.4	7.8 $\pm$ 0.5	Aug. 26
	Coker	3.7 $\pm$ 0.4	7.3 $\pm$ 0.4	Sep. 6
	Ladnier	5.1 $\pm$ 0.4	10.1 $\pm$ 0.7	Aug. 26
	GCREC	3.4 $\pm$ 0.3	8.1 $\pm$ 0.5	Aug. 12
	McDaniel	4.5 $\pm$ 0.4	8.9 $\pm$ 0.5	Sep. 6
2007	Brantley	6.4 $\pm$ 0.2	10.9 $\pm$ 0.7	Jul. 17
	Coker	5.1 $\pm$ 0.2	10.0 $\pm$ 0.6	Jul. 2
	GCREC	5.3 $\pm$ 0.2	10.4 $\pm$ 0.7	Sep. 6
	McDaniel	4.3 $\pm$ 0.2	8.1 $\pm$ 0.6	Aug. 24

<sup>a</sup> Mean of 25 flushes per sampling date (biweekly). Immatures = larvae + prepupae + pupae.

Note: immatures were detected as early as April 27 in 2006 and April 10 in 2007. In this and Table 2 and the figures, GCREC = Gulf Coast Research and Extension Center.

**Table 2. Captures of *Phyllocnistis citrella* males in delta and wing traps baited with synthetic sex pheromone lures during 2006 and 2007 in two Alabama satsuma orchards**

Location	Year	Trap	Date of first moth capture <sup>b</sup>	Mean ( $\pm$ SE) trap capture per sampling period <sup>a</sup>		Date of peak moth capture
				Seasonal capture	Peak capture	
GCREC	2006	Delta	Apr. 27	223 $\pm$ 97	972 $\pm$ 125	Oct. 19
		Wing	Apr. 27	213 $\pm$ 63	836 $\pm$ 273	Oct. 19
				$t = 0.44, df = 206$	$t = 0.628, df = 6$	
				$P = 0.66$	$P = 0.553$	
	2007	Delta	Mar. 12	252 $\pm$ 32	849 $\pm$ 94	Sep. 6
		Wing	Mar. 12	219 $\pm$ 33	769 $\pm$ 287	Sep. 6
			$t = 0.79, df = 150$	$t = 0.508, df = 6$		
			$P = 0.4298$	$P = 0.629$		
Coker	2006	Delta	Apr. 27	164 $\pm$ 114	663 $\pm$ 215	Oct. 4
		Wing	Apr. 27	194 $\pm$ 38	640 $\pm$ 175	Aug. 26
				$t = 0.69, df = 206$	$t = 0.063, df = 6$	
				$P = 0.49$	$P = 0.95$	
	2007	Delta	Mar. 12	175 $\pm$ 21	425 $\pm$ 43	Sep. 6
		Wing	Mar. 12	143 $\pm$ 20	527 $\pm$ 186	Sep. 6
			$t = 1.113, df = 150$	$t = 0.569, df = 6$		
			$P = 0.2673$	$P = 0.59$		

<sup>a</sup> Means of four replicates per trap type per sampling date (weekly in 2006 and biweekly in 2007). In this and other tables and figures, GCREC = Gulf Coast Research and Extension Center. Captures in delta and wing traps were not significantly different ( $P > 0.05$ , Student's  $t$  test).

<sup>b</sup> Date of the first moth capture in 2006 may not signal the beginning of activity of the pest since activity may have commenced prior to trap deployment in the field.

**Table 3. Beneficial arthropods observed in association with *P. citrella* in six Alabama satsuma citrus orchards in 2006**

Order	Family	Species	Feeding habit	Distribution <sup>a</sup>
Araneae	Anyphaenidae	<i>Hibana</i> sp.	Predator	+++++
	Araneidae	<i>Gasteracantha</i>	Web builder	++
		<i>cancriformis</i> (Linnaeus)		
	Filistatidae	<i>Kukulcania hibernalis</i>	Web builder	++
		(Hentz)		
	Lycosidae	<i>Schizocosa</i> sp.	Hunter	++
	Miturgidae	<i>Cheiracanthium</i> sp.	Predator	+++
	Oxyopidae	<i>Peucetia virescens</i>		+
		(Hentz)		
	Salticidae	<i>Hentzia</i> sp.	Hunter	+++
		<i>Hentzia palmarum</i>	Hunter	
		(Hentz)		
		<i>Sitticus</i> sp.	Hunter	+
		<i>Thiodina sylvana</i> (Hentz)	Hunter	+
Tetragnathidae	<i>Leucauge venusta</i>	Web builder	+	
	(Walckenaer)			
	<i>Nephila clavipes</i>	Web builder	+	
(Linnaeus)				
Theridiidae	<i>Latrodectes geometricus</i>	Web builder	++	

(Koch)				
Coleoptera	Coccinellidea	<i>Harmonia axyridis</i> Pallas	Predator	++++++
Hymenoptera	Encyrtidae	<i>Ageniaspis citricola</i>	Parasitoid	+
		Heppner		
	Eulophidae	<i>Cirrospilus</i> sp.	Parasitoid	++
	Formicidae	<i>Solenopsis invicta</i> Buren	Predator	++++++
<i>Brachymyrmex patagonicus</i> Mayr		Predator?	++	
Hemiptera	Anthocoridae	<i>Orius insidiosus</i> (Say)	Predator	++
Neuroptera	Chrysopidae	<i>Chrysoperia</i> sp.	Predator	+++++
Thysanoptera	Phlaeothripidae	Unknown species	Predator	++

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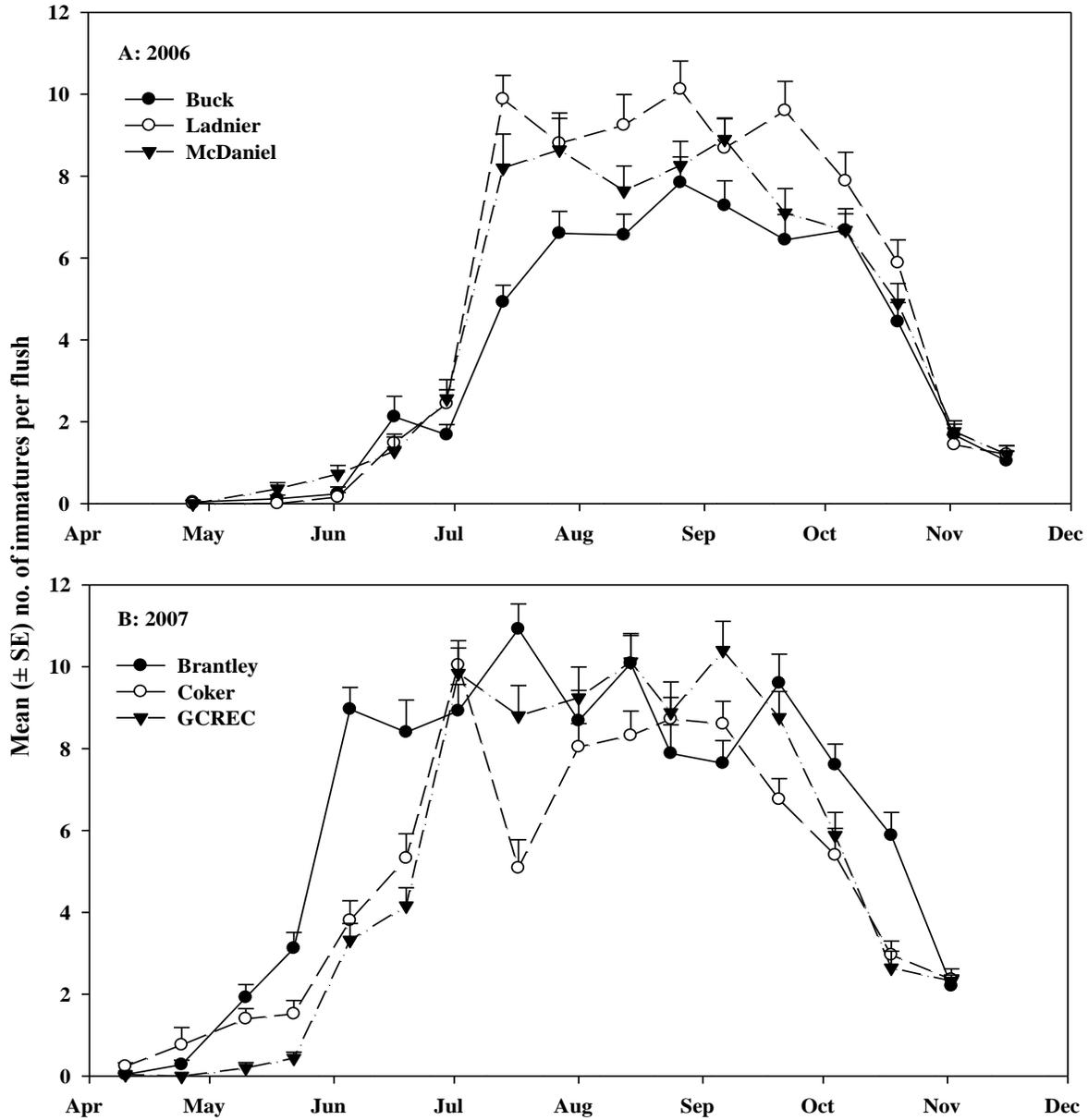
<sup>a</sup>Distribution in Alabama was computed based on the presence of beneficial species in the surveyed orchards (+ indicates presence in only one of the surveyed orchards while ++++++ indicates presence in six surveyed orchards).

**Table 4. Abundance of key beneficial arthropods observed in association with *P. citrella* in six Alabama satsuma citrus orchards in 2006**

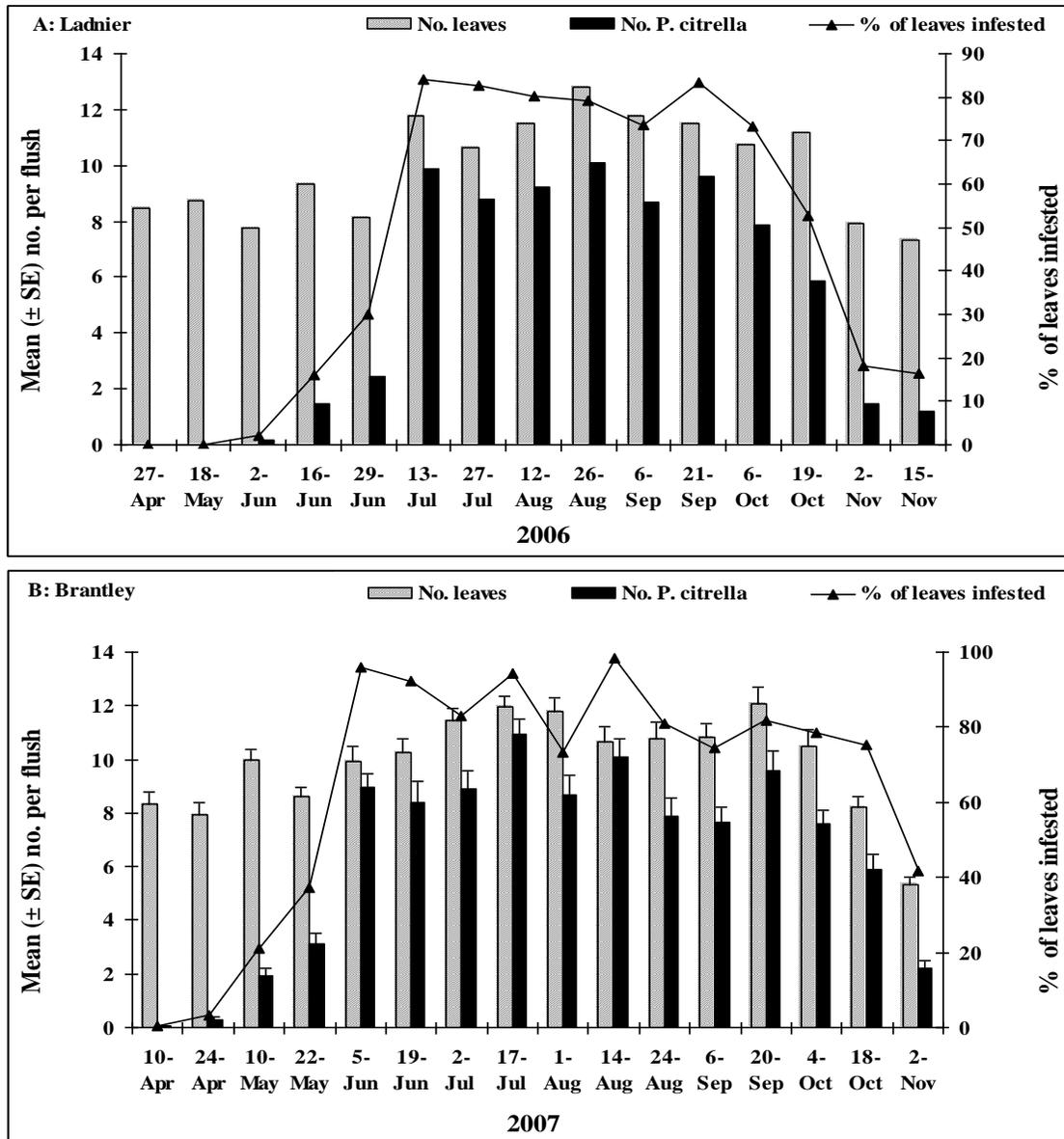
Location	May			July			October		
	SP	IP	PA	SP	IP	PA	SP	IP	PA
Brantley	6	1	0	8	2	0	2	1	1
Buck	1	1	0	1	0	0	0	0	0
Coker	4	3	0	6	3	2	0	4	1
Ladnier	1	0	0	0	2	0	0	0	0
GCREC	2	0	0	2	1	1	1	0	0
McDaniel	4	0	0	9	1	0	4	2	0

Table shows number of natural enemies of each type recorded on five trees per orchard per sampling date. SP: spiders; IP: insect predators (i.e. ants, lady beetles, lacewings, etc); PA: parasitoids

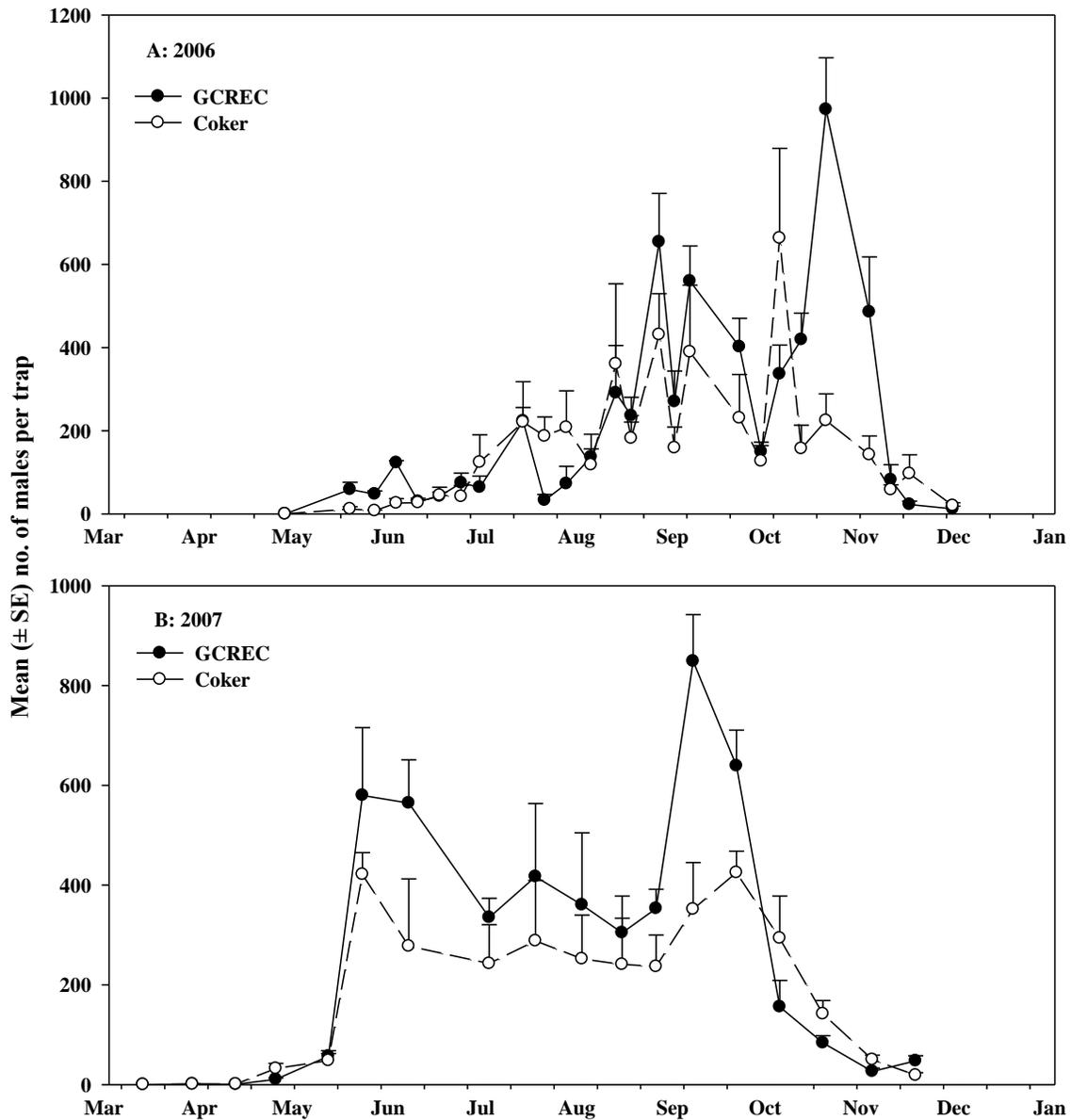
**Figure 1.** Seasonal abundance of *Phyllocnistis citrella* immatures in the Alabama satsuma orchards surveyed during 2006 (A) and 2007 (B). Figures shows mean ( $\pm$  SE) number of immatures per flush (~ 10 leaves) per sampling date ( $n = 25$  flushes). Immatures = larvae + prepupae + pupae. GCREC = Gulf Coast Research and Extension Center.



