FIELD EVALUATION OF PREDACIOUS MITES (ACARI: PHYTOSEIIDAE) FOR BIOLOGICAL CONTROL OF CITRUS RED MITE, *PANONYCHUS CITRI* (TROMBIDIFORMES: TETRANYCHIDAE)

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Abstract

We evaluated 3 species of predacious mites (Acari: Phytoseiidae), *Galendromus occidentalis* (Nesbitt), *Phytoseiulus persimilis* Athias-Henriot and *Neoseiulus californicus* (McGregor), as biological control agents for citrus red mite, *Panonychus citri* (McGregor) (Trombidiformes: Tetranychidae), on citrus in southern Alabama. Three separate experiments were carried out during 2008 and 2011 to evaluate various factors (i.e. release rate, release frequency and initial prey density) that may impact the performance of the predacious mites. In the first experiment conducted in 2008 on trees with moderate initial prey densities (i.e. < 4 *P. citri* motiles per leaf), one single release of *P. persimilis* or *G. occidentalis* at a rate of 100 or 200 per tree effectively prevented the prey from exceeding the economic threshold (5 motiles/leaf) for the entire duration (35 d) of the experiment. The result of the second experiment in 2008 on trees with high initial prey densities (i.e. ≥ 5 motiles per leaf) showed that 2 releases of *P. persimilis* or *G. occidentalis* at a rate of 100 or 200 per tree per release could not provide adequate suppression of *P. citri* below the economic threshold. In both experiments, *P. citri* densities were significantly lower in most predacious mite treatments compared to the control (no release). Also, lower *P. citri* densities were recorded at the higher release rate (200 per tree) compared to the lower rate, but this was only significant in a few cases. The third experiment conducted in 2011 in large plots on trees with low initial *P. citri* densities (i.e. < 1 motile per leaf) showed that 2 releases of *N. californicus* or *P. persimilis* at a rate of 200 per tree per release effectively maintained *P. citri* at low densities (< 1.5 motiles per leaf) throughout the duration (56 d) of the experiment. Limited observations in spring 2012 confirmed the establishment of the predacious mites released in the 2011 study. These results showed that all 3 phytoseiid species were effective in reducing *P. citri* densities on citrus. However, initial prey density may be an important factor influencing their performance.

Key Words: *Galendromus occidentalis; Neoseiulus californicus*; *Phytoseiulus persimilis*; *Panonychus citri;* biological control; satsuma citrus

Resumen

Se evaluaron tres especies de ácaros depredadores (Acari: Phytoseiidae), *Galendromus occidentalis* (Nesbitt), *Phytoseiulus persimilis* Athias-Henriot y *Neoseiulus californicus* (McGregor), como agentes de control biológico del ácaro rojo de los cítricos, *Panonychus citri* (McGregor) (Trombidiformes: Tetranychidae), en cítricos en el sur de Alabama. Tres experimentos fueron realizados durante los años 2008 y 2011 para evaluar varios factores (i.e. taza de liberación, frecuencia de liberaciones y la densidad inicial de la presa) que podrían afectar el desempeño de ácaros depredadores. En el primer experimento del 2008, en árboles con una densidad de presa inicial moderada (i.e. < 4 *P. citri* estadios móviles por hoja), una liberación de *P. persimilis* o *G. occidentalis* a una taza de 100 o 200 individuos por árbol, logró prevenir que *P. citri* excediera el nivel de daño económico (5 estadios móviles/ árbol) durante el todo el experimento (35 d). En el segundo experimento del2008, en árboles con una densidad inicial de presa alta (i.e. ≥ 5 estadios móviles por hoja), dos liberaciones de *P. persimilis* o *G. occidentalis* a una taza de 100 o 200 por árbol no lograron suprimir *P. citri* a niveles por debajo del nivel económico. En ambos experimentos las densidades de *P. citri* fueron significativamente menores en la mayoría de los tratamientos con ácaros depredadores comparados con los controles (sin liberación). Se registraron densidades más bajas de *P. citri* en los tratamientos donde se liberaron mas ácaros (200 por árbol) comparados con el nivel más bajo de liberación, pero esta diferencia fue significativa en pocas ocasiones. El tercer experimento en lotes grandes y árboles con densidades iniciales bajas de *P. citri* (i.e. < 1 estadios móviles por árbol) dos liberaciones de 200 *N. californicus* o *P. persimilis* por árbol mantuvienron a *P. citri* en bajas densidades (< 1.5 estadios móviles por hoja) durante todo el experimento (56 d). Finalmente, observaciones durante la primavera del 2012 confirmaron el establecimiento de los ácaros depredadores liberados en este estudio en 2011. Estos resultados muestran que las tres especies de phytoseiidos fueron efectivas reduciendo las densidades de *P. citri* en cítricos, sin embargo, el nivel inicial de la población puede ser un factor importante que afecta su desempeño.

Palabras Clave: *Galendromus occidentalis; Neoseiulus californicus*; *Phytoseiulus persimilis*; *Panonychus citri;* control biológico; cítricos satsuma

The citrus red mite, *Panonychus citri* (McGregor) (Trombidiformes: Tetranychidae) is a key pest of citrus (Sapindales: Rutaceae) in many parts of the world (Gotoh & 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 injury, reduced photosynthesis, premature shoot dieback and decreased plant vigor (Kranz et al. 1977). High infestations may also result in fruit feeding and injury (Childers et al. 2007).

Panonychus citri is also an important pest of satsuma mandarin (*Citrus unshiu* Marcovitch) in Alabama (English & Turnipseed 1940, Fadamiro et al. 2007, 2008). Although this citrus variety has been grown for over a century in Alabama and other parts of the Gulf Coast region of the United States (English & Turnipseed 1940), satsuma mandarin production is a growing industry in the region partly because of strong industry, state support and development of new markets (Campbell et al. 2004). 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).

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 & 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 & Enns 1975; McMurtry 1983; Childers 1994; Wood et al. 1994; Takano-Lee & Hoddle 2001, 2002; Pratt et al. 2002; Colfer et al. 2004; Opit et al. 2004; Jamieson et al. 2005; Rhodes et al. 2006; Fraulo & Liburd 2007; Arthurs et al. 2009; Cakmak et al. 2009). The key species evaluated in many systems include 3 commercially available phytoseiids, *Phytoseiulus persimilis* Athias-Henriot, *Galendromus occidentalis* (Nesbitt), and *Neoseiulus californicus* (McGregor). Some indigenous species have also been evaluated (Muma & Denmark 1970; Childers & Enns 1975; Childers 1994). A recent survey of the predacious mite fauna of satsuma mandarin in Alabama identified 29 species from 9 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 provide 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 (X. Y. & H. Y. F, 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 3 commercially available phytoseiids, *P. persimilis*, *G. occidentalis* and *N. californicus*, on *P. citri* (Xiao & Fadamiro 2010). *Phytoseiulus persimilis* is a specialist predator of *Tetranychus* spp., while *G. occidentalis* and *N. californicus* are selective predators of spider mites (McMurtry & Croft 1997; Blackwood et al. 2001; Fraulo & Liburd 2007). The laboratory 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 3 species were effective in reducing *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 linearly with prey availability up to a maximum (Xiao & Fadamiro 2010). These initial findings coupled with their favorable life history traits (McMurtry & Croft 1997; Pratt & Croft 2000), aided our selection of these commercially available phytoseiids for further evaluation in the field. In the present study, field experiments were conducted in 2008 and 2011 to evaluate the effectiveness of releases of the phytoseiid species at different rates and frequencies for suppression of *P. citri* in satsuma orchards in southern Alabama.