**Figure 2.** Associating seasonal abundance of *Phyllocnistis citrella* immatures with abundance of leaf flushes in two Alabama satsuma orchards (A: 2006 data for Ladnier, B: 2007 data for Brantley). No. leaves = number of leaves per flush, No. *P. citrella* = total number of *P. citrella* immatures (larvae + prepupae + pupae) counted; % infested leaves = % leaves with at least one *P. citrella* (calculated by dividing number of infested leaves per flush by the total number of leaves per flush).



**Figure 3.** Seasonal phenology of *Phyllocnistis citrella* moths in two Alabama satsuma orchards during 2006 and 2007. Figure shows mean ( $\pm$  SE) weekly (A: 2006) or biweekly (B: 2007) captures of males per Delta trap baited with the synthetic sex pheromone lure. GCREC = Gulf Coast Research and Extension Center.



## CHAPTER 5

# EXCLUSION EXPERIMENTS REVEALED RELATIVE CONTRIBUTION OF NATURAL ENEMIES TO MORTALITY OF CITRUS LEAFMINER, *PHYLLOCNISTIS* *CITRELLA*, (LEPIDOPTERA: GRACILLARIIDAE) IN ALABAMA SATSUMA CITRUS ORCHARDS

## INTRODUCTION

Satsuma mandarin (*Citrus unshiu* Marcovitch) production is a growing industry in southern Alabama and other parts of the Gulf Coast region of the United States (Campbell et al. 2004). Recent surveys have identified the citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) as a key pest of this specialty fruit crop in Alabama (Fadamiro et al. 2007, 2008). *Phyllocnistis citrella* originated in southeast Asia and has become a global pest of citrus, having been found in Africa, Australia, the Middle East, the Caribbean, Central, South, and North America, (Heppner 1993, Heppner and Dixon 1995, Peña et al. 1996, Perales-Gutierrez et al. 1996, Hoy and Nguyen 1997, Legaspi et al. 1999, Diez et al. 2006). In the United States, *P. citrella* was first recorded in 1993 in citrus nurseries in Dade County, Florida (Heppner 1993). The pest is now found throughout the state of Florida, as well as in several other citrus producing states including Alabama, Louisiana, Texas and California (Legaspi et al. 1999, Gill, 1999).

*Phyllocnistis citrella* attacks all varieties of citrus, other Rutaceae, and several ornamental species (Heppner 1993, Legaspi et al. 1999). Females lay eggs on host leaves and eclosing larvae feed on leaf epidermis ingesting sap and causing chlorosis and curled leaves (Heppner 1993, Legaspi et al. 1999). Larvae of *P. citrella* make characteristic serpentine mines under the leaf cuticle, which may reduce photosynthesis (Cook 1988) The feeding tunnels produced by *P. citrella* larvae on citrus leaves may facilitate infection by the citrus canker bacterium, *Xanthomonas axopodis* pv. *citri* (Sohi and Sandhu, 1968, Cook 1988, Gottwald et al. 1997, 2002). High population densities of *P. citrella* are usually recorded in spring and summer due to greater availability of leaf flushes and new shoots, as well as higher temperatures (Peña et al. 1996, Legaspi et al. 1999, Diez et al. 2006). *Phyllocnistis citrella* is an important pest in citrus nurseries and top-grafted trees (Diez et al. 2006), and heavy infestation can have significant impact on growth and yield (Peña et al. 2000, Browning et al. 2006).

Control of *P. citrella* is typically accomplished through multiple applications of conventional insecticides, which are often ineffective because the larvae are usually concealed within the mines and thus are protected from insecticide sprays (Legaspi et al. 2001). Biological control is generally regarded as the most economically sound and environmentally sustainable management practice for *P. citrella* (Knapp et al. 1995, Hoy and Nguyen 1997). The population dynamics of *P. citrella* and associated natural enemies have been documented in several countries and regions (Chen et al. 1989, Pena et al. 1996, Pena 1998, Urbaneja et al. 2000, Legaspi et al. 2001, Diez et al. 2006, Lapointe and Leal. 2007). Several predatory arthropods are known to feed on *P. citrella*, including lacewing larvae, ants, and hunting spiders (Argov et al. 1996, Pomerinke 1999, Amalin et al. 2001, Hoy et al. 2007, Xiao et al. 2007), and many studies have identified predation as the most important natural mortality factor acting on *P. citrella* in

many parts of the world (Chen et al. 1989, Amalin et al. 1996, 2001, 2002, Hoy et al. 2007, Xiao et al. 2007). In addition, many species of parasitoids have been reared from *P. citrella* worldwide (Hoy and Nguyen 1997, Schauff et al. 1998, Legaspi et al. 1999), however indigenous parasitoids were found to provide only minimal levels of parasitism in Florida (Pena et al. 1996). Consequently, *Ageniaspis citricola* Logvinovskaya (Hymenoptera: Encyrtidae), an exotic specialist endo-parasitoid of *P. citrella* larvae, was introduced in the 1990s for classical biological control of the pest in Florida and Texas (Hoy and Nguyen, 1997 Pomerinke and Stansly 1998).

In a recent study (Xiao and Fadamiro, in review), we documented the natural enemy fauna of *P. citrella* in southern Alabama as consisting of at least 21 species of beneficial arthropods, including various species of spiders (e.g., *Hibana* sp., *Cheiracanthium* sp. and *Hentzia* sp.) (Araneae), ants (Hymenoptera: Formicidae), *Chrysoperla* spp. (Neuroptera: Chrysopidae), and *Harmonia axyridis* (Coleoptera: Coccinellidae). Two parasitoid species were also detected in low numbers: *A. citricola* and *Cirrospilus* sp. (Hymenoptera: Eulophidae). *Cirrospilus* spp. are indigenous generalist ecto-parasitoids of *P. citrella* in the United States (Pena 1996, Schauff et al. 1998). However, little is known about the impact of these beneficial arthropods on *P. citrella* in Alabama. In this study, exclusion techniques (e.g., Smith and DeBach 1942, Xiao et al., 2007, Qureshi and Stansly 2009) were used to determine the relative contributions of key beneficial arthropods to natural mortality of *P. citrella* in Alabama satsuma orchards. The results should provide the baseline data necessary for development of an effective biological control program for managing *P. citrella* in the Gulf Coast region of the United States.

## MATERIALS AND METHODS

### Study Sites

Experiments were conducted in July 2007 and August 2008 in two satsuma orchards located in Baldwin County in southern Alabama: Coker orchard and the Gulf Coast Research and Extension Center orchard (GGREC). Both orchards were comprised mainly of satsuma mandarin, with a few sweet orange (*Citrus sinensis*), grapefruit (*C. paradise*), and kumquat (*Fortunella* spp.). The predominant cultivar of satsuma mandarin was ‘Owari’, with few trees each of ‘Armstrong Early’ and ‘Brown’s Select’. Both orchards were typically managed using conventional practices including routine applications of pesticides, but were not sprayed during this study.

### Exclusion Experiments

Experimental trees in both orchards were first pruned to induce new flushes necessary for *P. citrella* infestation. Newly hatched first instar larvae were then located using a hand lens and identified by marking the adjacent leaf surface with dark ink. Two types of exclusion techniques (sticky barrier and cage barrier) were used to evaluate the relative contribution of natural enemies to *P. citrella* mortality, following the procedures described by Xiao et al. (2007) and Qureshi and Stansly (2009), but with some minor modifications. The use of both exclusion techniques was timed in order to determine the impact of various biotic factors attacking larvae of *P. citrella* at the different development stages. The aim of the sticky barrier exclusion treatments was to evaluate *P. citrella* mortality factors due to ants and other crawling predators (e.g., lacewing and lady beetle larvae) by comparing mortalities in *P. citrella* cohorts protected by a sticky barrier versus unprotected (control) cohorts. The cage barrier exclusion treatments

were tested to separate the effect of other biotic factors (e.g., spiders and parasitoids) attacking larvae of *P. citrella*.

In the first experiment (July 2007), 30 pruned branches located on mature satsuma trees were selected in each orchard. Each branch contained expanding leaves and 15 first stage larvae of *P. citrella*. The branches were randomly distributed among the following six treatments (i.e. each treatment was replicated five times): i) branch protected with sticky barrier from day 1 to day 12 (sticky barrier 1-12 or complete sticky barrier); ii) branch protected with sticky barrier from day 1 to day 5 with the sticky barrier removed on day 6 when the larvae had become second instars (sticky barrier 1-5 or early sticky barrier); iii) branch protected with sticky barrier from day 6 to day 12 when the larvae had become pupae (sticky barrier 6-12 or later sticky barrier); iv) branch caged from day 1 to day 5 with the cage removed on day 6 when the larvae had become second instars (cage barrier 1-5 or early cage barrier); v) branch caged from day 6 to day 12 when the larvae had become pupae (cage barrier 6 - 12 or later cage barrier); vi) control (branch not protected with sticky barrier or cage throughout the experiment). For the sticky barrier treatments, a 5-cm diameter sticky trap strip (Trece Inc, Salinas, CA) was applied to the branch to preclude ants and other crawling predators from reaching the larvae. For the cage barrier treatments, a sleeve cage made of fine mesh organdy (35 × 15 cm diameter, white color made from screen netting) was used to exclude beneficial arthropods, such as spiders and parasitoids. The control (no sticky or cage barrier) ensured that no arthropods were excluded (Smith and DeBach, 1942).

The experiment was repeated in August 2008 but with some minor modifications. At each location, 28 pruned branches, each containing expanding leaves and 15 first instar larvae of *P. citrella*, were randomly distributed among seven treatments (i.e. four replicates per treatment).

The treatments consisted of the six treatments evaluated in 2007 plus a seventh treatment, a branch caged from day 1 to day 12 (cage barrier 1-12 or complete cage barrier). The average temperatures at both locations during the experiments in July 2007 and August 2008 were 29.3°C (minimum: 24.5°C, maximum: 34°C) and 28.0°C (minimum: 23.4°C, maximum: 32.5°C), respectively. Average rainfall (precipitation) for July 2007 and August 2008 was 5.12 and 5.33, respectively.

### **Evaluation of Mortality Factors**

Branches were inspected daily noting and recording the development and survival of the larvae, until all larvae had died or emerged. The number of dead larvae and the likely cause of the death were also noted. Pupae were checked for parasitism starting from day 10. Dead and missing larvae were classified according to the likely cause of death using the following criteria (Pomerinke 1999, Amalin et al. 2002, Xiao et al., 2007): (a) Spiders: spiders were observed to puncture immobile larvae over mines, sucking the body fluid or making a slit in the mine to remove larvae; (b) Ants: ants were observed to remove larvae from the mine through a small hole in the leaf cuticle, or through the back of the leaf over the pupa chamber; (c) Other crawling predatory insects (e.g., lacewing and lady beetle larvae) were observed to feed on body parts of *P. citrella* larvae without removing the entire cadaver; (d) Parasitism by ecto-parasitoids (e.g., *Cirrospilus* spp.): presence of larval or pupal parasitoid inside the mine or pupal chamber of *P. citrella*; (e) Parasitism by endo-parasitoids, (e.g., *A. citricola*): presence of multiple parasitoid pupae within the prepupae and inside pupal chamber of *P. citrella*; and (f) Physical mortality: absence of much of the cuticle over the mine.

## Statistical Analysis

Daily survivorship was calculated for larvae in the different treatments. Percentage cumulative larval mortality (at the end of the experiment) was calculated for each treatment (by mortality factor) and used for statistical analysis. Data obtained were first normalized by using the arcsine square-root transformation ( $\sqrt{x + 0.5}$ ) and then analyzed with one-way analysis of variance (ANOVA) followed by the Tukey-Kramer honestly significant difference (HSD) test to determine significant treatments effects ( $P < 0.05$ , JMP Version 7.01, SAS Institute, 2007). Data for each orchard and year were analyzed and presented separately.

## RESULTS

Daily survivorship of *P. citrella* larvae in the control and key exclusion treatments evaluated in 2007 and 2008 are shown in Figures 1 and 2. As expected, survivorship of larvae on unprotected (control) branches was lower than larval survival in the complete sticky barrier and cage exclusion treatments in which larvae were protected for the entire duration of the study. In general, significant differences in larval survival in the control versus exclusion treatments were recorded at GCREC orchard beginning as early as day 2 or 3 of the experiment. In 2007 at Coker orchard, however, similar larval survival rates were recorded from day 1 to day 6 of the experiment in the control versus the complete (1-12 days) sticky barrier treatment, after which larval survival in the control began to reduce significantly (Fig. 1A). The complete (1-12 days) cage barrier treatment was evaluated in 2008, which allowed for a comparison between the two complete exclusion (sticky barrier versus cage barrier) techniques. At both locations, daily larval survivorship was significantly greater in the complete cage barrier treatment than in the complete sticky barrier treatment beginning from day 2 until the end of the experiment (Fig. 2). For

instance, percent survival of larvae on day 12 in the complete cage barrier treatment was ~ 92 % in both orchards compared with ~ 65-67 % in complete sticky barrier treatment and < 50 % in the control. These results suggest that the cage exclusion method was more effective in excluding key natural enemies, and consequently in reducing larval mortality.

Percentage cumulative mortalities of *P. citrella* larvae recorded at the end of the experiment (12 days) in the different treatments are presented in Tables 1-4. In general, significant differences in mortalities were recorded among the treatments, with the highest mortalities recorded in the control. Overall mortality in the control was ~ 39-41 % in 2007 (Tables 1 and 2) and ~ 48-52 % in 2008 (Tables 3 and 4) in both orchards. In both orchards in 2007 (Tables 1 and 2), predation by spiders was the single most important mortality factor accounting for ~ 25-30 % mortality, whereas parasitism accounted for only ~ 1-3 % mortality in the control. All five exclusion (sticky barrier and cage barrier) treatments significantly reduced predation by spiders, which also resulted in significant reductions in overall larval mortalities compared with the control. However, the effects of the other mortality factors (i.e. ants, other crawling predatory insects and physical damage) were not significantly different between the control and any of the exclusion treatments. Furthermore, no significant differences in total larval mortalities were recorded among the five exclusion treatments, irrespective of whether the larvae were protected early (1-5 days), later (6-12 days) or throughout the experiment (1-12 days). Comparison among the three sticky barrier treatments showed that only the complete (1-12 days) sticky barrier treatment was very effective in excluding ants.

In 2008 at Coker orchard, spiders also were the most important mortality factor which accounted for ~ 25% mortality in the control. In contrast, predation by spiders was completely excluded (0%) in the complete (1-12 days) cage barrier treatment, and significantly reduced (~

13%) in the later (6-12 days) cage barrier treatment, but not significantly reduced in the other treatments compared with the control (Table 3). Similarly, predation by ants and other predatory insects was completely (0%) excluded in the complete (1-12 days) cage barrier treatment. Predation by ants was also completely excluded (0%) in the complete (1-12 days) sticky barrier treatment. Parasitism was generally low ranging from 0% in the later cage barrier treatment to ~ 5 % in the control. Compared with the control, overall mortality was significantly reduced in four of the five exclusion treatments, with the early (1-5 days) sticky barrier treatment being the exception. This suggests that the effect of predation by ants was greatest on early instar larvae. Among the three cage barrier treatments, the complete cage barrier treatment was the only treatment which effectively excluded spiders and ants (Table 3). No consistent differences were recorded between the early and the later cage barrier treatments, suggesting that *P. citrella* larvae are susceptible to predation by spiders and ants throughout their development. In general, similar results were recorded at the GCREC orchard: overall mortality was significantly reduced in five of the six exclusion treatments. The lowest mortality (~ 8 %) was recorded in the complete cage barrier treatment compared with 35 % mortality in the complete sticky barrier treatment and 48 % mortality in the control (Table 4).

## DISCUSSION

The results from both locations and years clearly showed that predation was the most significant biological effect, which accounted for ~ 87-96 % of all *P. citrella* larval mortalities in unprotected (control) trees in Alabama satsuma orchards. In particular, predation by spiders was the single most important natural mortality factor acting on early and late instars of *P. citrella*. The key predacious spider species recorded in our study included the yellow glost spider, *Hibana*

sp. (Anyphaenidae), the long-legged sac spider, *Cheiracanthium* sp. (Miturgidae), and *Hentzia* sp. (Salticidae). Several authors have also reported on the role of spiders and insect predators as a key or dominant natural mortality factor acting on *P. citrella* in many parts of the world (Amalin et al. 1996, 2001, 2002; Browning and Pěna 1995, Argov and Rosler 1996, Xiao et al. 2007, Hoy et al. 2007). Three species of sac spiders, *Chiracanthium inclusum* (Hentz) (Clubionidae), *Hibana velox* (Becker) (Anyphaenidae), and *Trachelas volutes* Gertsch (Corrinnidae) were reported to feed on *P. citrella* larvae and prepupae in Florida lime orchards (Amalin et al. 1996, 2001). Further studies on their predatory habit showed that they are nocturnal species with the ability to detect their concealed prey by sensing movements (vibrations) of *P. citrella* larvae and prepupae with the leaf epidermis (Amalin et al. 2001). Prey preference studies also confirmed that certain predacious spider species prefer to feed on Lepidopteran and Homopteran pests in orchards (Jackson et al. 1977, Nyffeler et al. 1987, Amalin et al. 2001, Brown et al. 2003, Stephen and Berg 2008). Spiders are known to feed on *P. citrella* in two ways. They may directly puncture mines or remove the prey through open slit in the mines (Amalin et al. 2001, Xiao et al. 2007). Thus, the specialized predatory habit and feeding behavior of spiders make them important predators of *P. citrella* and similar pests. In addition to directly feeding on the immature stages, spiders could disturb the larvae and cause them to drop from the plant, and the webs spun over the leaves may make them less suitable for oviposition and feeding by pests (Stephen and Berg 2008).

Predation by ants was the second most important mortality factor, which accounted for ~ 10-19 % of all *P. citrella* deaths on unprotected (control) branches. The key ant species recorded included *Solenopsis invicta* Buren and *Brachymyrmex patagonicus* Mayr, and both species were observed feeding on *P. citrella* larvae. Ants have also been reported as important predators of *P.*

*citrella* in many parts of the world (Huang et al. 1989, Pomerinke 1999, Amalin et al. 2001, Xiao et al. 2007), but their impact appears to vary by region or climate. For instance, ants were the key predators of *P. citrella* in China, in particular during the dry and hot summer and fall seasons (Huang et al. 1989). Also, predation by ants, such as *Pseudomyrmex gracilis* (Roger) and *Crematogaster ashmeadi* (Mayr), was the largest single cause of *P. citrella* mortality which accounted for > 30% of all mortality by natural enemies in a southwest Florida citrus grove (Xiao et al. 2007). In contrast, Urbaneja et al. (2004) reported no significant effect of ant exclusion on mortality of *P. citrella* in Spain. Ants have been observed to remove *P. citrella* larvae through a small hole made in the mine, resulting in missing cadavers (Amalin et al. 2001, Pomerinke 1999, Xiao et al. 2007). Predation by other predatory insects, such as larvae of lacewings (*Chrysoperia* sp.) and the multicolored Asian lady beetle (*H. axyridis*) accounted for ~ 3-27 % of all *P. citrella* mortalities, as has been reported by other authors (Chen et al. 1989, Amalin et al. 2002).

Two important parasitoids of *P. citrella*, *Cirrospilus* sp. and *A. citricola*, were recorded in this study. *Cirrospilus* spp., are indigenous generalist ecto-parasitoid of *P. citrella* in the U.S. (Pena 1996, Schauff et al. 1998), while *A. citricola* is an introduced parasitoid of *P. citrella* in Florida and Texas (Hoy and Nguyen 1997, Pomerinke and Stansly 1998). However, parasitism contributed only minimally (~ 0-10 %) to *P. citrella* mortality in the present study. Several authors have reported low to moderate rates of parasitism of *P. citrella* larvae in the field (Peña et al., 1996, Legaspi et al. 2001, Amalin et al. 2002, Diez et al. 2006, Xiao et al. 2007). Legaspi et al. (2001) recorded ~ 20 % parasitism in Mexico, with the dominant parasitoid being *Zagrammosoma multilineatum* (Ashmead) (Hymenoptera: Eulophidae). The introduced parasitoid, *A. citricola* was the dominant parasitoid recorded in southwest Florida, and accounted

for ~ 8 - 29 % of *P. citrella* natural mortality (Xiao et al. 2007). We are not aware of any purposeful introduction of *A. citricola* in Alabama, and its occurrence is likely due to accidental introductions through movement of rootstock materials from Florida or Texas, or range expansion by natural dispersal. The relatively minor impact of parasitism recorded in the present study may simply be a reflection of the low endemic population densities of the identified parasitoids in Alabama citrus orchards. Since *A. citricola* has been reported to perform well in humid regions (Neale et al. 1995, Hoy and Nguyen 1997, Xiao et al. 2007), field augmentation of this parasitoid species is likely to be successful in the humid southern Alabama conditions.

The sticky barrier and cage barrier exclusion methods evaluated in this study have been commonly used to assess the effectiveness and impact of natural enemies in the field (Grabenweger et al. 2005, Pomerinke 1999, Xiao et al. 2007, Qureshi and Stansly 2009). Our results which showed that predation by spiders was completely excluded in the complete cage barrier treatment, and reduced in the complete sticky barrier treatment, are not surprising given their behavior on trees (Stephen and Berg 2008). Spiders could move from branch to branch by ballooning and thus would not be that affected by a sticky barrier. As expected, predation by ants was excluded or significantly reduced in the complete sticky barrier treatment. Interestingly, predation by ants was also excluded or reduced in the cage barrier treatment. Similarly, key predatory larvae were also excluded or reduced in the complete cage barrier treatment but the numbers were sometimes too low to detect a significant effect. These results possibly indicate that the mesh size of the cage barrier was fine enough to exclude some ants (e.g., major ant workers) and predatory larvae. In general, the timed (early or later) barrier treatments were not as effective in excluding or reducing any of the key predatory arthropods. Also, parasitism rates were too low to detect a significant effect of the exclusion treatments.

In summary, our results showed that predation by spiders and ants are very important natural mortality factors acting on *P. citrella* in Alabama citrus. Conservation of these key predators through the judicious use of pesticides and augmentation of field populations of key natural enemies are essential to the development of a sustainable pest management strategy for the pest in Alabama.

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**Table 1. Mortality of *Phyllocnistis citrella* cohorts from first stage larvae to adult emergence on unprotected (control) branches of satsuma trees versus branches protected with different exclusion techniques (sticky barrier or cage) during different stages of development at Coker orchard in 2007.**

Exclusion treatments	Percentage mortality (mean $\pm$ SE) due to each mortality factor					
	Spiders	Ants	Other predators	Parasitoids	Physical	Total mortality
Control	29.3 $\pm$ 4.9a	6.7 $\pm$ 2.1	1.3 $\pm$ 1.3	2.7 $\pm$ 1.6	1.3 $\pm$ 1.3	41.3 $\pm$ 5.7a
Complete sticky barrier	13.3 $\pm$ 1.6b	0 $\pm$ 0	6.7 $\pm$ 2.1	1.3 $\pm$ 1.3	2.7 $\pm$ 1.6	24.0 $\pm$ 3.4b
Early sticky barrier	13.3 $\pm$ 0b	1.3 $\pm$ 1.3	5.3 $\pm$ 1.6	1.3 $\pm$ 1.6	1.3 $\pm$ 1.6	22.7 $\pm$ 1.6b
Later sticky barrier	9.3 $\pm$ 1.6b	2.7 $\pm$ 1.6	4.0 $\pm$ 1.6	2.7 $\pm$ 1.6	2.6 $\pm$ 1.6	21.3 $\pm$ 2.5b
Early cage barrier	9.3 $\pm$ 1.6b	1.3 $\pm$ 1.3	4.0 $\pm$ 1.6	4.0 $\pm$ 1.6	2.7 $\pm$ 1.6	21.3 $\pm$ 1.3b
Later cage barrier	10.7 $\pm$ 1.6b	4.0 $\pm$ 2.6	1.3 $\pm$ 1.3	1.3 $\pm$ 1.6	2.7 $\pm$ 1.6	20.0 $\pm$ 3.0b
ANOVA ( <i>F</i> , <i>P</i> )	<i>F</i> = 10.7,	<i>F</i> = 1.96,	<i>F</i> = 1.81,	<i>F</i> = 0.24,	<i>F</i> = 0.2,	<i>F</i> = 4.99,
df = 5, 24	<i>P</i> = 0.0001	<i>P</i> = 0.124	<i>P</i> = 0.14	<i>P</i> = 0.94	<i>P</i> = 0.95	<i>P</i> = 0.002

Means in the same column followed by the same or no letter are not significantly different ( $P > 0.05$ , Tukey-Kramer HSD test). In this and subsequent tables, complete sticky barrier = branch protected with sticky barrier from day 1 to day 12; early sticky barrier = branch protected with sticky barrier from day 1 to day 5; later sticky barrier = branch protected with sticky barrier from day 6 to day 12; complete cage barrier = branch caged from day 1 to day 12; early cage barrier = branch caged from day 1 to day 5; later cage barrier = branch caged from day 6 to day 12; and Control = branch not protected with sticky barrier or cage. Other predators = other crawling predacious insects (e.g., lacewing and lady beetle larvae).

**Table 2. Mortality of *Phyllocnistis citrella* cohorts from first stage larvae to adult emergence on unprotected (control) branches of satsuma trees versus branches protected with different exclusion techniques (sticky barrier or cage) during different stages of development at GCREC orchard in 2007.**

Exclusion treatments	Percentage mortality (mean $\pm$ SE) due to each mortality factor					
	Spiders	Ants	Other predators	Parasitoids	Physical	Total mortality
Control	25.3 $\pm$ 2.5a	4.0 $\pm$ 1.5	5.3 $\pm$ 1.3	1.3 $\pm$ 1.3	2.7 $\pm$ 1.3	38.6 $\pm$ 3.2a
Complete sticky barrier	14.7 $\pm$ 2.5b	0 $\pm$ 0	5.3 $\pm$ 1.3	0 $\pm$ 0	2.7 $\pm$ 1.3	22.7 $\pm$ 2.6b
Early sticky barrier	12.0 $\pm$ 1.5b	4.0 $\pm$ 1.6	8.0 $\pm$ 2.5	0 $\pm$ 0	2.7 $\pm$ 1.3	26.7 $\pm$ 3.6b
Later sticky barrier	10.0 $\pm$ 1.6bc	2.7 $\pm$ 1.6	8.0 $\pm$ 1.3	5.3 $\pm$ 2.5	2.7 $\pm$ 1.1	28.7 $\pm$ 2.6b
Early cage barrier	9.3 $\pm$ 2.6b	2.7 $\pm$ 1.6	5.3 $\pm$ 1.3	0 $\pm$ 0	2.7 $\pm$ 1.3	20.0 $\pm$ 2.9b
Later cage barrier	8.0 $\pm$ 1.3bc	2.7 $\pm$ 1.6	5.3 $\pm$ 2.5	2.7 $\pm$ 2.6	4.0 $\pm$ 1.6	22.7 $\pm$ 3.4b
ANOVA ( <i>F</i> , <i>P</i> )	<i>F</i> = 10.4,	<i>F</i> = 0.96,	<i>F</i> = 0.58,	<i>F</i> = 1.0,	<i>F</i> = 0.11,	<i>F</i> = 4.46,
df = 5, 24	<i>P</i> = 0.0001	<i>P</i> = 0.46	<i>P</i> = 0.71	<i>P</i> = 0.44	<i>P</i> = 0.98	<i>P</i> = 0.005

Means in the same column followed by the same or no letter are not significantly different ( $P > 0.05$ , Tukey-Kramer HSD test).

GCREC = Gulf Coast Research and Extension Center, Fairhope, AL.

**Table 3. Mortality of *Phyllocnistis citrella* cohorts from first stage larvae to adult emergence on unprotected (control) branches of satsuma trees versus branches protected with different exclusion techniques (sticky barrier or cage) during different stages of development at Coker orchard in 2008.**

Exclusion treatments	Percentage mortality (mean $\pm$ SE) due to each mortality factor					
	Spiders	Ants	Other predators	Parasitoids	Physical	Total mortality
Control	25.3 $\pm$ 1.7a	9.7 $\pm$ 1.8ab	10.0 $\pm$ 1.3ab	5.0 $\pm$ 3.2	1.6 $\pm$ 0.8	51.6 $\pm$ 1.7a
Complete sticky barrier	21.7 $\pm$ 1.7a	0 $\pm$ 0c	8.3 $\pm$ 1.7ab	3.3 $\pm$ 0.5	0 $\pm$ 0	33.3 $\pm$ 3.8b
Early sticky barrier	18.3 $\pm$ 1.7ab	1.7 $\pm$ 0.8	13.3 $\pm$ 1.9a	3.3 $\pm$ 3.3	1.7 $\pm$ 0.9	38.3 $\pm$ 3.2ab
Later sticky barrier	18.3 $\pm$ 2.3ab	10.0 $\pm$ 1.6ab	3.3 $\pm$ 1.9bc	5.0 $\pm$ 1.7	0 $\pm$ 0	36.6 $\pm$ 3.3b
Complete cage barrier	0 $\pm$ 0c	0 $\pm$ 0c	0 $\pm$ 0c	1.6 $\pm$ 0.5	6.7 $\pm$ 2.7	8.3 $\pm$ 3.2c
Early cage barrier	15.0 $\pm$ 1.7a	0 $\pm$ 0c	3.3 $\pm$ 1.3bc	6.7 $\pm$ 1.9	3.3 $\pm$ 1.9	28.3 $\pm$ 7.4b
Later cage barrier	13.3 $\pm$ 1.7b	6.7 $\pm$ 2.7bc	5.0 $\pm$ 0.5ab	0 $\pm$ 0	1.7 $\pm$ 0.8	26.7 $\pm$ 2.7b
ANOVA ( <i>F</i> , <i>P</i> )	<i>F</i> = 47.7,	<i>F</i> = 12.5,	<i>F</i> = 6.03,	<i>F</i> = 0.69,	<i>F</i> = 1.87,	<i>F</i> = 11.05,
df = 6, 21	<i>P</i> = 0.0001	<i>P</i> = 0.0001	<i>P</i> = 0.009	<i>P</i> = 0.65	<i>P</i> = 0.13	<i>P</i> = 0.0001

Means in the same column followed by the same or no letter are not significantly different ( $P > 0.05$ , Tukey-Kramer HSD test).

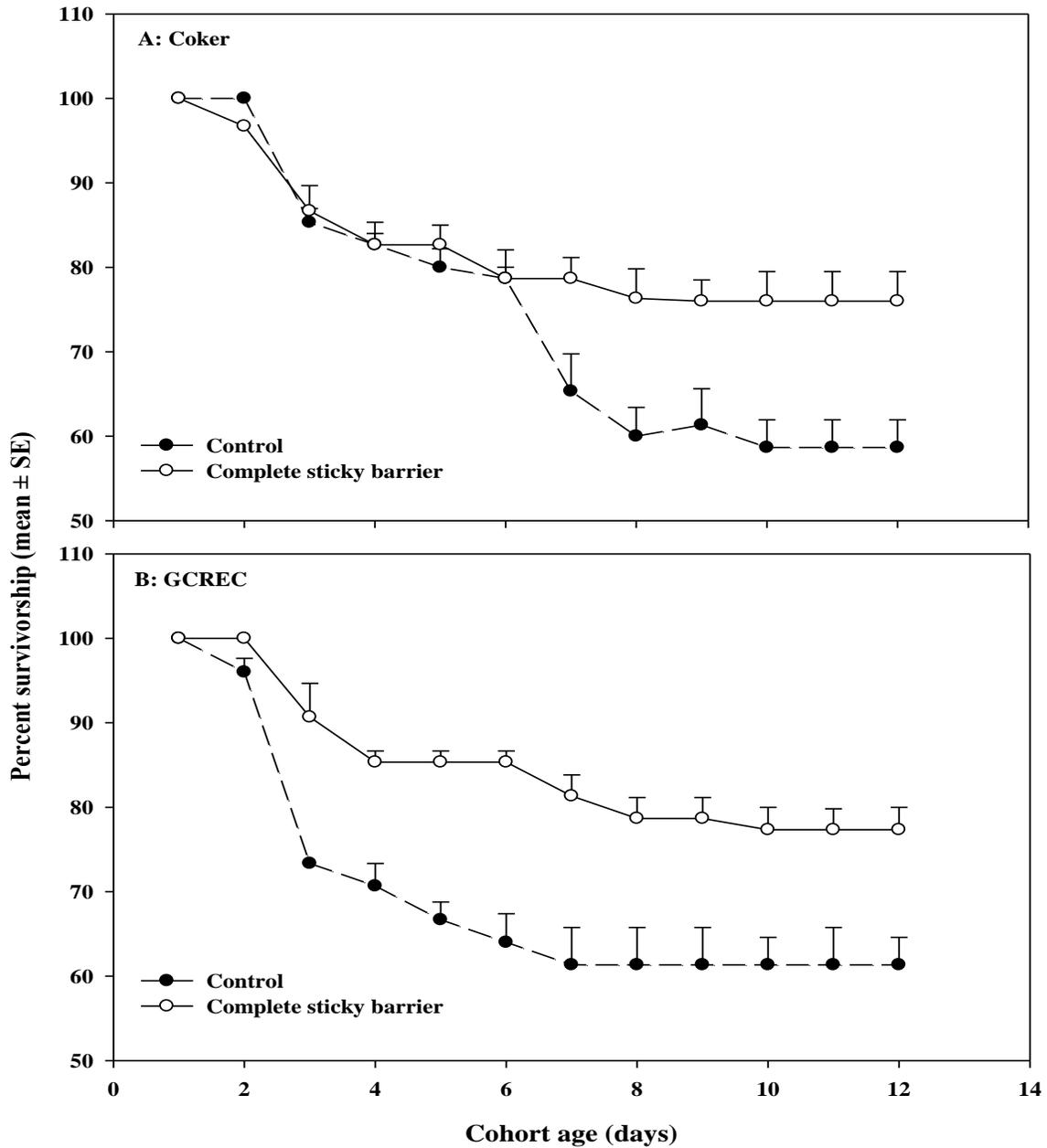
**Table 4. Mortality of *Phyllocnistis citrella* cohorts from first stage larvae to adult emergence on unprotected (control) branches of satsuma trees versus branches protected with different exclusion techniques (sticky barrier or cage) during different stages of development at GCREC orchard in 2008**

Exclusion treatments	Percentage mortality (mean $\pm$ SE) due to each mortality factor					
	Spiders	Ants	Other predators	Parasitoids	Physical	Total mortality
Control	25.0 $\pm$ 3.2a	8.3 $\pm$ 3.2a	13.3 $\pm$ 2.3a	0 $\pm$ 0	1.7 $\pm$ 1.7	48.3 $\pm$ 1.7a
Complete sticky barrier	20.0 $\pm$ 2.7a	0 $\pm$ 0b	10 $\pm$ 1.9ab	3.3 $\pm$ 1.9	1.7 $\pm$ 1.7	35.0 $\pm$ 1.7ab
Early sticky barrier	18.3 $\pm$ 1.7a	3.3 $\pm$ 1.9b	5.0 $\pm$ 1.7bc	0 $\pm$ 0	3.3 $\pm$ 1.9	29.9 $\pm$ 3.3b
Later sticky barrier	18.3 $\pm$ 1.7a	6.7 $\pm$ 0a	6.7 $\pm$ 2.7bc	0 $\pm$ 0	0 $\pm$ 0	31.7 $\pm$ 3.2b
Complete cage barrier	0 $\pm$ 0b	0 $\pm$ 0b	0 $\pm$ 0c	3.3 $\pm$ 1.9	5.0 $\pm$ 1.7	8.3 $\pm$ 3.2c
Early cage barrier	16.7 $\pm$ 1.9a	8.3 $\pm$ 1.7a	8.3 $\pm$ 1.7ab	0 $\pm$ 0	0 $\pm$ 0	33.3 $\pm$ 2.7b
Later cage barrier	18.3 $\pm$ 1.7a	6.7 $\pm$ 2.7a	3.3 $\pm$ 1.9bc	0 $\pm$ 0	1.7 $\pm$ 1.7	30.0 $\pm$ 4.3b
ANOVA ( <i>F</i> , <i>P</i> )	<i>F</i> = 14.4,	<i>F</i> = 4.18, <i>P</i>	<i>F</i> = 6.68,	<i>F</i> = 2.5,	<i>F</i> = 1.5,	<i>F</i> = 15.5,
df = 6, 21	<i>P</i> = 0.0001	= 0.006	<i>P</i> = 0.0005	<i>P</i> = 0.054	<i>P</i> = 0.225	<i>P</i> = 0.0001