MATERIAL AND METHODS

Study Sites

Three separate field experiments were conducted at 2 locations in Baldwin county, southern Alabama. The first 2 experiments were conducted at Coker orchard in 2008. This orchard had ~ 250 citrus trees comprised primarily of satsuma mandarin ('Owari' variety). The orchard has had a history of high infestations 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. Mean temperatures at this location during the experiments in spring (Mar-May) 2008 were 15.3-24.0 °C (min: 9.5 °C, max: 28.6 °C). Relative humidity was in the range of 65-75%. The third experiment was conducted in 2011 in a citrus orchard block at the Gulf Coast Research and Extension Center (GCREC), Fairhope, Alabama. This orchard block had ~ 176 citrus trees comprised mainly of satsuma mandarin ('Owari' variety), and was not conventionally sprayed during the trials. Mean temperatures at this location during the experiments in spring (Mar-May) 2011 were 10.1-21.3 °C (min: 5 °C, max: 26.3 °C). Relative humidity was in the range of 50-90%.

Predacious Mites

The phytoseiids, *P. persimilis*, *G. occidentalis* and *N. californicus*, were purchased from Biocontrol Network, Inc. (Brentwood, Tennessee) and kept separately in a cooler (4 °C) for 1-2 days, if the weather was unsuitable for immediate release. Only *P. persimilis* and *G. occidentalis* were tested in the 2008 experiments. However, *G. occidentalis* was not commercially available for the 2011 experiment and was, therefore, replaced with *N. californicus*.

General Procedure for Predacious Mite Release and Sampling

The study evaluated the effectiveness of releases of the phytoseiids at different rates and frequency in suppressing *P. citri* in 3 experiments. First, satsuma trees in each orchard were sampled for *P. citri*, using a protocol described by Fadamiro et al. (2008) to determine initial population densities. Three experiments were conducted on trees with different initial *P. citri* densities relative to the economic threshold of 5 motiles per leaf proposed by Childers et al. (2007). Experiment 1 was conducted on trees with initial *P. citri* densities slightly lower than the economic threshold (i.e. 3-4 motiles per leaf, abbreviated as moderate initial prey densities). Experiment 2 was conducted on trees with initial *P. citri* densities higher than the economic threshold (i.e. ≥ 5 motiles per leaf, abbreviated as high initial prey densities). Experiment 3 was conducted on trees with initial *P. citri* densities much lower than the economic threshold (i.e. < 1 motile per leaf, abbreviated as low initial prey densities). All selected trees were of similar size (canopy of 2 m high, 1.5 m diam) and had not previously been treated with pesticides during the season. In the first 2 experiments, treatments were applied in single-tree plots. Two test trees were separated by at least one "buffer" tree to minimize wind-aided dispersal of mites between test trees. The third experiment was conducted in larger plots each consisting of 12 trees. Each experiment was a randomized complete block design and trees were randomly assigned to the treatments.

Shipments of predacious mites from the supplier arrived in plastic vials (6 cm ht × 5 cm diam), each 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 checked to confirm identity and viability by observing subset samples of ~ 20 individuals in a Petri-dish for about 10 min. Viability was always > 95% for all species. The release rates evaluated (100 or 200 predatory mites per tree) were selected based on the results of a preliminary trial in 2007 that showed minimal efficacy at lower release rates. The tested release rates are also consistent with the supplier recommendation (i.e. ~ 2000/acre for field releases), and published rates (e.g., ~5000/ha in cotton, Colfer et al. 2004).

For each phytoseiid species, individuals, at the appropriate release rate, were evenly distributed into four 3.5 ml plastic containers (each with holes to allow predacious mites to exit and disperse), which were used as release devices. The 4 plastic containers were then hung on branches located on 4 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 24-27 °C, at RH 65-70%.

Experiment 1: Single Release of *G. occidentalis* or *P. persimilis* in Single-tree Plots with Moderate Initial Prey Densities

The first experiment was conducted in a block of Coker orchard in 2008 to evaluate single releases of *G. occidentalis* or *P. persimilis* at 2 release rates (100 or 200 per tree) on satsuma trees infested with *P. citri* at moderate initial densities (i.e 3-4 motiles per leaf). The following 5 treatments were evaluated on single trees: 1) one single release of *G. occidentalis* at a rate of 100 per tree (G100-1), 2) one single release of *G. occidentalis* at a rate of 200 per tree (G200-1), 3) one single release of *P. persimilis* at a rate of 100 per tree (P100-1), 4) one single release of *P. persimilis* at a rate of 200 per tree (P200-1), and 5) no-release control. Predacious mites were released at the same time in all treatments (with the exception of the no-release control) in late March. Each treatment was replicated 3 times. To evaluate *P. citri* density, 24 (6 per tree quadrant) randomly selected leaves were taken from each test tree at 0, 7, 21 and 35 d after predacious mite release. The leaves were collected in properly-labeled paper bags, held in a cooler and transported to the laboratory where they were examined under a dissecting microscope at 20 × magnification. The numbers of *P. citri* eggs and motiles (nymphs + adults) per leaf were counted and recorded. In all experiments, predacious mites were rarely observed on the leaves and thus were not recorded.

Experiment 2: Two Releases of *G. occidentalis* or *P. persimilis* in Single-tree Plots with High Initial Prey Densities

A second experiment was conducted in another block of Coker orchard in 2008 to evaluate 2 releases of *G. occidentalis* or *P. persimilis* at 2 release rates (100 or 200 per tree) on satsuma trees infested with *P. citri* at high initial densities (i.e. > 5 motiles per leaf). The following 5 treatments were evaluated on single trees: 1) 2 releases of *G. occidentalis* each at a rate of 100 per tree (G100-2), 2) 2 releases of *G. occidentalis* each at a rate of 200 per tree (G200-2), 3) 2 releases of *P. persimilis* each at a rate of 100 per tree (P100-2), 4) 2 releases of *P. persimilis* each at a rate of 200 per tree (P200-2), and 5) no-release control. Predacious mites were released at the same time in all treatments (with the exception of the no-release control), in early March (first release) and late March (second release), respectively. Each treatment was replicated 3 times. To evaluate *P. citri* density, 24 (6 per tree quadrant) randomly selected leaves were collected from each test tree at 0, 7, 14, 28, 35, 49, and 63 d after the first predacious mite release. The leaves were collected, handled and analyzed as described above for experiment 1.