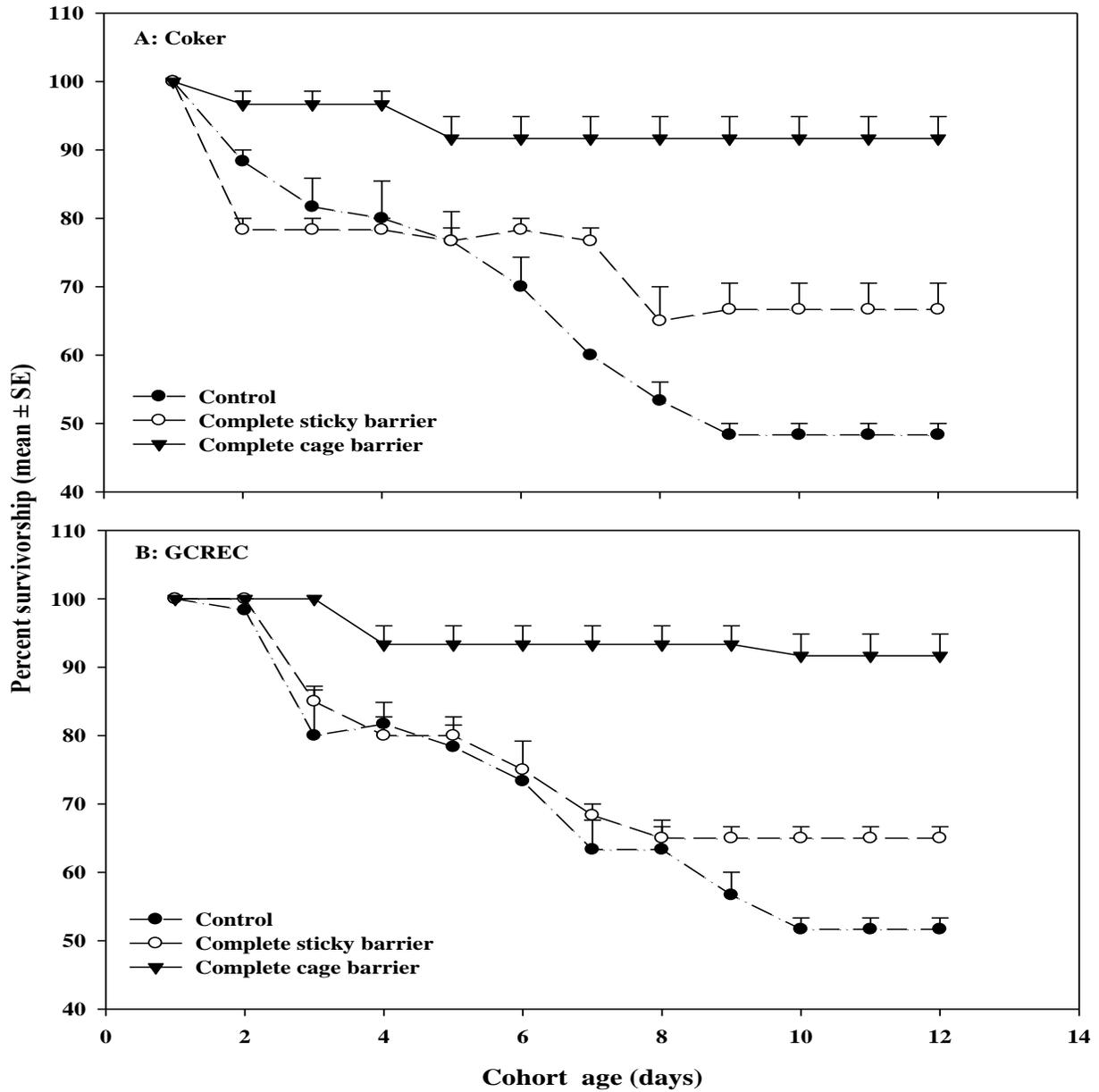
Means in the same column followed by the same or no letter are not significantly different ( $P > 0.05$ , Tukey-Kramer HSD test).

GCREC = Gulf Coast Research and Extension Center, Fairhope, AL.

**Figure 1.** Survivorship curve of *P. citrella* immature stage over whole development periods in natural and protected condition in the two orchards in 2007. Figure showed mean ( $\pm$  SE) numbers of *P. citrella*. A: Coker and B: GCREC (Gulf Coast Research and Extension Center, Fairhope, AL).



**Figure 2.** Survivorship curve of *P. citrella* immature stage over whole development periods in natural and protected condition in the two orchards in 2008. Figure showed mean ( $\pm$  SE) numbers of *P. citrella*. A: Coker and B: GCREC (Gulf Coast Research and Extension Center, Fairhope, AL).



## CHAPTER 6

### FUNCTIONAL RESPONSE AND PREY STAGE PREFERENCES OF THREE SPECIES OF PREDACEOUS MITES (ACARI: PHYTOSEIIDAE) WHEN OFFERRED CITRUS RED MITES, *PANONYCHUS CITRI* (ACARI: TETRANYCHIDAE)

#### INTRODUCTION

Citrus red mite, *Panonychus citri* (McGregor) (Acari: Tetranychidae), is a key pest of citrus in many parts of the world (Gotoh and Kubota 1997, Jamieson et al. 2005, Childers et al. 2007). The adults and immatures feed primarily on leaves producing tiny grey or silvery spots known as stippling damage, which may inhibit photosynthesis (Kranz et al. 1977). High infestations can result in premature leaf drop, shoot dieback and decreased plant vigor, as well as fruit feeding and damage (Kranz et al. 1977, Jamieson et al. 2005). *Panonychus citri* is a major pest of satsuma mandarin (*Citrus unshiu* Marcovitch.) in Alabama (English and Turnipseed 1940, Fadamiro et al. 2007, 2008). Satsuma mandarin production is a growing industry in southern Alabama and other parts of the Gulf Coast region of the United States (Campbell et al. 2004). The results of recent field surveys identified *P. citri* as one of the most numerically abundant pests in Alabama satsuma orchards (Fadamiro et al. 2007, 2008). Two to three generations per year were recorded for *P. citri* in southern Alabama, and the pest was most abundant in the spring (Fadamiro et al. 2008), when population densities were typically greater than the economic threshold of five motiles per leaf, proposed by Childers et al. (2007). The

results also showed that *P. citri* was more abundant in the exterior canopy than in the interior canopy, and infestations were more severe in conventionally sprayed orchards than in unsprayed orchards (Fadamiro et al. 2008).

Predacious mites, in particular those belonging to the families Phytoseiidae and Stigmaeidae, have been widely used for biological control of pest mites in fruits and other crops worldwide (Childers et al. 1975, McMurtry 1983, Childers 1994, Wood et al. 1994, Opit et al. 2004, Jamieson et al. 2005, Carmak et al. 2009, Authur et al. 2009), and may present an alternative to chemical control of *P. citri* in Alabama. A recent survey of the predacious mite fauna of satsuma mandarin in Alabama identified a total of 29 species from nine families, including 18 species in the family Phytoseiidae and one species in the family Stigmaeidae (Fadamiro et al. 2009). The dominant predacious mite species recorded were *Typhlodromalus peregrinus* Muma and *Proprioseiopsis mexicanus* (Garman) (Phytoseiidae), and *Agistemus floridanus* Gonzalez (Stigmaeidae) (Fadamiro et al. 2009). However, these predacious mites were recorded in the orchards at densities too low for effective suppression of *P. citri* (Fadamiro et al. 2009). Furthermore, recent attempts to mass rear *T. peregrinus* and other indigenous predacious mites for augmentative releases against *P. citri* have not been successful (Xiao and Fadamiro, unpublished data), prompting our interest in the evaluation of some commercially available phytoseiids (e.g., *Phytoseiulus persimilis*, *Galendromus occidentalis*, and *Neoseiulus californicus*) as potential biological control agents of *P. citri*. An assessment of the functional and numerical responses and prey-stage preferences of these phytoseiids when offered *P. citri* is a critical first step in determining their ability to regulate the prey.

The functional response concept first described by Holling (1959) has been widely utilized to evaluate effectiveness of predacious mites (Laing and Osborn 1974, Everson 1980,

Sabelis 1985, Badii et al. 2004, Reis et al. 2003, 2007). In general, the functional response of a predator to a prey could follow one of three mathematical models: linear, convex or sigmoid. In the linear (type I) model, the number of prey consumed increases linearly with prey availability up to a maximum. In the convex (type II) model, the number of prey consumed increases with prey availability but begins to decrease when a maximum point is reached. In the sigmoid (type III) model, the relationship between numbers of prey consumed and prey availability follow a sigmoid form up to a maximum (Holling 1959, 1961). This study was carried out to determine the potential of three commercially available phytoseiid species, *P. persimilis*, *G. occidentalis* and *N. californicus*, as biological control agents of *P. citri* by evaluating their functional responses and prey-stage preferences. Additional experiments were conducted to test the effect of starvation on the predation potential and prey-stage preferences of the three phytoseiid species.

## MATERIALS AND METHODS

### Rearing of *P. citri* (the prey)

The starting colony of *P. citri* used as prey was collected from satsuma orchards in southern Alabama in spring 2008. Mixed stages of *P. citri* were reared on lima bean (*Phaseolus lunatus* L.) and/or satsuma leaves in transparent plastic containers (25 × 10 × 7 cm) with the top covered by screening net. Each container had about 10 fully expanded *P. citri*-infested lima bean or satsuma leaves placed ventral side up on a moistened paper towel, and then placed over a water-saturated sponge. This arrangement prevented mite escape and maintained leaf freshness for up to two weeks. The moistened paper towel and sponge were kept wet by adding water as

necessary. All rearing and experiments were conducted under laboratory conditions at  $25 \pm 2^\circ\text{C}$ ,  $60 \pm 10\%$  RH and a photoperiod of 14:10 (L: D) h.

### **Rearing of Predacious Mites**

The starting colonies of all three phytoseiid species (*P. persimilis*, *G. occidentalis*, and *N. californicus*) were purchased from Biocontrol Network, Inc., (Brentwood, TN) and reared on mixed stages of *P. citri* on satsuma leaves for two generations before the bioassays. The rearing method was as described above for *P. citri*. Adult females (~ 2 day old) of each species previously starved for 8 h were used for the bioassays. All experiments were conducted in 2008.

### **Experiment 1. Functional Response**

The functional response of each of the three phytoseiid species on *P. citri* was investigated in separate bioassays using a protocol similar to that described by Reis et al. (2003). For each species, an adult female was confined for seven days in a 3-cm diameter satsuma leaf disc arena, placed on a moistened filter paper and over a thin wet sponge, inside a Petri dish (8.0 cm diameter  $\times$  1.5 cm depth). *P. citri* nymphs (motile immatures) were introduced as prey onto the leaf disc inside the Petri-dish at densities of 2, 5, 10, 20, 30, 40, 50, 75, 100, and 150 per arena. The Petri dish was then sealed with parafilm to prevent mite escape. Each density treatment was replicated four times. The controls consisted of arenas with the same densities of *P. citri* nymphs but without predacious mite. The numbers of *P. citri* larvae killed and eggs laid by the predator were recorded daily for seven days. To maintain appropriate *P. citri* densities in the arenas, dead *P. citri* were replaced with live ones daily.

## Experiment 2. Prey-Stage Preference

Experiments were conducted to determine prey stage (egg or nymph) is preferred by each phytoseiid species. The experimental protocol used was similar to that described by Blackwood et al. (2001). For each species, an adult female was confined in a 3-cm diameter satsuma leaf disc arena (as described above) with *P. citri* eggs and nymphs at one of five ratios: 0:1, 1:0, 1:1, 1:2 and 2:1 eggs: nymphs. The last two ratios (1:2 and 2:1) were tested to evaluate prey-stage switching. A total of 60 prey per arena was tested for the first three ratios and 90 prey per arena for the 1:2 and 2:1 ratios. The experiment was replicated 10 times per ratio per species. The numbers of each prey-stage consumed by the predator were recorded after 24 h.

## Experiment 3. Effect of Starvation

Using the protocol described above, an adult female of each phytoseiid species was starved (held without food) for 0, 24, or 48 h before tests, and then introduced into a satsuma leaf arena containing 1:1 ratio of eggs and nymphs of *P. citri* (60 total prey per arena). The numbers of each prey stage consumed were recorded after 24 h. The experiment was replicated 10 times per species.

## Preference Quantification Methods

Prey-stage preferences were quantified with the index  $\beta$  (Manly et al., 1972), as described by Blackwood et al. (2001).

$$\beta = \left[ \frac{\ln(N' / N_c')}{\ln(N / N_c)} + 1 \right]^{-1}$$

$N$  (eggs) and  $N'$  (larvae) are the numbers of each prey type provided, and  $N_c$  (eggs) and  $N_c'$  (larvae) are the numbers of each prey type consumed. This index assigns preference values from 0 to 1, where values 0.5 represent no preference. The  $\beta$  value was calculated for each replicate and averaged to determine the mean  $\beta$  value for each treatment.

## **Statistical Analysis**

Data from the functional response experiment were first analyzed using regression analysis to determine the best fit model (Sigmpilot 8, Systat Software, 2007). Data were then normalized by using the square-root transformation ( $\sqrt{x + 0.5}$ ) and analyzed with analysis of variance (ANOVA) followed by Tukey-Kramer honest significant difference (HSD) test to determine significant differences among the three species ( $P < 0.05$ , JMP Version 7.0.1, SAS Institute Inc., 2007). Data from the experiments on prey-stage preference and effect of starvation were first normalized by using square-root transformation ( $\sqrt{x + 0.5}$ ) and then analyzed with ANOVA. Differences among treatments in mean numbers of prey killed or mean preference index ( $\beta$ ) were determined by using the Tukey-Kramer HSD test or Student's t-test ( $P < 0.05$ , JMP Version 7.0.1, SAS Institute Inc., 2007).

## **RESULTS**

### **Experiment 1. Functional Response**

The functional responses of the three phytoseiid species on *P. citri* are shown in Table 1 and Fig.1. Natural mortality of *P. citri* observed in the control was minimal: 4.6%, 9.3 %, and 6.7 % for *P. persimilis*, *G.occidentalis*, and *N. californicus*, respectively. This suggests that

predation by the predacious mites was the dominant source of *P. citri* mortality recorded in this experiment.

***P. persimilis.*** The number of *P. citri* killed per day increased from ~ 0.2 at a prey density of 2 per arena to a maximum of ~ 14 (~ 19 % predation) at a prey density of 75 per arena (Table 1, Fig. 1A). The percentage of prey killed (or percentage of predation) ranged from ~ 11 % at the lowest prey density (2 per arena) to 24 % at the 5 prey per arena density. The generally low percentage of predation suggests that most of the prey provided were not consumed by the predator. The lowest percentage of predation recorded at the lowest prey density was probably related to the difficulty in finding the prey at very low densities. A plot of the relationship between number of prey provided and number killed by female *P. persimilis* revealed a positive and highly significant correlation ( $R^2 = 0.9467$ ;  $F = 151.06$ ;  $P = 0.0001$ ; Fig. 1A). The number of prey killed increased as a function of prey density up to a maximum point (75 prey per arena) and then started to decrease, resulting in a convex (type II) functional response (Fig. 1A).

***G. occidentalis.*** The number of *P. citri* killed per day also rose as a function of prey density from ~ 1 at a prey density of 2 to a maximum of 12 (~ 17 % predation) at a prey density of 75 (Table 1, Fig. 1B). The percentage of predation ranged from ~ 7 % at the highest prey density (150 per arena) to 37 % at a prey density of 10 per arena. The number of *P. citri* killed was positively correlated to the number provided ( $R^2 = 0.921$ ;  $F = 41.23$ ;  $P = 0.0001$ ), and was expressed by a convex (type II) functional response curve (Fig. 1B).

***N. californicus.*** A similar trend was observed in the functional response of *N. californicus* on *P. citri*. The number of prey killed per day increased with density from 0.3 at a prey density of 2 per arena to a maximum of ~ 10 (~10 % predation) at a prey density of 100 (Table 1, Fig. 1C). The percentage of predation ranged from ~ 6 % at the highest prey density

(150 per arena) to 22 % at a prey density of 10 per arena. In general, predation by *N. californicus* was slightly lower than predation by *P. persimilis* and *G. occidentalis*. However, *N. californicus* demonstrated similar predation efficiency as the other two species at the lower prey densities of 30 or less per arena (Table 1 and Fig.1C). As with the other two species, a positive association ( $R^2 = 0.99$ ;  $F = 874.1$ ;  $P = 0.0001$ ) was recorded between the number of prey killed and the number provided up to a maximum point (100 prey per arena), which resulted in a convex (type II) functional response (Fig. 1C).