Experiment 3: Two Releases of *P. persimilis* or *N. californicus* in Large Plots on Trees with Low Initial Prey Densities

A third experiment was conducted at the GREC, Fairhope, AL in 2011 in relatively larger orchard plots each consisting of 12 satsuma trees. The spacing between the trees and tree rows were 4.5 m and 6 m, respectively. The experiment was arranged as a 3 x 3 randomized complete block design (3 treatments, 3 replicates). A buffer of 1 tree row was left between the block and 2 tree rows between plots in each block. The test trees were infested with *P. citri* at low initial densities (i.e. < 1 motile per leaf). The following 3 treatments were evaluated: 1) 2 releases of *P. persimilis* at a rate of 200 per release per tree (P200-2), 2) 2 releases of *N. californicus* at a rate of 200 per release per tree (N200-2), and 3) no predacious mite release control. The procedures were similar to those described for the previous experiments but with some modifications. In each plot, 6 inner trees were selected and tagged for predacious mite release. Predacious mites were released at the same time in all treatments (with the exception of the no-release control), in mid February (first release) and mid March (second release), respectively. *Panonychus citri* density was evaluated in each plot by collecting 24 randomly selected leaves (6 per tree quadrant) from each of the 6 test trees (for a total of 144 leaves per plot) at 0 and 7 d after the first predacious mite release. To ensure timely processing of the leaf samples before they dried up, the sample size was subsequently reduced by half. Thus, 12 randomly selected leaves (3 per tree quadrant) were taken from each of the 6 test trees (for a total of 72 leaves per plot) at 14, 21, 28, 35, 42, 49, and 56 d after the first predacious mite release. The leaves were collected, handled and analyzed as described above for experiment 1. To control for the different sample sizes, data were analyzed and presented as number of *P. citri* per leaf.

Statistical Analyses

For all experiments, mean numbers of *P. citri* eggs, and motiles per leaf per sampling date were computed for each treatment and used for statistical analyses. Data were not normally distributed and thus were transformed using √ x + 0.5 and then analyzed by repeated measures multivariate analysis of variance (MANOVA) on the 2 main factors (sampling date and treatment), with time as the repeated measures factor (Ott & Longnecker 2001; Norman & Streiner 2008; Frank et al. 2011). Repeated measures MANOVA was used to account for the possibility of pseudoreplication in the experimental design (Lazic 2010; Frank et al. 2011). The assumption of equal variances and correlations across time in the response variable and appropriateness of using the unadjusted univariate *F*-test values and the sphericity square root transformation (√) test were based on the sphericity of the model. Where the sphericity test, which is part of the within-subject analysis, was significant, we reported the adjusted *F*-test values including adjusted degrees of freedom from the Wilks' λ test. The Wilks' λ is a test statistic used in multivariate analysis of variance (MANOVA) to test whether there are differences between the means of identified groups of subjects on a combination of dependent variables. When the results of repeated measures MANOVA showed significant effects of sampling date, treatment and a significant sampling date* treatment interaction, the data was further analyzed by using one-way analysis of variance (ANOVA) followed by the Tukey-Kramer HSD comparison test to determine significant differences among the treatments on each sampling date ($P < 0.05$; JMP® 7.0.1, SAS Institute 2007).

RESULTS

Experiment 1: Single Release of *G. occidentalis* or *P. persimilis* in Single-tree Plots with Moderate Initial Prey Densities

Repeated measures MANOVA showed significant effects of sampling date (Wilks' λ = 0.144, Adj. df = 6, $P < 0.0001$), treatment (Wilks' λ = 0.149, Adj. df = 8, $P < 0.0001$), and sampling date* treatment interaction (Wilks' λ = 0.084, Adj. df = 24, $P < 0.0001$), on the numbers of *P. citri* eggs and motiles. Thus, the data was analyzed by sampling date using one-way ANOVA. No significant differences were recorded among the treatments in the numbers of *P. citri* eggs or motiles at 0 (pre-release) and 7 days after release (DAR). However, significant differences were recorded among the treatments at 21 and 35 DAR (Table 1). Numbers of *P. citri* eggs and motiles were significantly higher in the control (no release) than in the predacious mite treatments at 21 DAR (Table 1, Figs. 1A and 1B). Similarly, numbers of *P. citri* eggs were significantly higher in the control than in the predacious mite treatments at 35 DAR, with the exception of the treatment in which *G. occidentalis* was released at a rate of 100 per tree (G100-1). The sharp decline in the population density of *P. citri* in the control at the end of the experiment (35 DAR) was likely due to a general population crash induced by rainfall. In general, lower densities of the prey were recorded at the higher release rate (200 per tree) compared to the lower rate, although this was only significant at 21 DAR for eggs (Fig. 1A). No significant differences were recorded in the ability of both phytoseiid species to suppress the prey.

Experiment 2: Two Releases of *G. occidentalis* or *P. persimilis* in Single-tree Plots with High Initial Prey Densities

Repeated measures MANOVA showed significant effects of sampling date (Wilks' λ = 0.062, Adj. df = 12, $P < 0.0001$), treatment (Wilks' λ = 0.295, Adj. df = 8, $P < 0.0001$), and sampling date * treatment interaction (Wilks' = 0.122, Adj. df = 48, $P < 0.0001$) on the numbers of *P. citri* eggs and motiles. One-way ANOVA showed no significant differences among the treatments in the pre-release densities of *P. citri*. However, significant differences were recorded among the treatments in the numbers of *P. citri* eggs at 7, 14, 28, 35, and 63 DAR (Table 2, Fig. 2A). Similarly, the numbers of *P. citri* motiles were significantly different among the treatments at 7, 35, 49 and 63 DAR (Table 2, Fig. 2B). In general, lower numbers of *P. citri* eggs and motiles were recorded in the predacious mite treatments compared to the control (no release) on most sampling dates. As observed in the first experiment, the steep decline in the population density of *P. citri* in the control at the end of the experiment (35 DAR) was likely induced by rainfall. Among the predacious mite treatments, numerically lower numbers of *P. citri* eggs were recorded at the higher release rate (200 per tree) compared to lower release rate (100 per tree), but a significant effect of release rate was recorded only at 7 DAR (*P. persimilis*) and 63 DAR (both species) (Fig. 2A). No significant differences were recorded in the performance of the phytoseiid species.

Experiment 3: Two Releases of *P. persimilis* or *N. californicus* in Large Plots on Trees with Low Initial Prey Densities

Significant effects of sampling date (Wilks' λ = 0.935, Adj. df = 16, $P < 0.0001$), treatment (Wilks' λ = 0.989, Adj. df = 4, $P < 0.0001$), and sampling

TABLE 1. ONE-WAY ANOVA VALUES FOR EXPERIMENT 1: DENSITIES OF *P. CITRI* IN SINGLE-TREE PLOTS WITH MODERATE INITIAL PREY DENSITIES TESTING SINGLE RELEASE OF EITHER *G. OCCIDENTALIS* OR *P. PERSIMILIS* VERSUS THE CONTROL (NO RELEASE).