## **Experiment 2. Prey-Stage Preference**

Prey-stage preferences of the three predacious mite species are summarized in Table 2 and Fig. 2. When eggs and nymphs of *P. citri* were simultaneously presented, all three species fed on both stages but highly preferred nymphs, irrespective of the prey ratio (Table 2). This result was confirmed by the very high ( $> 0.65$ ) preference index ( $\beta$ ) recorded for all three species (Fig. 3):  $\beta$  values greater than 0.5 represent preference for nymphs while those less than 0.5 represent preference for eggs (Blackwood et al., 2001). When only eggs were provided, *P. persimilis* consumed more eggs (3.4 eggs) than *G. occidentalis* (2 eggs) and *N. californicus* (0.4 eggs). *Phytoseiulus persimilis* also consumed more nymphs than the other two species, when only this prey stage was provided (Table 2). Similar results were recorded when the number of each prey stage consumed was pooled across all ratios and compared among the three species. *Phytoseiulus persimilis* consumed significantly greater number of nymphs ( $F = 45.7$ ;  $df = 2, 87$ ;  $P = 0.0001$ ) than the other two species, and also significantly greater number of eggs than *N. californicus* ( $F = 5.7$ ;  $df = 2, 87$ ;  $P = 0.0047$ ) (Fig. 2). Also, the total number of prey (eggs + nymphs) consumed by *P. persimilis* was significantly greater than that consumed by the other

two species ( $F = 51.08$ ;  $df = 2, 87$ ;  $P = 0.0001$ ). The preferences of each species when offered eggs and nymphs of *P. citri* at ratios of 1:1, 1:2 and 2:1 were compared to determine prey-switching behavior, indicated by a significant change in  $\beta$ . Significant prey-switching was recorded for *G. occidentalis* ( $F = 5.50$ ;  $df = 2, 27$ ;  $P = 0.0099$ ), and *N. californicus* ( $F = 7.31$ ;  $df = 2, 27$ ;  $P = 0.0029$ ), but not for *P. persimilis* ( $F = 0.619$ ;  $df = 2, 27$ ;  $P = 0.546$ ). Both *G. occidentalis* and *N. californicus* exhibited negative switching, indicated by an increased preference for nymphs in response to an increase in the relative abundance of eggs (Fig. 3).

### **Experiment 3. Effect of Starvation**

No significant effect of starvation was recorded on prey consumption by the phytoseiids (*P. persimilis*: eggs:  $F = 0.135$ ,  $P = 0.874$ , nymphs:  $F = 1.77$ ,  $P = 0.189$ , total prey:  $F = 1.25$ ,  $P = 0.30$ ; *G. occidentalis*: eggs:  $F = 2.99$ ,  $P = 0.067$ , nymphs:  $F = 0.51$ ,  $P = 0.604$ , total prey:  $F = 1.33$ ,  $P = 0.28$ ; *N. californicus*: eggs:  $F = 0.345$ ,  $P = 0.71$ , nymphs:  $F = 2.71$ ,  $P = 0.084$ , total prey:  $F = 3.04$ ,  $P = 0.064$ ;  $df = 2, 27$ ) (Table 3). In other words, similar numbers of eggs and nymphs were consumed in the different starvation treatments. Furthermore, starvation had no significant effect on prey-stage preferences of phytoseiids: all three species showed similar preference for nymphs, irrespective of starvation duration. These results were also confirmed by the lack of significant change in  $\beta$  with starvation (*P. persimilis*:  $F = 0.211$ ,  $P = 0.81$ ; *G. occidentalis*:  $F = 0.965$ ,  $P = 0.39$ ; *N. californicus*:  $F = 0.764$ ,  $P = 0.48$ ;  $df = 2, 27$ ) (Fig. 4).

## **DISCUSSION**

The experimental results demonstrated the predation potential of the three

phytoseiids, *P. persimilis*, *G. occidentalis*, and *N. californicus*, on *P. citri*. The functional responses of all three species followed the type II convex model in which the number of prey consumed increased with prey availability, but began to decrease when a maximum point was reached. These results are in agreement with previous studies in which the relationship between most predacious mite species and their prey was reported to follow the type II convex functional response model (Holling 1961, Mori and Chant 1966, Sandness and McMurtry 1970, Laing and Osborn 1974, Santos 1975, Ryoo 1986, Reis et al. 2003, Badii et al. 2004). For instance, Laing and Osborn (1974) reported a functional type II response curve for *P. persimilis* and *G. occidentalis* feeding on *Tetranychus urticae* Koch. Chant (1961) also reported a type II curve for *P. persimilis* on *T. urticae*, but observed a type I linear curve for *G. occidentalis* on the same prey. Interference or disturbance by prey has been proposed by various authors to explain the functional and numerical responses of predacious mites on pest mites (Mori and Chant 1966, Sandness and McMurtry 1970, Reis et al. 2003). For instance, Mori and Chant (1966) suggested that high prey densities may result in the disturbance of predacious mites and thus decrease their functional or numerical responses. On the other hand, absence of “interference-stimulation” may explain the low predation efficiency recorded at low prey densities since contact with prey may have a stimulatory effect on predacious mites (Mori and Chant 1966, Sandness and McMurtry 1970, Reis et al. 2003).

Our results on prey-stage preference showed that all three phytoseiids (*P. persimilis*, *G. occidentalis*, and *N. californicus*) preferred nymphs to eggs of *P. citri*, and the nymphs were most often the first prey attacked by the predators. To our knowledge, this is the first documented evaluation of the predation potential of the three phytoseiids when offered *P. citri* as prey. However, previous studies in which the above or related phytoseiid species were evaluated

on *T. urticae* have produced varied results (Burnett 1971, Takafuji and Chant, 1976, Fernando and Hassell 1980, Blackwood et al. 2001, 2004). Blackwood et al. (2001) reported that adult females of *P. persimilis* preferred *T. urticae* eggs over the nymphs, whereas *G. occidentalis*, and *N. californicus* showed no prey-stage preference. In contrast, Popov and Kondryakov (2008) reported that adult females of *P. persimilis* and *G. occidentalis* consumed more nymphs and males of *Tetranychus* spp. than the eggs or females. These contrasting results may be related to differences in experimental design and number of prey provided. A comparison of our results with those in which a *Tetranychus* spp. was used as prey (e.g., Blackwood et al. 2001, Popov and Kondryakov 2008) suggest that prey-stage preference of predacious mites may vary with different prey species. Our observed preference of the three phytoseiids for *P. citri* nymphs over eggs may be related to differences in the size and mobility of the two stages. The phytoseiids may have preferred the motile nymphs, possibly because they are larger and active, thus increasing the possibility of being encountered by a predacious mite (Sabelis 1985). Our results may also be explained by possible differences in the nutritional benefits of prey nymphs versus eggs. Previous work on the relative nutritional value of *T. urticae* eggs and nymphs suggest that eggs may be a more profitable prey stage for some predacious mites (Burnett 1971, Croft and McMurty 1972, McMurty and Rodriguez 1987). For instance, Croft and McMurty (1972) showed that *G. occidentalis* females fed on eggs or nymphs of *Tetranychus pacifus* McGregor consumed twice as many nymphs as eggs but with similar fecundities, suggesting that the predator obtained more nutritional benefits from eggs. Thus, only a minimal consumption of eggs in a diet of mixed-prey stages may be necessary to achieve maximum fecundity (McMurty and Rodriguez 1987), which may explain the minimal consumption of *P. citri* eggs in our experiments. In contrast, consumption of nymphs may actually be more profitable than egg

consumption. Zaher and Shehata (1971) reported that *Typhlodromus pyri* Scheuten females had higher fecundity when feeding on mobile immatures of *Tetranychus cinnabarinus* Biosduval than on the eggs. Thus, our results may also suggest that *P. citri* nymphs are more profitable than the eggs to all three phytoseiids in terms of nutritional benefit and handling time. While no significant prey-switching was recorded for *P. persimilis*, both *G. occidentalis* and *N. californicus* exhibited negative prey switching, indicated by an increased preference for nymphs in response to an increase in the relative abundance of eggs. Blackwood et al. (2001) also reported significant prey-switching for some phytoseiid species on *T. urticae*, and suggested that this phenomenon may have been mediated by fixed preferences for particular ratios of prey stages.

The data which showed no significant effect of starvation on the total number of prey consumed by the three phytoseiids or their prey-stage preferences, are in agreement with the report by Blackwood et al. (2001), in which starvation had no effect on the prey-stage preference of *Neoseiulus fallacis* (Garman). In contrast, starvation had a significant effect on the foraging behavior of *P. persimilis* (Zhang and Sanderson 1992). The lack of significant changes in the foraging behavior of the phytoseiids even after 48 h of food deprivation may suggest that the phytoseiids can survive a few days of starvation, or that the risk of starvation-induced death is not high after two days of food deprivation (Pratt et al. 1999, Blackwood et al. 2001). The ability to survive a few to several days of food deprivation in the field may be an important survival strategy for predacious mites, in particular those species with specialized diets. However, food deprivation for a day or more may have a negative impact on the fecundity of predacious mites (Ohnesorge, 1981), but this was not tested in this study.

All three phytoseiids shared similar behavior and demonstrated strong potential in regulating *P. citri*. However, *P. persimilis* appeared to have a greater predation potential than the remaining two species. This conclusion is supported by a preponderance of evidence from the experiments which pointed to slightly greater predation efficiency for *P. persimilis*. For instance, *P. persimilis* consumed significantly greater number of prey than the other two species, in particular at high prey densities. However, very few eggs were laid by the phytoseiids, which made it difficult to compare their reproductive capabilities on *P. citri*.

Our results on the predation potential of the three phytoseiids on *P. citri* may be explained by their feeding habits and degree of diet specialization. Predacious mites in the family Phytoseiidae can be classified into four categories based on their feeding habits and related biological and morphological traits (McMurtry and Croft 1997). Type I phytoseiids (e.g., *Phytoseiulus* spp.) are specialized predators of spider mites, *Tetranychus* spp. Type II phytoseiids are selective predators of *Tetranychus* species. Examples include *Galendromus* spp. and some *Neoseiulus* spp. Type III phytoseiids (e.g., *Amblyseius* spp.) are generalist predators, while Type IV phytoseiids (e.g., *Euseius* spp) are specialized pollen feeders/generalist predators. Thus, *P. persimilis* is a type I phytoseiid, while *G. occidentalis*, and *N. californicus* are type II phytoseiids (McMurtry and Croft 1997). It is therefore not surprising that *P. persimilis*, which is relatively more specialized on spider mites, showed a slightly greater predation potential than *G. occidentalis*, and *N. californicus*, which are less specialized type II predators. The relative size of the phytoseiids and their prey consumption rates may also play a role. *Phytoseiulus* species are relatively large and both nymphs and adults have a high prey consumption rate compared to most species in other genera (Gilstrap and Friese 1985, McMurtry and Croft 1997). In addition, we also observed that *P. persimilis* was more active than *G. occidentalis* and *N. californicus*, a

behavior that may increase its probability of encountering a prey.

In conclusion, our results demonstrated for the first time the potential of the commercially available phytoseiids, *P. persimilis*, *G. occidentalis*, and *N. californicus*, as biological control agents of *P. citri*. Studies are ongoing to evaluate small scale releases of the phytoseiids against *P. citri* in Alabama satsuma orchards.

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**Table 1. Predation capacity of three species of predacious mites at different densities of the prey, *P. citri* under laboratory conditions.**

Densities / arena	Mean $\pm$ SE) number or percent per day								
	<i>P. persimilis</i>			<i>G. occidentalis</i>			<i>N. californicus</i>		
	No. prey killed	(%)	No. eggs laid	No. prey killed	(%)	No. eggs laid	No. prey killed	(%)	No. eggs laid
2	0.2 $\pm$ 0.2	10.7 $\pm$ 11.2	0 $\pm$ 0	0.9 $\pm$ 0.6	46.4 $\pm$ 31.0	0 $\pm$ 0	0.3 $\pm$ 0.3	12.5 $\pm$ 14.4	0 $\pm$ 0
5	1.2 $\pm$ 0.6	23.7 $\pm$ 13.2	0 $\pm$ 0	1.7 $\pm$ 0.8	34.6 $\pm$ 16.1	0 $\pm$ 0	0.9 $\pm$ 0.3	18.1 $\pm$ 6.7	0 $\pm$ 0
10	2.1 $\pm$ 0.8	21.3 $\pm$ 8.6	0 $\pm$ 0	3.7 $\pm$ 1.3	37.0 $\pm$ 13.2	0 $\pm$ 0	2.2 $\pm$ 0.8	21.8 $\pm$ 7.7	0 $\pm$ 0
20	4.3 $\pm$ 0.7	21.7 $\pm$ 3.5	0.1 $\pm$ 0.1	3.0 $\pm$ 1.1	14.8 $\pm$ 5.4	0 $\pm$ 0	3.3 $\pm$ 0.9	16.4 $\pm$ 4.6	0 $\pm$ 0
30	4.4 $\pm$ 2.3	14.9 $\pm$ 7.6	0.6 $\pm$ 0.3	4.7 $\pm$ 1.1	15.7 $\pm$ 3.7	0 $\pm$ 0	3.9 $\pm$ 0.8	12.9 $\pm$ 2.8	0.1 $\pm$ 0.1
40	7.5 $\pm$ 1.3	18.8 $\pm$ 3.2	0.1 $\pm$ 0.1	6.3 $\pm$ 3.2	15.8 $\pm$ 7.9	0.3 $\pm$ 0.2	5.1 $\pm$ 1.6	12.8 $\pm$ 3.9	0.1 $\pm$ 0.1
50	10.8 $\pm$ 2.2	21.6 $\pm$ 4.3	0.4 $\pm$ 0.3	7.2 $\pm$ 1.2	14.4 $\pm$ 2.4	0 $\pm$ 0	7.0 $\pm$ 2.0	14.0 $\pm$ 4.0	0.2 $\pm$ 0.1
75	14.3 $\pm$ 3.5	19.1 $\pm$ 4.7	0.7 $\pm$ 0.3	12.4 $\pm$ 2.1	16.5 $\pm$ 2.8	0.4 $\pm$ 0.3	7.6 $\pm$ 2.7	10.2 $\pm$ 3.6	0.1 $\pm$ 0.1
100	13.8 $\pm$ 3.2	13.8 $\pm$ 3.2	0.7 $\pm$ 0.3	10.2 $\pm$ 2.9	10.2 $\pm$ 2.9	0.3 $\pm$ 0.2	9.8 $\pm$ 2.3	9.8 $\pm$ 2.3	0.3 $\pm$ 0.2
150	10.7 $\pm$ 3.1	7.2 $\pm$ 2.1	0.4 $\pm$ 0.3	9.9 $\pm$ 2.4	6.6 $\pm$ 1.6	0.6 $\pm$ 0.3	8.7 $\pm$ 1.7	5.8 $\pm$ 1.1	0.3 $\pm$ 0.3

**Table 2. Prey-stage preference of three species of predacious mites when offered eggs and nymphs of *P. citri* at different ratios for 24 h under laboratory conditions.**

Species	Ratio (Eggs: Nymphs)	Mean ( $\pm$ SE) number of <i>P. citri</i> killed		<i>t</i> test
		Eggs	Nymphs	<i>t</i> , <i>P</i>
<i>P. persimilis</i>	1:0	3.4 $\pm$ 0.4	0 $\pm$ 0	-
	0:1	0 $\pm$ 0	6.7 $\pm$ 0.8	-
	1:1	0.9 $\pm$ 0.3b	7.3 $\pm$ 0.5a	10.2, 0.0001
	1:2	1.2 $\pm$ 0.5b	8.1 $\pm$ 0.4a	9.07, 0.0001
	2:1	1.0 $\pm$ 0.3b	7.6 $\pm$ 0a	9.31, 0.0001
<i>G. occidentalis</i>	1:0	2.0 $\pm$ 0.5	0 $\pm$ 0	-
	0:1	0 $\pm$ 0	4.7 $\pm$ 0.6	-
	1:1	0.6 $\pm$ 0.3b	4.4 $\pm$ 0.5a	7.08, 0.0001
	1:2	0.5 $\pm$ 0.2b	3.7 $\pm$ 0.3a	8.01, 0.0001
	2:1	0.5 $\pm$ 0.2b	5.1 $\pm$ 0.6a	8.74, 0.0001
<i>N. californicus</i>	1:0	0.4 $\pm$ 0.3	0 $\pm$ 0	-
	0:1	0 $\pm$ 0	4.0 $\pm$ 0.6	-
	1:1	0.0 $\pm$ 0.0b	1.6 $\pm$ 0.4a	9.01, 0.0001
	1:2	0.3 $\pm$ 0.2b	3.0 $\pm$ 0.7a	3.68, 0.0001
	2:1	0.3 $\pm$ 0.2b	4.6 $\pm$ 0.6a	8.78, 0.0001

For each species, means in the same row followed by different letters are significantly different ( $P < 0.05$ , t-test). Note that numbers of eggs versus nymphs consumed were not compared at the first two ratios (0:1 and 1:0)