	P. citri eggs			*P. citri* motiles		
Day after Release (DAR)	*F*	df	*P*	*F*	df	*P*
0	0.10	4, 8	0.9807	0.23	4, 8	0.9163
7	0.29	4, 8	0.8793	1.80	4, 8	0.2227
21	39.63	4, 8	<0.0001	29.00	4, 8	<0.0001
35	7.27	4, 8	0.0090	5.03	4, 8	0.0337

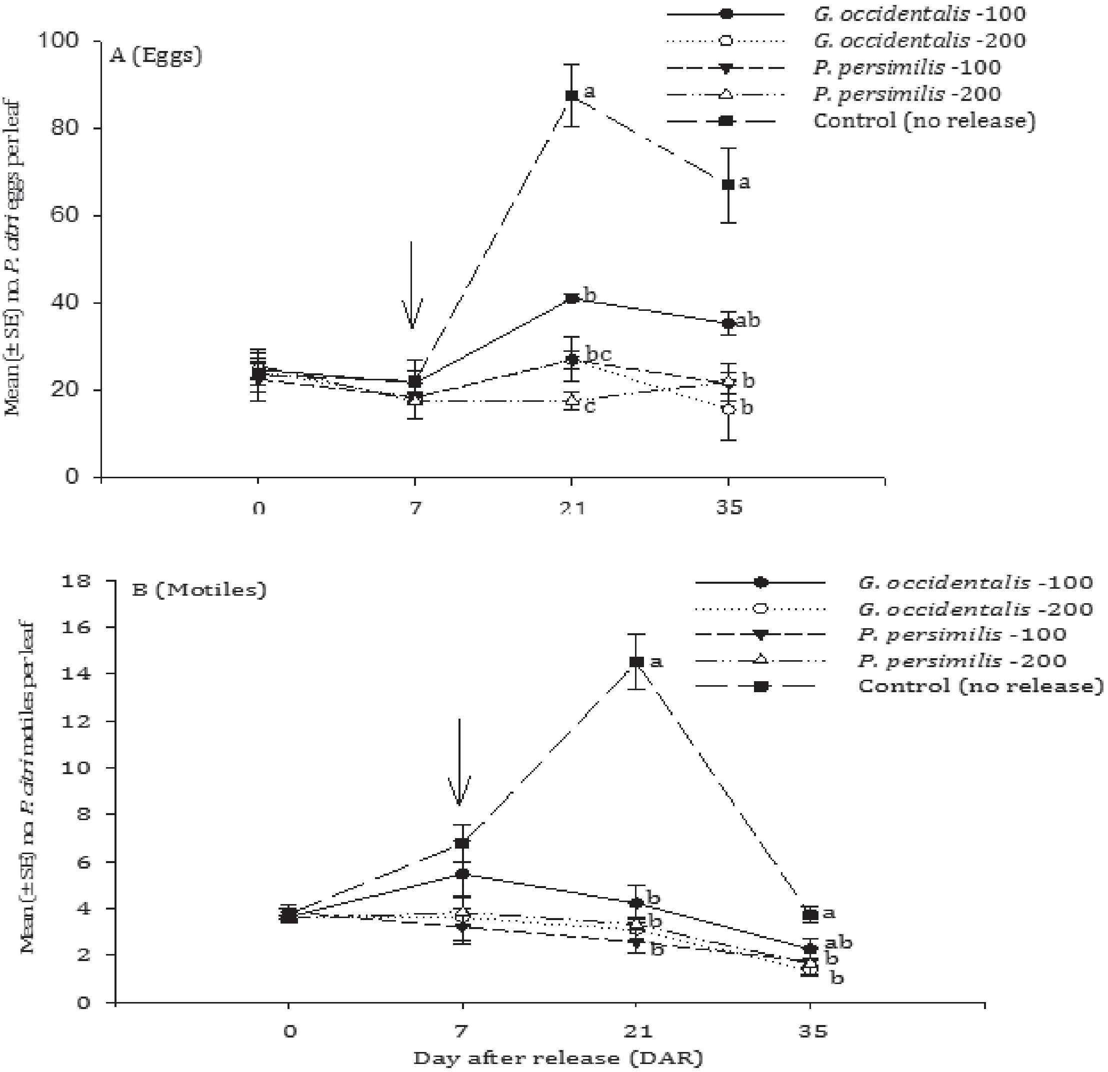

Fig. 1. Mean (± SE) number of *P. citri* eggs. (A) and motiles; (B) recorded in the test involving single release of *G. occidentalis* or *P. persimilis* in single-tree plots with moderate initial prey densities (Experiment 1). *G. occidentalis* - 100: one release of *G. occidentalis* at the rate of 100/tree; *G. occidentalis* - 200: one release of *G. occidentalis* at the rate of 200/tree; *P. persimilis* - 100: one release of *P. persimilis* at the rate of 100/tree; *P. persimilis* - 200: one release of *P. persimilis* at the rate of 200/tree; Control: no release. Arrows indicate date (29 Mar 2009) when predacious mites were released. Means having no letter in common are significantly different ($P < 0.05$).

date * treatment interaction (Wilks' $\lambda = 0.972$, Adj. df = 32, $P < 0.0001$) were recorded. Further analysis of the data using one-way ANOVA showed no significant differences in the number of *P. citri* eggs among the treatments at 0 (pre-release), 35, and 42 DAR. However, significant treatment effects were recorded at 7, 14, 21, 28, 49 and 56 DAR (Table 3, Fig. 3A). For *P. citri* motiles, significant treatment effects were recorded at 7, 14, 21, 28, 49, and 56 DAR (Table 3, Fig. 3B). In general, *P. citri* densities were significantly higher in the control than in the predacious mite treatments on most of the sampling dates. The only significant difference between the species was recorded at 56 DAR when a significantly lower numbers of *P. citri* eggs were recorded in the *P. persimilis* treatment compared to *N. californicus* (Fig. 3A).

DISCUSSION

The results of the 3 experiments showed that the phytoseiids, *P. persimilis, G. occidentalis,* and *N. californicus*, were effective in reducing *P. citri*

TABLE 2. ONE-WAY ANOVA VALUES FOR EXPERIMENT 2: DENSITIES OF *P. CITRI* IN SINGLE-TREE PLOTS WITH HIGH INITIAL PREY DENSITIES TESTING TWO RELEASES OF EITHER *G. OCCIDENTALIS* OR *P. PERSIMILIS* VERSUS THE CONTROL (NO RELEASE).

	P. citri eggs			*P. citri* motiles		
Day after Release (DAR)	*F*	df	*P*	*F*	df	*P*
0	0.37	4, 8	0.8262	0.11	4, 8	0.9717
7	17.01	4, 8	0.0006	10.63	4, 8	0.0027
14	15.76	4, 8	0.0007	3.02	4, 8	0.0857
28	13.14	4, 8	0.0014	1.59	4, 8	0.2658
35	7.78	4, 8	0.0073	21.83	4, 8	0.0001
49	1.08	4, 8	0.4269	9.24	4, 8	0.0043
63	17.78	4, 8	0.0005	22.20	4, 8	0.0002