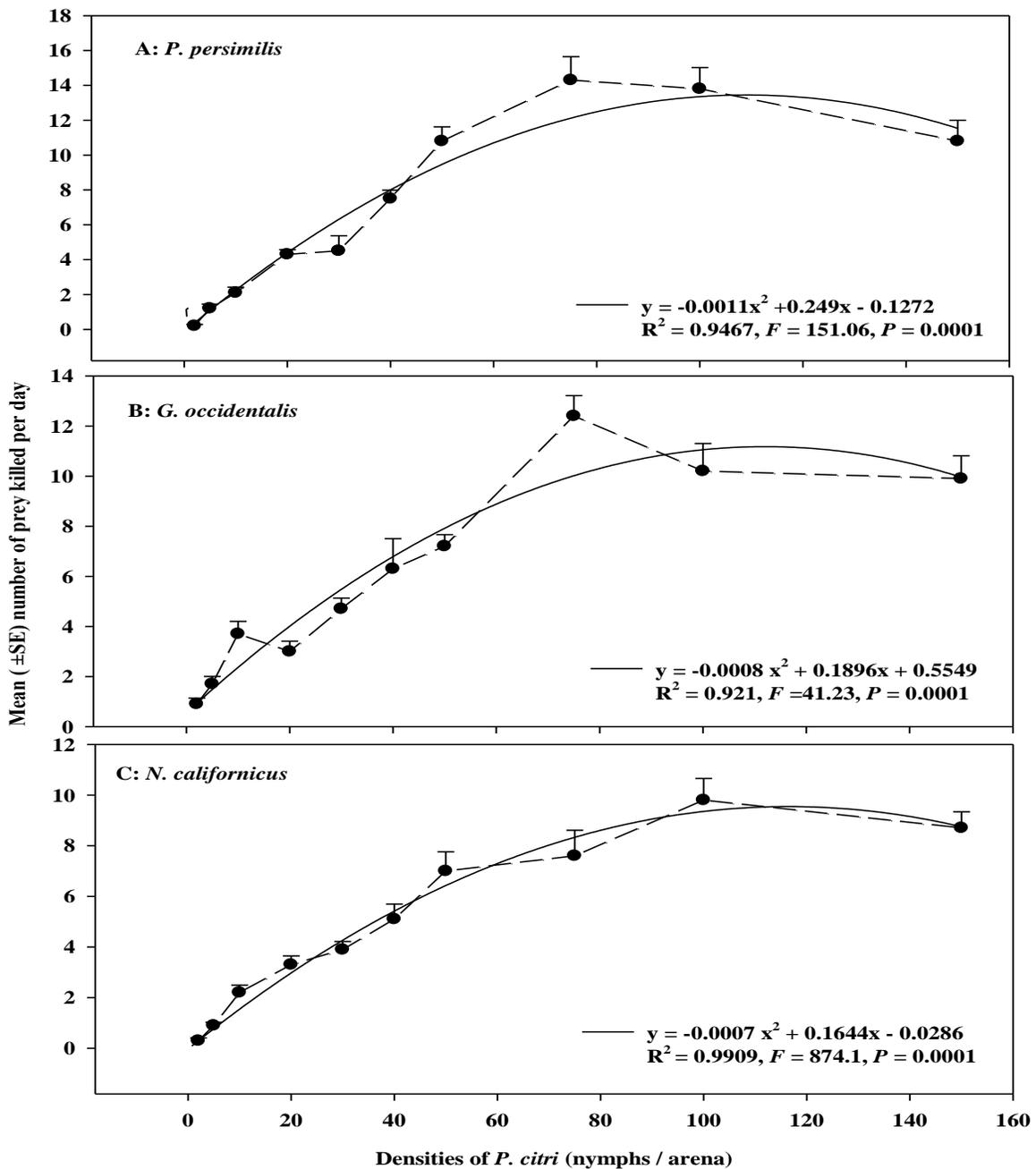
**Table 3. Effect of starvation on the predation capability of three species of predacious mites when offered a 1:1 ratio of *P. citri* eggs and nymphs.**

PM species	Starvation duration (h)	Mean ( $\pm$ SE) number of <i>P. citri</i> killed <sup>a</sup>		
		Eggs	Nymphs	Total
<i>P. persimilis</i>	0	1.4 $\pm$ 0.3	5.2 $\pm$ 0.4	6.6 $\pm$ 0.5
	24	1.5 $\pm$ 0.3	6.5 $\pm$ 0.7	8.0 $\pm$ 0.5
	48	1.4 $\pm$ 0.5	5.8 $\pm$ 0.6	7.2 $\pm$ 0.8
<i>G. occidentalis</i>	0	1.0 $\pm$ 0.3	6.0 $\pm$ 0.4	7.0 $\pm$ 0.6
	24	0.3 $\pm$ 0.2	5.2 $\pm$ 0.6	5.5 $\pm$ 0.7
	48	0.3 $\pm$ 0.2	5.8 $\pm$ 0.8	6.1 $\pm$ 0.8
<i>N. californicus</i>	0	0.5 $\pm$ 0.3	4.5 $\pm$ 0.6	5.0 $\pm$ 0.7
	24	0.7 $\pm$ 0.3	6.3 $\pm$ 0.5	7.0 $\pm$ 0.5
	48	0.3 $\pm$ 0.2	5.7 $\pm$ 0.6	6.0 $\pm$ 0.5

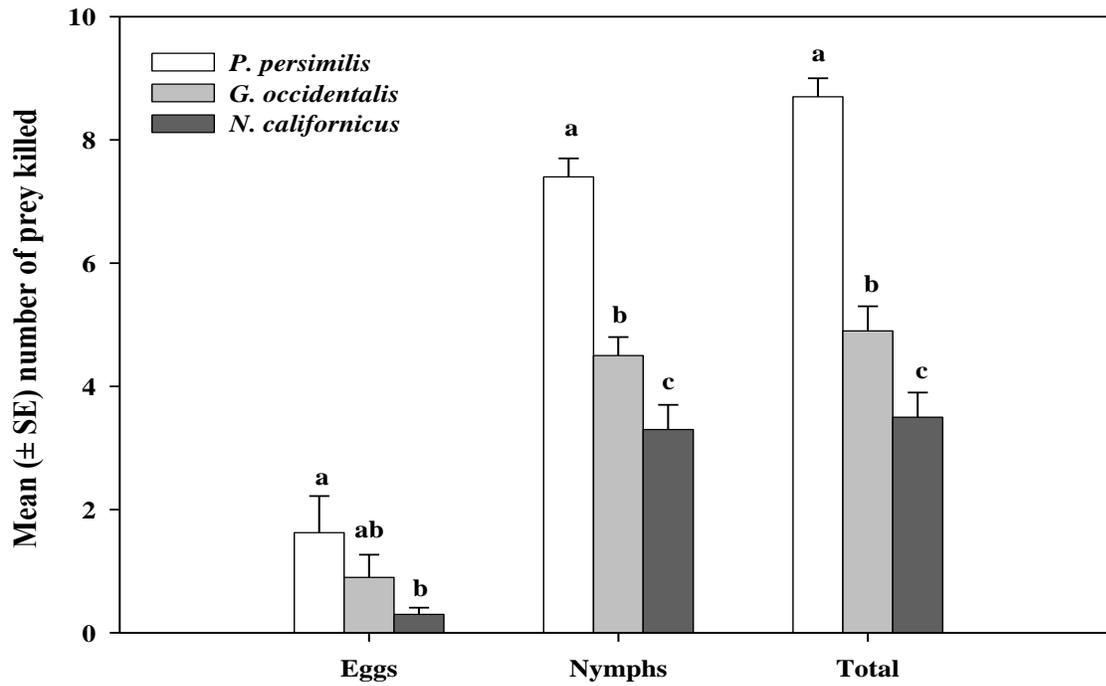
For each species, means in the same column followed by same or no letters are not significantly different ( $P < 0.05$ , Tukey HSD test).

**Figure 1.** Relationship between numbers of *P. citri* preyed on by a female of *P. persimilis* (A), *G. occidentalis* (B), or *N. californicus* (C) and the density of *P. citri* (nymphs) provided per day.

For all three species, the data followed the type II convex functional response model in which the number of prey consumed increased with prey availability but began to decrease when a maximum point was reached.

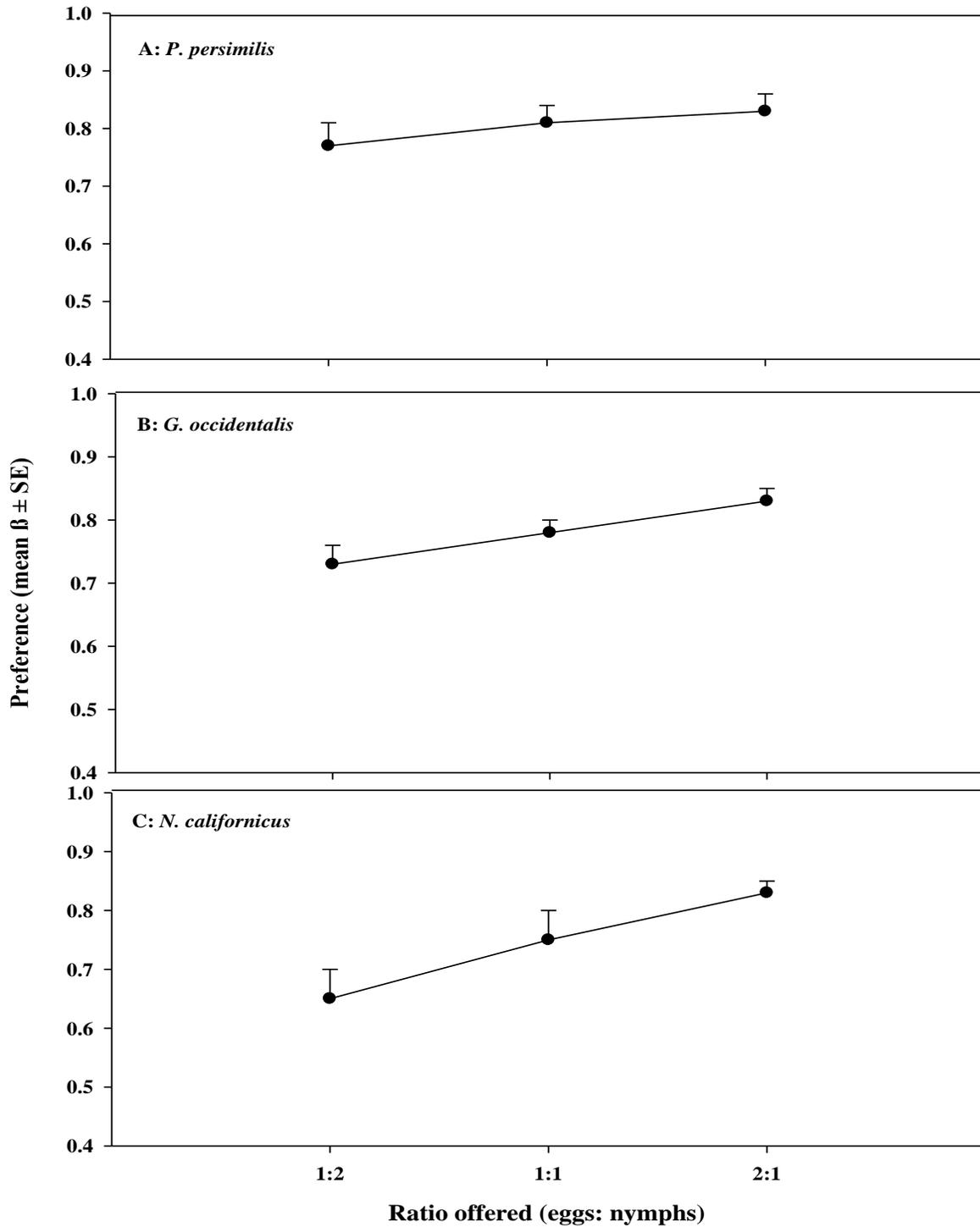


**Figure 2.** Predation potential of three phytoseiid species on *P. citri*. Figure shows mean ( $\pm$  SE) number of eggs, nymphs, and total number of prey killed by *P. persimilis*, *G. occidentalis*, and *N. californicus* in 24 h.

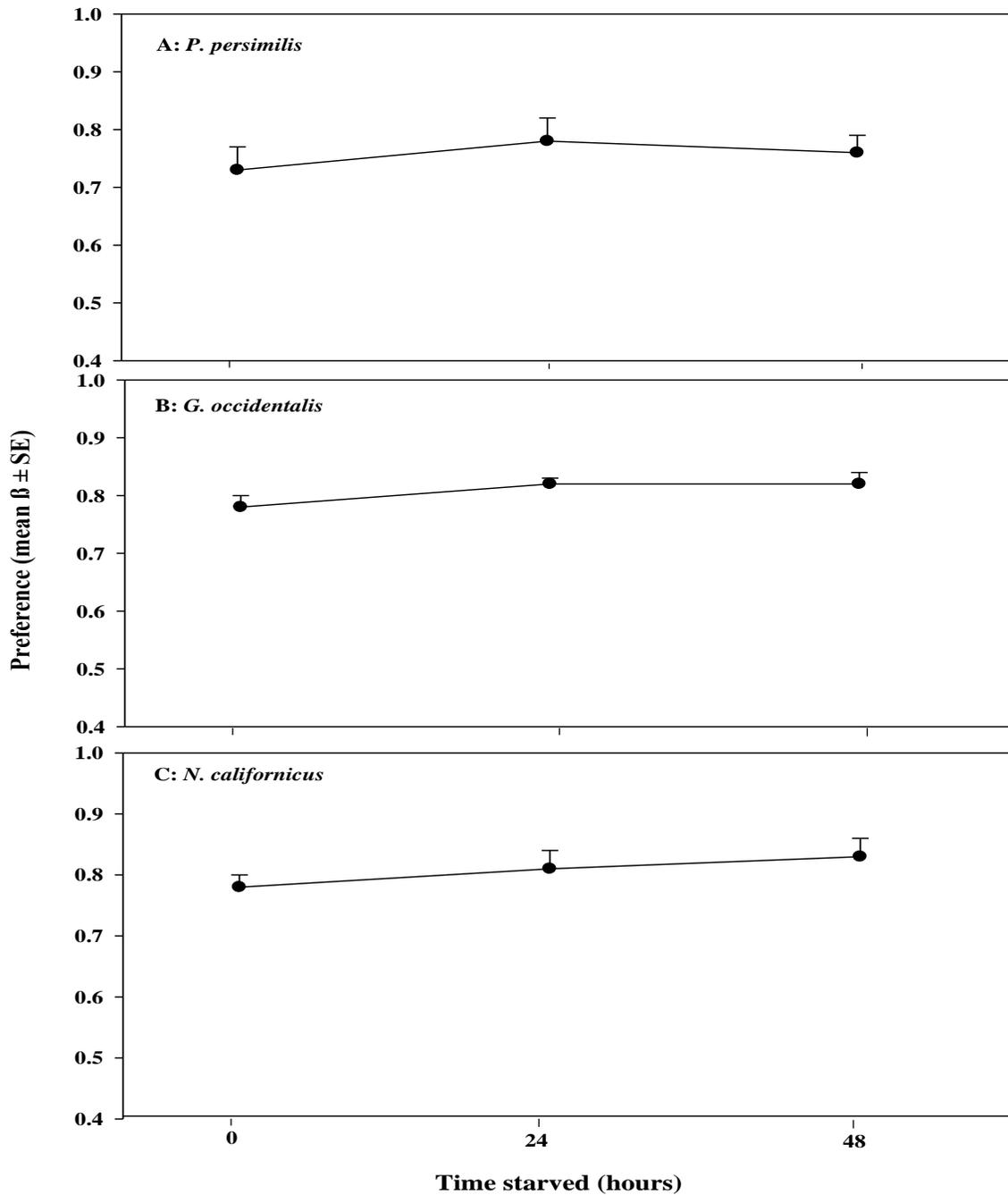


**Figure 3.** Prey-stage preferences of female *P. persimilis* (A), *G. occidentalis* (B), and *N. californicus* (C) when provided varying ratios (1:1, 1:2 or 2:1) of eggs and nymphs of *P. citri*.

Figure shows mean ( $\pm$  SE) preference index ( $\beta$ ).



**Figure 4.** Effect of starvation on the prey-stage preferences of three species of female *P. persimilis* (A), *G. occidentalis* (B), and *N. californicus* (C) when provided varying starvation level of 0, 24 h, and 48 h at a ratio (1:1) of eggs and nymphs of *P. citri*. Figure shows mean ( $\pm$  SE) preference index ( $\beta$ ).



## CHAPTER 7

# EVALUATION OF SMALL SCALE RELEASE OF THE PREDACIOUS MITES, *PHYTOSEIULUS PERSIMILIS* AND *GALENDROMUS OCCIDENTALIS* (ACARI: PHYTOSEIIDAE) FOR BIOLOGICAL CONTROL OF CITRUS RED MITES, *PANONYCHUS CITRI* (ACARI: TETRANYCHIDAE) IN ALABAMA SATSUMA CITRUS ORCHARDS

## INTRODUCTION

Satsuma mandarin (*Citrus unshiu* Marcovitch) production is a growing industry in southern Alabama and other parts of the Gulf Coast region of the United States (Campbell et al. 2004). This crop, which has been grown for over a century in Alabama (English and Turnipseed 1940), has received renewed interests in the last two decades, partly because of strong industry and state support and development of new markets (Campbell et al. 2004). The citrus red mite, *Panonychus citri* (McGregor) (Acari: Tetranychidae) was first reported as an important pest of satsuma mandarin in Alabama by English and Turnipseed (1940). In recent surveys, *P. citri* was identified as one of the most abundant pests in Alabama satsuma orchards, in particular conventionally sprayed orchards (Fadamiro et al. 2007, 2008). In Alabama, *P. citri* is typically a spring pest (Fadamiro et al. 2008), and population densities of the pest during this period are usually above the economic threshold of 5 motiles per leaf, proposed by Childers (1994). *Panonychus citri* is also a key pest of citrus in many other parts of the world (Gotoh and Kubota

1997, Jamieson et al. 2005, Childers et al. 2007). Both immatures and adults feed on citrus leaves. Severe leaf infestation may result in grey or silvery spots known as stippling damage, reduced photosynthesis, premature shoot dieback and decreased plant vigor (Kranz et al. 1977). High infestations may also result in fruit feeding and damage (Childers et al. 2007).

Historically, *P. citri* has been controlled in the Gulf Coast region and other parts of the world through applications of conventional acaricides (Childers 1994, Jamieson et al. 2005, Childers et al 2007). However, use of broad spectrum acaricides may speed up phytophagous mite resistance (Shanks et al. 1992, Omoto et al. 1995, Bergh et al. 1999), disrupt predators (Thistlewood 1991, Welty 1995, Antonelli et al. 1997, Jamieson et al. 2005, Urbaneja et al. 2008), and intensify food safety concerns. These concerns have resulted in renewed interests in the use of predacious mites for biological control of spider mites (McMurtry 1983, McMurtry and Croft 1997, Jamieson et al. 2005, Rhodes et al. 2006).

Predacious mites, in particular those belonging to the families Phytoseiidae and Stigmaeidae, have been widely evaluated in the laboratory and field for biological control of phytophagous mites in many crop systems (Childers et al. 1975, McMurtry 1983, Childers 1994, Wood et al. 1994, Takano-Lee and Hoddle 2001, 2002; Colfer et al. 2004, Opit et al. 2004, Pratt et al. 2002, Jamieson et al. 2005, Rhodes et al. 2006, Fraduo and Liburd 2007, Arthur et al. 2009, Cakmak et al. 2009). The key species evaluated in many systems include three commercially available phytoseiids, *Phytoseiulus persimilis* Athias-Henriot, *Galendromus occidentalis* (Nesbitt), and *Neoseiulus californicus* (McGregor), but some indigenous species have also been evaluated (Muma and Denmark 1970, Childers et al. 1975, Childers 1994). Childers (1994) reported on the potential use of certain indigenous species of predacious mites for biological control of pest mites on citrus in Florida, including *Typhlodromalus peregrinus* (Phytoseiidae)

and *Agistemus floridanus* (Stigmaeidae). A recent catalogue of the predacious mite fauna of satsuma mandarin in Alabama identified 29 species from nine families, with the dominant species being *Typhlodromalus peregrinus* Muma and *Proprioseiopsis mexicanus* (Garman) (Phytoseiidae), and *Agistemus floridanus* Gonzalez (Stigmaeidae) (Fadamiro et al. 2009). Many of these species (e.g., *T. peregrinus* and *P. mexicanus*) were found in association with *P. citri* (Fadamiro et al. 2008, 2009). However, abundance of predacious mites in local orchards was generally too low to effect significant suppression of phytophagous mites (Fadamiro et al. 2009). Attempts to mass rear the key indigenous predacious mites for augmentative releases against *P. citri* in Alabama orchards have not been successful (Xiao and Fadamiro, unpublished data), and thus our interest in evaluating commercially available phytoseiids as potential biological control agents for *P. citri*.

In a recent laboratory study, we evaluated the predation potential of three commercially available phytoseiids, *P. persimilis*, *G. occidentalis*, and *N. californicus*, on *P. citri* (Xiao and Fadamiro in review). *Phytoseiulus persimilis* is a type I specialist predator of spider mites, while *G. occidentalis*, and *N. californicus* are type II selective predators of spider mites (McMurtry and Croft 1997, Blackwood et al. 2001, Croft and Jung 2001, Fraduo and Liburd 2007). Our study included a series of experiments to assess the numerical and functional responses and prey-stage preferences of the phytoseiids when offered *P. citri* as prey. The results showed that all three species were effective in regulating *P. citri* density, preferred *P. citri* nymphs over eggs, and showed a functional type II (convex) response in which the number of prey consumed increased with prey availability up to a maximum point after which it slowly began to decrease. However, the two most effective species were *P. persimilis* and *G. occidentalis* (Xiao and Fadamiro, in review). These initial findings coupled with their favorable life history traits (McMurtry and

Croft 1997, Pratt and Croft 2000), aided our selection of both species for further evaluation in the field.

In this study, field experiments were conducted to evaluate the effectiveness of small-scale releases of *P. persimilis* or *G. occidentalis* at different rates and frequencies for suppression of *P. citri* in satsuma orchards in southern Alabama.

## MATERIALS AND METHODS

### Study Sites

Field trials were conducted in Coker orchard (N 30° 33.598', W 87° 48.026') located in Baldwin county, south Alabama during 2007 and 2008. The orchard had ~ 250 citrus trees comprised primarily of satsuma mandarin, with a few sweet orange (*Citrus sinensis* (L.) Osbeck), grapefruit (*C. paradisi* Macfad), and kumquat (*Fortunella* spp.). The predominant cultivar of satsuma mandarin was 'Owari'. The orchard has a history of high infestation of *P. citri* and is typically managed using conventional practices including routine applications of pesticides. However, no pesticides were applied in the orchard during this study. Temperatures at this location during the experiments in spring (March-May) 2007 and 2008 were 17.1- 24 °C (minimum: 10 °C, maximum: 29.4 °C), and 15.3 - 24.0°C (minimum: 9.5 °C, maximum: 28.6 °C), respectively. Relative humidity in both years was around 65 -75 %.

### Predacious Mites

The phytoseiids, *P. persimilis* and *G. occidentalis* were purchased from Biocontrol Network, Inc. (Brentwood, TN) and kept separately in a cooler (4 °C) for 1-2 days, if the weather was unsuitable for immediate release.

## General Procedure for Predacious Mite Release and Sampling

Four separate field experiments were conducted during 2007 and 2008 in the test orchard to determine the effectiveness of releases of *P. persimilis* or *G. occidentalis* at different rates and frequency in suppressing *P. citri*. First, satsuma trees in the orchard were sampled for *P. citri* using a protocol described by Fadamiro et al. (2008), to determine initial population densities. The trees were then classified into one of two groups based on the *P. citri* threshold proposed by Childers et al. (2007): i) trees with high initial *P. citri* density (i.e. > 120 motiles per 24 leaves, or > 5 motiles per leaf), and ii) trees with moderate initial *P. citri* density (i.e. ~ 72-96 motiles per 24 leaves or 3-4 motiles per leaf). The first three experiments were conducted on trees with high initial densities of *P. citri*, whereas the fourth experiment was conducted on trees with moderate initial prey densities. All selected trees were of similar size (canopy of 2 m height, 1.5 m diameter) and had not previously been treated with pesticides during the season. Two test trees were separated by at least one “buffer” tree to minimize wind-aided dispersal of mites between test trees. The experiment was a randomized complete block design and trees were randomly assigned to the different treatments.

Each shipment of predacious mites from the supplier arrived in a plastic vial (6 cm high × 5 cm diameter) consisting of 500-1000 individuals on a carrier. The average number of predacious mites per carrier was estimated by rolling the vial to evenly disperse mites within the carrier, placing samples into a petri dish, and counting the number of predacious mites under a stereo dissecting microscope (20×). Prior to release, the predacious mites were tested for viability and activity by observing subset samples of ~ 20 individuals in a Petri-dish for about 10 min. The releases rates evaluated were based on the supplier recommendation (i.e. ~ 2000/acre for field releases), published rates (e.g., ~5000/ha in cotton, Colfer et al. 2004), and cost

considerations. For each species, individuals at the appropriate release rate were evenly distributed into four plastic containers (each with holes to allow predacious mites to exit and disperse), which were used as the release device. The four plastic containers were then hung on branches located on four sides (one container per side) of a test tree at ~ 1.5 m above the ground. Predacious mite releases were performed around 11 am to 2 pm when temperature was around 25 °C, at RH 65-70 %.

To evaluate efficacy of predacious mite releases, 24 randomly selected leaves (6 per side) were taken from each test tree (trees on which predacious mites were released) per sampling date. The leaves were collected in properly-labeled paper bags, held in a cooler and transported to the laboratory where they were examined under a microscope at 20 × magnification. The numbers of *P. citri* eggs and motiles (nymphs + adults) per leaf were counted and recorded. The total number of *P. citri* was calculated as the sum of eggs and motiles (i.e. all stages). Predacious mites were rarely observed on the leaves and thus were not recorded.

### **Experiment 1: *G. occidentalis* releases in 2007**

The first trial in 2007 evaluated releases of *G. occidentalis* on satsuma trees infested with high densities (> 5 motiles per leaf) of *P. citri*, based on pre-release samples collected on February 28, one day before the first predacious mite release (March 1). The following five treatments testing releases of *G. occidentalis* at different rates and frequencies were evaluated: 1) one single release (in early March) at a rate of 25 *G. occidentalis* per tree (G25-1); 2) one single release (in early March) at a rate of 50 *G. occidentalis* per tree (G50-1); 3) two releases (in early March and late March, respectively) at a rate of 25 *G. occidentalis* per release per tree (G25-2); 4) two releases (in early March and late March, respectively) at a rate of 50 *G. occidentalis* per

release per tree (G50-2); and 5) no-release control. Each treatment was replicated three times and data were collected at 14, 28, 42, and 56 days after the first predacious mite release (DAR).

### **Experiment 2: *G. occidentalis* releases in 2008**

A second experiment was conducted in 2008 to further evaluate releases of *G. occidentalis* on satsuma trees infested with high densities of *P. citri*. Higher release rates of the predacious mites were tested based on the results from the 2007 trial, which showed minimal efficacy at low release rates. The following treatments were evaluated: 1) two releases (in early March and late March, respectively) at a rate of 50 *G. occidentalis* per release per tree (G50-2); 2) two releases (in early March and late March, respectively) at a rate of 100 *G. occidentalis* per release per tree (G100-2); 3) two releases (in early March and late March, respectively) at a rate of 200 *G. occidentalis* per release per tree (G200-2); 4) one release (in early March) at a rate of 200 *G. occidentalis* per release per tree (G200-1); and 5) no-release control. Each treatment was replicated three times and data were collected at 7, 14, 28, 35, 49, and 63 days after the first predacious mite release.

### **Experiment 3: *P. persimilis* releases in 2008**

Similar to the *G. occidentalis* releases described in experiment 2, a third trial was conducted in 2008 to evaluate releases of *P. persimilis* on satsuma trees infested with high densities of *P. citri*. The following treatments were evaluated: 1) two releases (in early March and late March, respectively) at a rate of 50 *P. persimilis* per release per tree (P50-2); 2) two releases (in early March and late March, respectively) at a rate of 100 *P. persimilis* per release per tree (P100-2); 3) two releases (in early March and late March, respectively) at a rate of 200 *P.*

*persimilis* per release per tree (P200-2); 4) one release (in early March) at a rate of 200 *P.*

*persimilis* per release per tree (P200-1); and 5) no-release control. Each treatment was replicated three times and data were collected at 7, 14, 28, 35, 49, and 63 days after the first predacious mite release.

#### **Experiment 4: Releases of *G. occidentalis* or *P. persimilis* in 2008**

A fourth experiment was conducted in 2008 to evaluate single releases of *G. occidentalis* or *P. persimilis* at two release rates (low and high) on satsuma trees infested with moderate densities (3-4 motiles per leaf) of *P. citri*. The following treatments were evaluated: 1) one single release of *G. occidentalis* (in late March) at a rate of 100 per tree (G100-1); 2) one single release of *G. occidentalis* (in late March) at a rate of 200 per tree (G200-1); 3) one single release of *P. persimilis* (in late March) at a rate of 100 per tree (P100-1); 4) one single release of *P. persimilis* (in late March) at a rate of 200 per tree (P200-1); and 5) no-release control. Each treatment was replicated three times and data were collected at 7, 21 and 35 days after predacious mite release.

#### **Statistical analysis**

Data for each experiment and predacious mite species were analyzed and presented separately. Mean number of *P. citri* (eggs, motiles and total) per sampling data were computed for each treatment and used for statistical analysis. Data were not normally distributed and thus were normalized by using the square-root transformation ( $\sqrt{x + 0.5}$ ). The transformed data were then analyzed with one-way analysis of variance (ANOVA) followed by the Tukey-Kramer honestly significant difference (HSD) comparison test to test for significant treatment effect on each sampling data ( $P < 0.05$ , JMP Version 7.0.1, SAS Institute Inc. 2007).

## RESULTS

### Experiment 1: *G. occidentalis* Releases in 2007

Pre-release sampling of *P. citri* on February 28 showed no significant differences among the treatments in the number of *P. citri* eggs, motiles, and the total number of all stages (Table 1). The first release of *G. occidentalis* was made on March 1. Data collected 14 days (March 14) after the first release (DAR) of *G. occidentalis* showed no significant differences among the treatments. However, significant differences were recorded among the treatments at 28 DAR. Significantly lower numbers of *P. citri* eggs and motiles were recorded in the treatment in which *G. occidentalis* was released twice at a rate of 50 per tree (G50-2) than in the control (Table 1), but no differences were recorded among the treatments at 42 (for motiles) and 56 DAR. Note that the second release of predacious mites was made in the appropriate treatments (G25-2 and G50-2) on March 28. Although densities of *P. citri* were lower in some treatments than in the control, high densities above the economic threshold (> 5 motiles per leaf) were recorded in all treatments in most sampling dates, with the exception of the last sampling date (56 DAR) when relatively lower densities (were recorded across the board (Table 1).

### Experiment 2: *G. occidentalis* Releases in 2008

Data collected during the pre-release sampling of *P. citri* on February 29 showed no significant differences among the treatments in the density of *P. citri* (Table 2). The first release of *G. occidentalis* was made on March 1. Significant differences were recorded among the treatments as early as 7 DAR. Significant differences were also recorded at 14 DAR for eggs, 28 DAR for eggs, 35 DAR, 49 DAR for motiles, and 63 DAR for eggs. In general, lower *P. citri* densities were recorded in all the treatments compared to control. For instance, the numbers of *P.*

*citri* eggs were significantly lower in all the *G. occidentalis* treatments than in the control at 7, 14, 28, and 35 DAR. At 63 DAR, significantly lower numbers of *P. citri* eggs were recorded in the G200-1 and G200-2 treatments than in the remaining treatments, suggesting that release rate of 200 *G. occidentalis* per tree was more effective than the lower release rates (Table 2). The G200-2 treatment also had the lowest number of *P. citri* motiles at 49 DAR. Note that the second release of *G. occidentalis* was made in the appropriate treatments (G50-2, G100-2 and G200-2) on March 28.

### **Experiment 3: *P. persimilis* Releases in 2008**

The trends for the *P. persimilis* releases were generally similar to those recorded for *G. occidentalis* in Experiment 2. Pre-release sampling was conducted on February 29, followed by the first predacious mite release on March 1. The pre-release data showed no significant effect among treatments on *P. citri* density, but significant differences were recorded among the treatments at 7 (only for eggs), 14, 28, 35, 49, and 63 DAR (Table 3). In almost all the cases, densities of *P. citri* in the control were significantly greater than in the treatments. In general, the lowest densities were recorded in the P200-2 treatment, suggesting that this treatment may be more effective than the others. Surprisingly, the lowest number of *P. citri* motiles was recorded in the control at 63 DAR, but this may be due to a general crashing of *P. citri* populations at that time of the season. Note that the second release of *P. persimilis* was made in the appropriate treatments (G50-2, G100-2 and G200-2) on March 28.

### **Experiment 4: Releases of *G. occidentalis* or *P. persimilis* in 2008**

In this experiment the two predacious mite species were released (in separate treatments) once on trees with initial moderate densities of *P. citri*. Pre-release data were collected on March 28, and the first predacious mite release was made on March 29. No significant differences were recorded among the treatments in the pre-release density of *P. citri*, and also at 7 DAR. However, significant differences in the numbers of *P. citri* eggs and motiles were recorded among the treatments at 21 and 35 DAR. In general, *P. citri* densities were higher in the control than in the treatments. No clear differences were recorded between *G. occidentalis* and *P. persimilis* releases, suggesting a similar efficacy for both predacious mite species.

### **Population Dynamics of *P. citri* on Experimental Trees**

Data from Experiments 2 (*G. occidentalis* releases) and 3 (*P. persimilis* releases) were presented to illustrate the population dynamics of *P. citri* in the different treatments (Fig. 1). In general, *P. citri* density (total count of all stages) in the control increased from ~ 50 per leaf at the beginning of the experiment on March 6 to a high ~ 360 per leaf on April 4, and then declined to ~ 57 per leaf by the last sampling on May 2. In general, a similar trend in population dynamics of *P. citri* was recorded in the predacious mite treatments, only that densities of *P. citri* were much lower in some treatments (Fig. 1).

## **DISCUSSION**

Several conclusions can be drawn based on the results from all four experiments. First, varying levels of success were achieved with releases of either *G. occidentalis* or *P. persimilis* on trees with moderate to high initial densities of *P. citri*, with release rate and frequency being the key factors determining the ability of the predacious mites to maintain effective long-term

suppression of the prey. Second, predacious mites were more effective at high release rates than at lower rates. Third, releases were more successful at moderate than at high prey densities. Fourth, both predacious mite species showed similar efficacy in suppressing *P. citri*, but were detected at very low population densities throughout the trial, indicative of poor establishment and reproduction. Other authors have also reported that release rate/ratio, frequency and timing of release, and initial prey density are critical factors that may impact successful biological control of phytophagous mites by predacious mites (Hamlen and Lindquist 1981, Hoddle et al. 2000, Pratt and Croft 2002, Shrewsbury and Hardin 2003, Opit et al. 2004, Fraulo and Liburd 2007). In general, predacious mites were more effective at higher release rates and frequencies and at low initial prey densities. Furthermore, Fraulo and Liburd (2007) reported that release rate and frequency had a greater impact than release timing, on the ability of *Neoseiulus californicus* (McGregor) to provide effective and season-long suppression of spider mites on strawberry.

Although results from the first three experiments suggest that the predacious mites were not consistently effective when released on trees with high population densities of *P. citri*, data from the last experiment using trees with moderate *P. citri* density showed that one single releases of *G. occidentalis* or *P. persimilis* at a rate of 100 or more per tree provided effective, long-term suppression of *P. citri* below the economic threshold, whereas densities on control trees dramatically increased during this period. These results are consistent with the reports which showed that small to large scale releases of *G. occidentalis*, *P. persimilis* or other phytoseiids provided effective suppression of spider mite populations in many crop systems, including strawberries (McMurtry 1982, 1991, Van de Vrie and Price 1994, Fraulo and Liburd 2007, Cakmak et al. 2009), avocado (Hoddle et al. 2000, Takano-Lee and Hoddle 2001), hops (Strong and Croft 1995, 1996), ivy geranium (Opit et al. 2004), greenhouse vegetable crops

(McMurtry 1991, Arthurs et al. 2009), and ornamental plants (Hamlen and Lindquist 1981, Pratt and Croft 1998). In contrast, a few authors reported little or no efficacy of predacious mite releases in suppression of spider mites in some crops (Shrewsbury and Hardin 2003, Colfer 2004). Shrewsbury and Hardin (2003) reported that predator releases were more expensive and provided less effective suppression of spruce spider mites, *Oligonychus ununguis* (Jacobi) on juniper, compared with conventional pesticides. Similarly, Colfer et al. (2004) reported that large-scale releases of *G. occidentalis* did not provide economic control of spider mite in cotton.