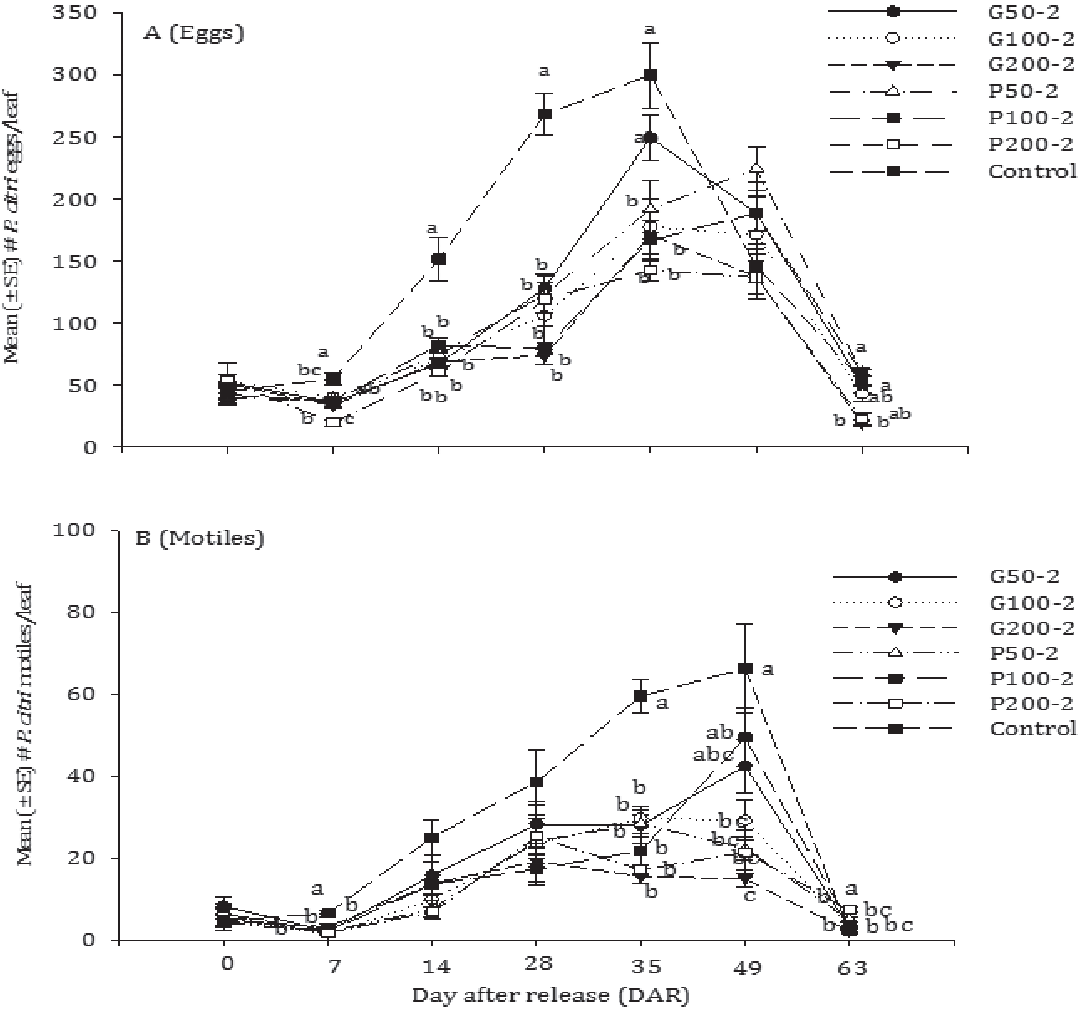

Fig. 2. Mean (± SE) number of *P. citri* eggs (A) and motiles (B) recorded in the test involving 2 releases of *G. occidentalis* or *P. persimilis* in single-tree plots with high initial prey densities (Experiment 2). *G. occidentalis* - 100: 2 releases of *G. occidentalis* at the rate of 100/tree; *G. occidentalis* - 200: 2 releases of *G. occidentalis* at the rate of 200/tree; *P. persimilis* - 100: 2 releases of *P. persimilis* at the rate of 100/tree; *P. persimilis* - 200: 2 releases of *P. persimilis* at the rate of 200/tree; Control: no release. Arrows indicate dates (1st release: 6 Mar 2009; 2nd release: 29 Mar 2009) when predacious mites were released. Means having no letter in common are significantly different ($P < 0.05$).

TABLE 3. ONE-WAY ANOVA VALUES FOR EXPERIMENT 3: DENSITIES OF *P. CITRI* IN LARGE PLOTS ON TREES WITH LOW INITIAL PREY DENSITIES TESTING TWO RELEASES OF EITHER *N. CALIFORNICUS* OR *P. PERSIMILIS* VERSUS THE CONTROL (NO RELEASE).

	P. citri eggs			*P. citri* motiles		
Day after Release (DAR)	*F*	df	*P*	*F*	df	*P*
0	2.18	2, 1291	0.1134	5.03	2, 1291	0.0066
7	13.53	2, 1291	<0.0001	18.33	2, 1291	<0.0001
14	13.75	2, 643	<0.0001	7.71	2, 643	0.0005
21	3.37	2, 643	0.0349	8.76	2, 643	0.0002
28	18.37	2, 643	<0.0001	37.15	2, 643	<0.0001
35	0.74	2, 643	0.4784	0.04	2, 643	0.9641
42	2.35	2, 643	0.0962	2.93	2, 643	0.0541
49	16.25	2, 643	<0.0001	14.41	2, 643	<0.0001
56	15.15	2, 643	<0.0001	14.50	2, 643	<0.0001

densities on citrus. However, their efficacy appeared to be influenced by release rate and initial prey density. In the first experiment, conducted on trees with moderate initial prey densities (i.e., < 4 *P. citri* motiles per leaf), one single release of *P. persimilis* or *G. occidentalis* at a rate of 100 or 200 per tree effectively prevented *P. citri* from exceeding the economic threshold (5 motiles/leaf) for the entire duration (35 d) of the experiment. The results were similar for both release rates, although numerically lower prey densities were achieved at the higher release rate. The results of the second experiment, conducted on trees with high initial prey densities (i.e. ≥ 5 motiles per leaf) of *P. citri*, were not as promising. Although *P. citri* densities were significantly lower in most predacious mite treatments compared to the control (no release), the data showed that 2 releases of *P. persimilis* or *G. occidentalis* at a rate of 100 or 200 per tree per release could not provide adequate suppression of *P. citri* below the economic threshold. As in the first experiment, lower prey densities were recorded at the higher release rate compared to the lower rate, but this was only significant in a few cases. Together, these results suggest that initial prey density may be an important factor affecting the ability of the phytoseiids to effectively control *P. citri*. The results of the large plot experiment conducted on trees with low initial *P. citri* densities (i.e. < 1 motile per leaf) showed that 2 releases of *P. persimilis* or *N. californicus* at a rate of 200 per tree per release effectively maintained *P. citri* at low densities (< 1.5 motiles per leaf) throughout the duration (56 d) of the experiment. It is worth noting that *P. citri* densities in the control plots also remained below the economic threshold, suggesting that treatment of plots with initial mite densities of < 1 motile per leaf using miticides or biological control agents may not be necessary.

Katayama et al. (2006) identified *N. californicus* as a major predator of *P. citri* in satsuma citrus groves in central Japan but we are not aware of previously published systematic field studies that evaluated *P. persimilis, G. occidentalis,* or *N. californicus* against *P. citri* in citrus orchards/groves. However, studies have reported that the phytoseiids were effective in suppressing *Panonychus ulmi* in apples (Monetti & Fernandez 1995), and *Tetranychus urticae* in citrus (Grafton-Cardwell et al. 1997; Abad-Moyano et al. 2010). Several authors have also reported their efficacy in suppressing *Tetranychus* spp. in many other crop systems, including strawberries (McMurtry 1982, 1991; Van de Vrie & Price 1994; Rhodes et al. 2006; Fraulo & Liburd 2007; Cakmak et al. 2009), avocado (Hoddle et al. 2000; Takano-Lee and Hoddle 2001), hops (Strong & Croft 1995, 1996), ivy geranium (Opit et al. 2004), greenhouse vegetable crops (McMurtry 1991; Arthurs et al. 2009), and ornamental plants (Hamlen & Lindquist 1981; Pratt & Croft 1998). Many of the above studies demonstrated that release rate/frequency and initial prey density are critical factors that may impact successful biological control of phytophagous mites by the phytoseiids (Hamlen & Lindquist 1981; Hoddle et al. 2000; Pratt & Croft 2000; Opit et al. 2004; Fraulo & Liburd 2007). For instance, Fraulo & Liburd (2007) reported that release rate and frequency had a great impact on the ability of *N. californicus* to provide effective and season-long suppression of spider mites on strawberry.

These results demonstrated that one or 2 releases of *P. persimilis, G. occidentalis*, or *N. californicus* at release rates of 100 or more per tree could provide effective suppression of *P. citri* on citrus, in particular at low-moderate initial prey densities. Both the eggs and nymphs of *P. citri* were suppressed by the phytoseiids in this study. However, the data showed relatively higher suppression of nymphs, consistent with a recent laboratory finding that the 3 phytoseiid species prefer nymphs to eggs of *P. citri* (Xiao & Fadamiro 2010). Previous studies with these phytoseiids suggest that they can survive periods of starvation in the

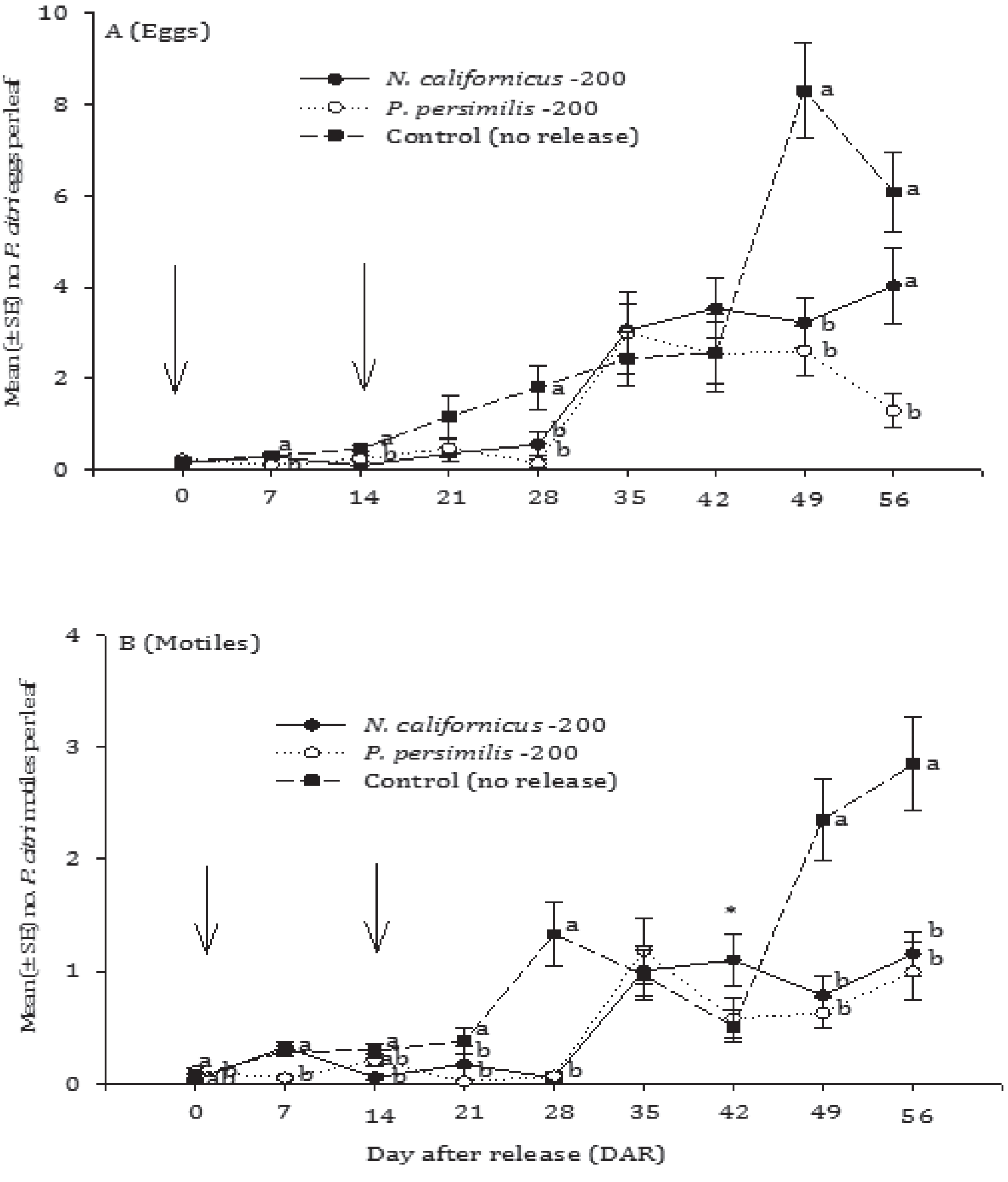

Fig. 3. Mean (± SE) number of *P. citri* eggs (A) and motiles (B) recorded in the test involving 2 releases of *N. californicus* or *P. persimilis* in large tree plots with low initial prey densities (Experiment 3). *N. californicus* - 200: 2 releases of *N. californicus* at the rate of 200/tree; *P. persimilis* - 200: 2 releases of *P. persimilis* at the rate of 200/tree; Control: no release. Arrows indicate dates (1st release: 28 Feb 2011; 2nd release: 14 Mar 2011) when predacious mites were released. * indicates marginal significant difference ($P = 0.054$). Means having no letter in common are significantly different ($P < 0.05$).

laboratory (Xiao & Fadamiro 2010) and tolerate high field temperatures (McMurtry & Croft 1997). Moreover, they are highly active with high prey searching efficiency (Pratt & Croft 2000, Blackwood et al. 2001), and adapted to disturbed habitats, such as intensively-managed orchards (McMurtry & Croft 1997). These factors suggest that all 3 phytoseiid species may do well in the severe southern Alabama climate. This is supported by our limited observations in 2012 at the GREC

(Fairhope) location which confirmed the establishment of the predacious mites (*P. persimilis* and *N. californicus*) previously released in 2011. In a small sample collected in spring 2012 at this location, the predacious mites were detected both on the trees on which they had been released in 2011 as well as on control trees, suggesting their spread or dispersal throughout this orchard. Further field evaluations including cost analysis are necessary to determine the economic feasibility of large-scale biological control of *P. citri* with these predacious mites.