Our results which showed the field efficacy of both predacious mite species in suppressing *P. citri*, in particular at high release rates and moderate prey densities, are consistent with the results of a recent laboratory study which also demonstrated the ability of both species to regulate the prey (Xiao and Fadamiro, in review). The data which showed reduced efficacy of both species at high prey densities are also in agreement with previous reports by other authors (e.g., Shrewsbury and Hardin 2003, Colfer et al. 2004). Several factors may explain of the lack of efficacy of both predatory mite species recorded at high prey densities. In Alabama satsuma orchards, *P. citri* is typically a major pest in early spring (February to April) with populations starting to decline in late April and eventually crashing by early summer (mid-May) (Fadamiro et al. 2007, 2008). Thus, this temporal (8-9 weeks) seasonal occurrence may have provided limited time and opportunity for the establishment and reproduction of predacious mites (McMurtry and Croft 1997), and this may also explain our observation of very low population densities of both predacious mite species on the test trees throughout the trial. Shrewsbury and Hardin (2003) also attributed the inability of multiple releases of *N. fallacies* and *G. occidentalis* to control *O. ununguis* on juniper, to the temporal seasonal abundance of the pest. Secondly, our data showing poor efficacy of both predatory mite species at high prey densities may be related to low predator:

prey release ratios (Greco et al. 2005, Easterbrook et al. 2001, Opit et al. 2004, Fraudo and Liburd 2007). For example, Sato et al. (2007) reported that three releases of *N. californicus* without pesticides were not sufficient to significantly reduce populations of *T. urticae*, possibly because of low initial predator: prey release ratio. In addition, the observed poor efficacy of both predacious mites at high prey densities may possibly be due to intense interference between predator and prey, which may have negatively impacted establishment and performance of the predacious mites (Mori and Chant 1966, Pratt and Croft 2002, Shrewsbury and Hardin 2003).

Although predacious mite releases at high rates resulted in lower densities of *P. citri* motiles, densities of prey eggs were consistently high in all treatments, suggesting that the predacious mites had minimal effects of *P. citri* eggs. These results are consistent with our findings in the laboratory which showed that both species preferred nymphs to eggs of *P. citri* (Xiao and Fadamiro in review). The fact that suppression of *P. citri* was achieved without any evidence of establishment of the predacious mites in the orchard is interesting and may suggest influence of other factors or interactions, other than predator release. Colfer et al. (2004) reported a similar result in which releases of *G. occidentalis* in cotton did enhance populations of the predator but resulted in lower spider mite densities, nonetheless. The authors proposed that the reduction in spider mite densities in plots in which the predator was released was probably caused by a chance event or a complicated set of effects which was triggered by predator mite releases (Colfer et al. 2004).

In conclusion, the results of this study demonstrated efficacy of small-scale releases of *P. persimilis* or *G. occidentalis* at release rates of 100 or more predacious mites per tree, in suppression of *P. citri* at moderate initial densities. The two species showed similar efficacy against *P. citri*, although *P. persimilis* was slightly more effective in at least one trial. Previous

studies suggest that both species can survive low to moderate starvation in the laboratory (Xiao and Fadamiro in review) and tolerate high field temperatures (McMurtry and Croft 1997). Moreover, both species are highly active with high prey searching efficiency (Pratt and Croft 2000, Blackwood et al. 2001), and appeared adapted to disturbed habitats, such as intensively-managed orchards (McMurtry and Croft 1997). These factors suggest that both species may fair well in the severe southern Alabama climate. Further field evaluations including cost analysis are necessary to determine the economic feasibility of large-scale biological control of *P. citri* with predacious mites. In this study, we evaluated releases of single predator species rather than multiple predator species. Previous studies that compared the effectiveness of single versus multiple predator species have produced contrasting results, ranging from negative effect (Rosenheim et al. 1995; Schausberger and Walzer 2001) to neutral (Denoth et al. 2002, Chow et al. 2008) or positive effect (Cakmak et al. 2009). Schausberger and Walzer (2001) found that interaction such as competition, interguild predation, and cannibalism affected the development and coexistence of mixed predator populations. In contrast, Cakmak et al. (2009) reported that combined releases of *N. californicus* and *P. persimilis* provided greater suppression of *Tetranychus cinnabarinus* (Boisd.) (Acari: Tetranychidae) on strawberry compared to releases of each predator species alone. Future studies will determine the efficacy of combined release of *G. occidentalis* and *P. persimilis*, as well as integration of predacious mites with petroleum oils (e.g., FC 435-66 oil) and other effective reduced-risk acaricides (Fadamiro et al. 2005), for managing *P. citri* in Alabama citrus orchards.

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**Table1. Effect of *G. occidentalis* releases on density of *P. citri* on satsuma trees (experiment 1, spring 2007)**

<i>P. citri</i> stage	Treatment	Mean ( $\pm$ SE) number of <i>P. citri</i> per 24 leaves				
		Pre-release (Feb 28)	14 DAR (Mar 14)	28 DAR (Mar 28)	42 DAR (Apr 11)	56 DAR (Apr 25)
Eggs	G25-1	1438 $\pm$ 416	3308 $\pm$ 776	7383 $\pm$ 785a	3025 $\pm$ 744a	506 $\pm$ 167
	G25-2	814 $\pm$ 216	1612 $\pm$ 626	5059 $\pm$ 848ab	732 $\pm$ 174b	316 $\pm$ 247
	G50-1	816 $\pm$ 174	2393 $\pm$ 785	3807 $\pm$ 874ab	2523 $\pm$ 587a	620 $\pm$ 100
	G50-2	984 $\pm$ 164	1244 $\pm$ 414	2976 $\pm$ 848b	2629 $\pm$ 390a	571 $\pm$ 90
	Control	921 $\pm$ 191	777 $\pm$ 389	6225 $\pm$ 654ab	1729 $\pm$ 185ab	380 $\pm$ 253
		$F = 0.871,$ $P = 0.514$	$F = 2.564,$ $P = 0.103$	$F = 4.840,$ $P = 0.019$	$F = 5.080,$ $P = 0.017$	$F = 0.792,$ $P = 0.556$
Motiles	G25-1	204 $\pm$ 77	516 $\pm$ 132	889 $\pm$ 235a	343 $\pm$ 52	218 $\pm$ 159
	G25-2	132 $\pm$ 45	271 $\pm$ 107	350 $\pm$ 85ab	293 $\pm$ 33	48 $\pm$ 31
	G50-1	209 $\pm$ 41	187 $\pm$ 98	430 $\pm$ 33ab	551 $\pm$ 85	69 $\pm$ 4
	G50-2	97 $\pm$ 18	217 $\pm$ 44	289 $\pm$ 79b	263 $\pm$ 79	118 $\pm$ 36
	Control	104 $\pm$ 21	202 $\pm$ 132	735 $\pm$ 58a	340 $\pm$ 81	99 $\pm$ 39
		$F = 1.270,$ $P = 0.344$	$F = 1.350,$ $P = 0.318$	$F = 5.090,$ $P = 0.017$	$F = 0.509,$ $P = 0.731$	$F = 0.661,$ $P = 0.634$

Means within the same column (sampling date) for each stage with different letters are significantly different ( $P < 0.05$ , HSD,  $df = 4, 10$ ). G 25-1: one release of *G. occidentalis* at the rate of 25/tree; G25-2: two releases at the rate of 25/tree; G50-1: one release at the rate of 50/tree; G50-2: two releases at the rate of 50/tree; Control: no releases. Note: First and second releases of PM were made on March 1 and Mar 28, respectively. DAR: days after the first release of predacious mites.

**Table 2. Effect of *G. occidentalis* releases on density of *P. citri* on satsuma trees (experiment 2, spring 2008)**

<i>P. citri</i> stage	Treatment	Mean ( $\pm$ SE) number of <i>P. citri</i> per 24 leaves						
		Pre-release (Feb 29)	7 DAR (Mar 7)	14 DAR (Mar 14)	28 DAR (Mar 28)	35 DAR (Apr 4)	49 DAR (Apr 18)	63 DAR (May 2)
Eggs	G50-2	1253 $\pm$ 205	879 $\pm$ 121b	1603 $\pm$ 129b	3069 $\pm$ 160b	5984 $\pm$ 379b	4524 $\pm$ 758	1210 $\pm$ 156a
	G100-2	1282 $\pm$ 377	956 $\pm$ 99b	1684 $\pm$ 140b	2536 $\pm$ 529b	4257 $\pm$ 420b	4110 $\pm$ 883	1022 $\pm$ 159a
	G200-1	925 $\pm$ 80	929 $\pm$ 102b	1388 $\pm$ 236b	2657 $\pm$ 426b	3757 $\pm$ 619b	3365 $\pm$ 303	524 $\pm$ 94b
	G200-2	1225 $\pm$ 61	801 $\pm$ 44b	1642 $\pm$ 99b	1763 $\pm$ 136b	4088 $\pm$ 542b	3299 $\pm$ 345	453 $\pm$ 38b
	Control	1092 $\pm$ 141	1586 $\pm$ 100a	3659 $\pm$ 456a	5473 $\pm$ 539a	7218 $\pm$ 729a	3503 $\pm$ 419	1309 $\pm$ 126a
		$F = 0.534,$ $P = 0.714$	$F = 11.08,$ $P = 0.0011$	$F = 14.32,$ $P = 0.0004$	$F = 10.74,$ $P = 0.0012$	$F = 6.445,$ $P = 0.007$	$F = 0.735,$ $P = 0.588$	$F = 12.86,$ $P = 0.0006$
Motiles	G50-2	201 $\pm$ 69	53 $\pm$ 39	379 $\pm$ 144	678 $\pm$ 132	672 $\pm$ 146b	1019 $\pm$ 170ab	76 $\pm$ 4
	G100-2	121 $\pm$ 73	38 $\pm$ 8	237 $\pm$ 54	569 $\pm$ 268	714 $\pm$ 26b	702 $\pm$ 131bc	78 $\pm$ 3
	G200-1	155 $\pm$ 5	49 $\pm$ 8	220 $\pm$ 137	242 $\pm$ 58	592 $\pm$ 142b	591 $\pm$ 127bc	82 $\pm$ 24
	G200-2	112 $\pm$ 24	77 $\pm$ 21	329 $\pm$ 52	460 $\pm$ 39	390 $\pm$ 154b	357 $\pm$ 123c	53 $\pm$ 4
	Control	116 $\pm$ 37	156 $\pm$ 63	569 $\pm$ 126	953 $\pm$ 226	1399 $\pm$ 210a	1649 $\pm$ 312a	52 $\pm$ 9
		$F = 0.584,$ $P = 0.681$	$F = 3.042,$ $P = 0.07$	$F = 1.410,$ $P = 0.297$	$F = 2.830,$ $P = 0.083$	$F = 9.050,$ $P = 0.002$	$F = 9.581,$ $P = 0.002$	$F = 1.250,$ $P = 0.349$

Means within the same column for each stage with different letters are significantly different ( $P < 0.05$ , HSD,  $df = 4, 10$ ). G50-2: two releases of *G. occidentalis* at the rate of 50/tree; G100-1: one release at the rate of 100/tree; G200-1: one release at the rate of 200/tree; G200-2: two releases at the rate of 200/tree; Control: no releases. Note: First and second releases of PM were made on March 1 and March 28, respectively. DAR: days after the first release of predacious mites.

**Table 3. Effect of *P. persimilis* releases on density of *P. citri* on satsuma trees (experiment 3, spring 2008)**

<i>P. citri</i> stage	Treatment	Mean ( $\pm$ SE) number of <i>P. citri</i> per 24 leaves						
		Pre-release (Feb 29)	7 DAR (Mar 7)	14 DAR (Mar 14)	28 DAR (Mar 28)	35 DAR (Apr 4)	49 DAR (Apr 18)	63 DAR (May 2)
Eggs	P50-2	930 $\pm$ 64	915 $\pm$ 37b	1788 $\pm$ 76b	2947 $\pm$ 401b	4594 $\pm$ 591b	5393 $\pm$ 238a	1446 $\pm$ 75a
	P100-2	1009 $\pm$ 223	868 $\pm$ 89b	1967 $\pm$ 139b	1899 $\pm$ 259b	4002 $\pm$ 336b	4525 $\pm$ 196ab	1430 $\pm$ 184a
	P200-1	983 $\pm$ 90	843 $\pm$ 22b	1316 $\pm$ 58b	1986 $\pm$ 144b	4240 $\pm$ 303b	4108 $\pm$ 611ab	1418 $\pm$ 51a
	P200-2	1276 $\pm$ 167	463 $\pm$ 52c	1437 $\pm$ 70b	2848 $\pm$ 620b	3417 $\pm$ 312b	3279 $\pm$ 459b	539 $\pm$ 123b
	Control	1077 $\pm$ 136	1309 $\pm$ 100a	3636 $\pm$ 456a	6437 $\pm$ 539a	7198 $\pm$ 729a	3501 $\pm$ 318b	1276 $\pm$ 96a
			$F = 0.854,$ $P = 0.523$	$F = 17.34,$ $P = 0.0001$	$F = 24.14,$ $P = 0.0001$	$F = 10.35,$ $P = 0.001$	$F = 8.770,$ $P = 0.003$	$F = 4.860,$ $P = 0.019$
Motiles	P50-2	103 $\pm$ 8	49 $\pm$ 14	182 $\pm$ 23b	581 $\pm$ 105ab	715 $\pm$ 79ab	537 $\pm$ 55bc	121 $\pm$ 16ab
	P100-2	155 $\pm$ 35	50 $\pm$ 3	327 $\pm$ 10ab	414 $\pm$ 62b	523 $\pm$ 133b	1156 $\pm$ 154ab	95 $\pm$ 11bc
	P200-1	150 $\pm$ 15	43 $\pm$ 18	172 $\pm$ 3b	401 $\pm$ 22b	537 $\pm$ 194b	450 $\pm$ 40c	136 $\pm$ 210ab
	P200-2	136 $\pm$ 96	43 $\pm$ 5	167 $\pm$ 13b	606 $\pm$ 44ab	410 $\pm$ 49b	514 $\pm$ 274c	175 $\pm$ 194a
	Control	114 $\pm$ 9	157 $\pm$ 44	602 $\pm$ 132a	924 $\pm$ 208a	1426 $\pm$ 186a	1492 $\pm$ 286a	55 $\pm$ 9c
			$F = 0.526,$ $P = 0.720$	$F = 3.038,$ $P = 0.070$	$F = 5.250,$ $P = 0.015$	$F = 3.780,$ $P = 0.039$	$F = 5.797,$ $P = 0.011$	$F = 11.84,$ $P = 0.0001$

Means within the same column for each stage with different letters are significantly different ( $P < 0.05$ , HSD,  $df = 4, 10$ ). P50-2: two releases of *P. persimilis* at the rate of 50/tree; P100-1: one release at the rate of 100/tree; P200-1: one release at the rate of 200/tree; P200-2: two releases at the rate of 200/tree; Control: no releases. Note: First and second releases of PM were made on March 1 and March 28, respectively. DAR: days after the first release of predacious mites.

**Table 4. Effect of *G. occidentalis* or *P. persimilis* releases on density of *P. citri* on satsuma trees (experiment 4, spring 2008)**

<i>P. citri</i> stage	Treatment	Mean ( $\pm$ SE) number of <i>P. citri</i> per 24 leaves			
		Pre-release (Mar 28)	7 DAR (Apr 4)	21 DAR (Apr 18)	35 DAR (May 2)
Eggs	G100-1	595 $\pm$ 70	522 $\pm$ 142	986 $\pm$ 29b	848 $\pm$ 99ab
	G200-1	586 $\pm$ 92	438 $\pm$ 12	649 $\pm$ 73bc	375 $\pm$ 172b
	P100-1	540 $\pm$ 106	442 $\pm$ 18	649 $\pm$ 85bc	518 $\pm$ 20b
	P200-1	615 $\pm$ 110	421 $\pm$ 91	422 $\pm$ 9c	529 $\pm$ 69b
	Control	562 $\pm$ 92	531 $\pm$ 55	2097 $\pm$ 210a	1610 $\pm$ 229a
		$F = 0.103,$ $P = 0.978$	$F = 0.332,$ $P = 0.851$	$F = 45.22,$ $P = 0.0001$	$F = 7.84,$ $P = 0.0004$
Motiles	G100-1	87 $\pm$ 4	131 $\pm$ 38	101 $\pm$ 23b	54 $\pm$ 10ab
	G200-1	87 $\pm$ 7	86 $\pm$ 20	74 $\pm$ 9b	33 $\pm$ 4b
	P100-1	92 $\pm$ 11	76 $\pm$ 20	61 $\pm$ 19b	41 $\pm$ 14b
	P200-1	85 $\pm$ 7	91 $\pm$ 32	79 $\pm$ 7b	39 $\pm$ 3b
	Control	90 $\pm$ 2	162 $\pm$ 16	347 $\pm$ 26a	89 $\pm$ 9a
		$F = 0.401,$ $P = 0.804$	$F = 2.180,$ $P = 0.144$	$F = 25.46,$ $P = 0.0001$	$F = 5.47,$ $P = 0.013$

Means within the same column for each stage with different letters are significantly different ( $P < 0.05$ , HSD,  $df = 4, 10$ ). G100-1: one release of *G. occidentalis* at the rate of 100/tree; G200-1: one release of *G. occidentalis* at the rate of 200/tree; P100-1: one release of *P. persimilis* at the rate of 100/tree; P200-1: one release of *P. persimilis* at the rate of 200/tree; Control: no releases. Note: Predacious mites were released on March 29. DAR: days after release of predacious mites.

**Figure 1.** Population dynamics of *P. citri* on control trees and trees on which predacious mites were released in spring 2008. (A): Data from experiment 2 for *G. occidentalis* releases; (B): Data from experiment 3 for *P. persimilis* releases. G50-2: two releases of *P. persimilis* at the rate of 50/tree; G100-1: one release at the rate of 100/tree; G200-1: one release at the rate of 200/tree; G200-2: two releases at the rate of 200/tree; Control: no releases. Total number of *P. citri* = eggs + motiles (nymphs and adults). Arrows indicate predacious mite release dates.