In this study, we evaluated releases of single predator species rather than multiple predator species. Studies that compared the effectiveness of single versus multiple predator species have produced varying results, ranging from negative (Rosenheim et al. 1995; Schausberger & Walzer 2001) to neutral (Denoth et al. 2002; Chow et al. 2008; Cakmak et al. 2009) effect. Future studies will determine the efficacy of combined releases of 2 or more phytoseiid species, 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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References Cited

Abad-Moyano, R., Pina, T., Pérez-Panadés, J., Carbonell, E., and Urbaneja, A. 2010. Efficacy of *Neoseiulus californicus* and *Phytoseiulus persimilis* in suppression of *Tetranychus urticae* in young clementine plants. Exp. Appl. Acarol. 50: 317-328.

Antonelli, A. L., Shanks, C. H., and Congdon, B. D. 1997. Impact of insecticides on the spider mite destroyer and two-spotted spider mite on red raspberries in Washington. Washington State Univ. Res. Bull. XB1034.

Arthurs, S., Mckenzie, C. L. Chen, J. J., Dogramaci M., Brennan, M., Houben, K., and Osborne, L. 2009. Evaluation of *Neoseiulus cucumeris* and *Amblyseius swirskii* (Acari: Phytoseiidae) as biological control agents of chili thrips, *Scirtothrips dorsalis* (Thysanoptera: Thripidae) on pepper. Biol. Control 49: 91-96.

Bergh, J. C, Rugg, D., Jansson, R. K., Mccoy, C. W., and Robertson, J. L. 1999. Monitoring the susceptibility of citrus rust mite (Acari: Eriophyidae) populations to abamectin. J. Econ. Entomol. 92: 781-787.

Blackwood, J. S., Schausberger, P., and Croft, B. A. 2001. Prey stage preferences in generalist and specialist phytoseiid mites (Acari: Phytoseiidae) when offered *Tetranychus urticae* (Acari: Tetranychidae) eggs and larvae. Environ. Entomol. 30: 1103-1111.

Cakmak, I., Janssen, A., Sabelis, W. M., and Baspinar, H. 2009. Biological control of an acarine pest by single and multiple natural enemies. Biol. Control 50: 60-65.

Campbell, B. L, Nelson, R. G., Ebel Dozier, R. C., Adrian, J. L., and Hockema, B. R. 2004. Fruit quality characteristics that affect consumer preferences for satsuma mandarins. Hort. Science 39: 1664-1669.

Childers, C. C., and Enns, W. R. 1975. Predaceous arthropods associated with spider mites in Missouri apple orchards. J. Kansas Entomol. Soc. 48: 453-471.

Childers, C. C. 1994. Biological control of phytophagous mites on Florida citrus utilizing predatory arthropods, *In* D. Rosen, F. D. Bennett, and J. Capinera [eds.], Pest Management in the Subtropics: Biological Control-A Florida Perspective. Andover, UK.

Childers, C. C., Mccoy, C. W, Nigg, H. N., P. A. Stansly, and Rogers, M. E. 2007. Florida pest management guide: rust mites, spider mites, and other phytophagous mites. Univ. Florida Coop. Ext. Serv., IFAS. Available via DIALOG. http://edis.ifas.ufl.edu/CG002. Cited 1 Mar 2007.

Chow, A., Chau, A., and Heinz, K. M. 2008. Compatibility of *Orius insidiosus* (Hemiptera: Anthocoridae) with *Amblyseius (Iphiseius) degenerans* (Acari: Phytoseiidae) for control of *Frankliniella occidentalis* (Thysanoptera: Thripidae) on greenhouse roses. Biol. Control 44: 259-270.

Colfer, R. G., Rosenheim, J. A, Godfrey, L. D., and Hsu, C. L. 2004. Evaluation of large scale releases of predatory mite for spider mite control in cotton. Biol. Control 30: 1-10.

Denoth, M., Frid, L., and Myers, J. H. 2002. Multiple agents in biological control: improving the odds? Biol. Control 24: 20-30.

English, L. L., and Turnipseed, G. F. 1940. Control of major pest of satsuma orange in south Alabama. Alabama Agric. Expt. Stn. Bull. pp 248.

Fadamiro, H. Y., Hargroder, T., and Nesbitt, M. 2005. Evaluation of acaricides for control of citrus red mite on Satsuma mandarin. Arthropod Manag. Tests. Vol. 30 Rept. No. D2.

Fadamiro, H. Y., Nesbitt, M, and Wall, C. 2007. Crop profile for satsuma mandarin in Alabama. Available via DIALOG. http://www.aces.edu/anr/ipm/old/crop_profiles/a_satsuma_citrus.pdf. Cited 2 May 2008.

Fadamiro, H. Y., Xiao, Y. F., Hargroder, T., Nesbitt, M., Umeh, V., and Childers, C. C. 2008. Seasonal occurrence of key arthropod pests and associated natural enemies in Alabama satsuma citrus. Environ. Entomol. 37: 555-567.

Fadamiro, H. Y., Xiao, Y. F., Nesbitt, M., and Childers, C. C. 2009. Diversity and seasonal abundance of predacious mites in Alabama satsuma citrus. Ann. Entomol. Soc. Am. 102: 617-628.

Fraulo, A. B., and Liburd, O. E. 2007. Biological control of two spotted spider mite, *Tetranychus urticae*, with predatory mite, *Neoseiulus californicus*, in strawberries. Exp. Appl. Acarol. 43: 109-119.

Frank, D. L., Brewster, C. C., Leskey, T. C., and Bergh, J. C. 2011. Factors Influencing the Temporal and spatial patterns of dogwood borer (Lepidoptera: Sesiidae) infestations in newly planted apple orchards. Environ. Entomol. 40: 173-183.

Gotoh, T., and Kubota, M. 1997. Population dynamics of the citrus red mite, *Panonychus citri* (McGregor) (Acari: Tetranychidae) in Japanese pear orchards. Exp. Appl. Acarol. 21: 343-356.

GRAFTON-CARDWELL, E. E., OUYANG, Y., AND STRIGGOW, R. A. 1997. Predaceous mite (Acari: Phytoseiidae) for control of spider mites (Acari: Tetranychidae) in nursery citrus. Environ. Entomol. 26: 121-130.

HAMLEN, R. A., AND LINDQUIST, R. K. 1981. Comparison of two *Phytoseiulus* species as predators of two-spotted spider mites on greenhouse ornamentals. Environ. Entomol. 10: 524-527.

HODDLE, M. S., ROBINSON, L., AND VIRZI, J. 2000. Biological control of *Oligonychus perseae* (Acari: Tetranychidae) on avocado: III. Evaluating the efficacy of varying release rates and release frequency of *Neoseiulus californicus* (Acari: Phytoseiidae). Int. J. Acarol. 26: 203-214.

JAMIESON, L. E., CHARLES, J. G., STEVENS, P. S., MCKENNA, C. E., AND BAWDEN, R. 2005. Natural enemies of the citrus red mites (*Panonychus citri)* in citrus orchards. New Zealand Plant Prot. 58: 299-305.

KATAYAMA, H., MASUI, S., TSUCHIYA, M., TATARA, A., DOI, M., KANEKO, S., AND SAITO, T. 2006. Density suppression of the citrus red mite *Panonychus citri* (Acari: Tetranychidae) due to the occurrence of *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) on Satsuma mandarin. Appl. Entomol. Zool. 41: 679-684.

KRANZ, J., SCHMUTTERER, H., AND KOCH, W. 1977. Diseases, pests, and weeds in tropical crops. Paul Parey, Berlin, Germany.

LAZIC, S. E. 2010. The problem of pseudoreplication in neuroscientific studies: is it affecting your analysis? BMC Neurosci. 11: 5 (http://www.biomedcentral.com/1471-2202/11/5).

MCMURTRY, J. A. 1982. The use of phytoseiids for biological control: progress and future prospects *In* M. A. Hoy [ed.], Recent Advances in Knowledge of the Phytoseiidae. Berkeley, Univ. California Press. CA.

MCMURTRY, J. A. 1983. Phytoseiid predators in orchard systems: a classical biological control success story *In* M. A. Hoy, G. L. Cunningham and L. Knutson [eds.], Biological control of pests by mites. Berkeley, ANR Publications, University of California, CA.

MCMURTRY, J. A. 1991. Augmentative releases to control mites in agriculture *In* F. Dusbabek and V. Bukva [eds.] Modern Acarology, Prague: Academia; The Hague: SPB Academic Publ. pp 151-157.

MCMURTRY, J. A, AND CROFT, B. A. 1997. Life-styles of phytoseiid mites and their role in biological control. Ann. Rev. Entomol. 42: 291-321.

MONETTI, L. N., AND FERNANDEZ, N. A. 1995. Seasonal population dynamics of the European red mite (*Panonychus ulmi*) and its predator *Neoseiulus californicus* in a sprayed apple orchard in Argentina (Acari: Tetranychidae: Phytoseiidae). Acarologia 36**:** 325-331.

MUMA, M. H., AND DENMARK, H. A. 1970. Phytoseiidae of Florida. Arthropods of Florida and neighboring areas. Florida Dept. Agric. Cons. Serv. Vol 6.

NORMAN, G. R., AND STREINER, D. L. 2008. Biostatistics. The bare essentials. B.C. Decker Inc., Hamilton, Ontario, Canada.

OMOTO, C., DENNEHY, T. J., MCCOY, C. W., CRANE, S. E., AND LONG, J. W. 1995. Management of citrus rust mite (Acari: Eriophyidae) resistance to dicofol in Florida citrus. J. Econ. Entomol. 88: 1120-1128.

OPIT, G. P., NECHOLS, J. R., AND MARGOLIES, D. C. 2004. Biological control of two spotted spider mites, *Tetranychus urticae* Koch (Acari: Tetranychidae), using *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseidae) on ivy geranium: assessment of predator release ratios. Biol. Control 29: 445-452.

OTT, R. L., AND LONGNECKER, M. 2001. An introduction to statistical methods and data analysis, 5th ed. Duxbury, Thompson Learning Inc., Pacific Grove, CA.

PRATT, P. D, AND CROFT, B. A. 1998. *Panonychus citri* (Acari: Tetranychidae) on ornamental *Skimmia* in Oregon, with assessment of predation by native phytoseiid mites. Pan-Pacific Entomol. 74: 163-168.

PRATT, P. D., AND CROFT, B. A. 2000. Screening of predatory mites as potential control agents of pest mites in landscape plant nurseries of the Pacific Northwest. J. Environ. Hort. 18: 218-223.

PRATT, P. D., ROSETTA, R., AND CROFT, B. A. 2002. Plant-related factors influence the effectiveness of *Neoseiulus fallacies* (Acari: Phytoseiidae), a biological control agent of spider mites on landscape ornamental plants. J. Econ. Entomol. 95: 1135-1141.

RHODES, E. M., LIBURD, O. E., KELTS, C., RONDON, S. I., AND FRANCIS, R. R. 2006. Comparison of single and combination treatments of *Phytoseiulus persimilis, Neoseiulus californicus*, and Acramite (bifenazate) for control of two spotted spider mites in strawberries. Exp. Appl. Acarol. 39: 213-225.

ROSENHEIM, J. A., KAYA, H. K., EHLER, L. E., MAROIS, J. J., AND JAFFEE, B. A. 1995. Intraguild predation among biological-control agents: theory and evidence. Biol. Control 5: 303-335.

SAS INSTITUTE. 2007. JMP Statistics and Graphics Guide, Version 7.0.1 SAS Institute, Cary, NC

SCHAUSBERGER, P., AND WALZER, A. 2001. Combined versus single species release of predaceous mites: predator-predator interactions and pest suppression. Biol. Control 20: 269-278.

SHANKS, C. H., ANTONELLI, L. A., AND CONGDON, B. D. 1992. Effect of pesticides on two spotted spider mite (Acarina: Tetranychidae) population on red raspberries in western Washington. Agric. Ecosys. Environ. 38: 159-165.

STRONG, W. B., AND CROFT, B. A. 1995. Inoculative release of phytoseiid mites (Acarina: Phytoseiidae) into the rapidly expanding canopy of hops for control of *Tetranychus urticae* (Acarina: Tetranychidae). Environ. Entomol. 24: 446-453.

STRONG, W. B., AND CROFT, B. A. 1996. Release strategies and cultural modifications for biological control of two spotted spider mite by *Neoseiulus fallacis* (Acari: Phytoseiidae) on hops. Environ. Entomol. 25: 529-535.

TAKANO-LEE, M., AND HODDLE, M. S. 2001. Biological control of *Oligonychus perseae* (Acari: Tetranychidae) on avocado: IV. Evaluating the efficacy of a modified mistblower to mechanically dispense *Neoseiulus californicus* (Acari: Phytoseiidae). Intl. J. Acarol. 27: 157-169.

TAKANO-LEE, M., AND HODDLE, M. 2002. *Oligonychus perseae* (Acari:Tetranychidae) response to cultural control attempts in an avocado. Florida Entomol. 85: 216-226.

THISTLEWOOD, H. M. A. 1991. A survey of predatory mites in Ontario apple orchards with diverse pesticide programs. Canadian Entomol. 123: 1163-1174.

URBANEJA, A., PASCUAL-RUIZ, S., AND PINA, T. 2008. Efficacy of some acaricides against *Tetranychus urticae* (Acari: Tetranychidae) and their side-effects on selected natural enemies occurring in citrus orchards. Pest Mgt. Sci. 64: 834-842.

Van De Vrie, M., and Price, J. F. 1994. Manual for biological control of two spotted spider mites on strawberry in Florida. Univ. Florida Agric. Exp. Stn. Rep. DOV, pp 10

Welty, C. 1995. Survey of predators associated with European red mite (*Panonychus ulmi* (Acari: Tetranychidae) in Ohio apple orchards. Great Lakes Entomol. 28: 171-184.

Wood, L., Raworth, D. A., and Mackauer, M. 1994. Biological control of the two spotted spider mite in raspberries with the predator mite, *Phytoseiulus persimilis.* J. Entomol. Soc. British Columbia 91: 59-61.

Xiao, Y. F., and Fadamiro, H. Y. 2010. Functional responses and prey-stage preferences of 3 species of predacious mites (Acari: Phytoseiidae) on citrus red mite, *Panonychus citri* (Acari: Tetranychidae). Biol. Control 53: 345-352.