Functional responses and prey-stage preferences of three species of predacious mites (Acari: Phytoseiidae) on citrus red mite, *Panonychus citri* (Acari: Tetranychidae)

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ABSTRACT

The citrus red mite, *Panonychus citri* (McGregor) (Acari: Tetranychidae), is a pest of citrus worldwide, and a major pest of satsuma mandarin (*Citrus unshiu* Marcovitch) in Alabama. This study was conducted to evaluate the potential of three commercially available predacious mite species (Acari: Phytoseiidae), *Phytoseiulus persimilis* Athias-Henriot, *Galendromus occidentalis* (Nesbitt), and *Neoseiulus californicus* (McGregor), as biological control agents of *P. citri*. The functional responses (at densities ranging from 2 to 150 *P. citri* immature per leaf disc arena) and prey-stage preferences (when offered varying ratios of *P. citri* eggs and nymphs) of the phytoseiids were investigated in laboratory experiments. All three species were modestly effective in regulating *P. citri* nymph density, but very few eggs were laid by each on the prey. Logistic regression analysis suggests a Type II (convex) functional 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. The maximum daily predation was recorded at a prey density of 75 *P. citri* nymphs per arena for female *P. persimilis* and *G. occidentalis*, and at a density of 100 nymphs per arena for female *N. californicus*. Results from experiments on prey-stage preferences showed that all three phytoseiids preferred nymphs to eggs of *P. citri*. Food deprivation for up to 48 h had no significant effect on the number of prey consumed or the prey-stage preferences of the phytoseiids. Among the three species, *P. persimilis* had the highest predation potential, in particular at high prey densities. These results are discussed in relation to the feeding habits and specialization of the phytoseiids and their potential for biological control of *P. citri* in citrus orchards.

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1. 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 immature 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). High infestations of *P. citri* are now associated with allergens to citrus workers (Fernández-Caldas and Calvo, 2007). *P. 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 and Enns, 1975; McMurtry, 1983; Childers, 1994; Wood et al., 1994; Opit et al., 2004; Jamieson et al., 2005; Cakmak et al., 2009; Arthurs 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*, possibly due to factors such as lack of suitable pollen sources and overwintering plants in local orchards, and pesticide use (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 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 insect and mites (Laing and Osborn, 1974; Everson, 1980; Sabelis, 1985; Trexler et al., 1988; De Clercq et al., 2000; Badii et al., 2004; Reis et al., 2003, 2007; Timms et al., 2008). In general, the functional response of a predator to a prey could follow one of three mathematical models: Type I (linear), Type II (convex) or Type III (sigmoid). In the Type I model, the proportion of prey consumed increases linearly with prey availability up to a maximum. In the Type II model, the proportion of prey consumed declines monotonically with prey density. Type III model depicts a sigmoid relationship in which the proportion of prey consumed is positively density-dependent over some region of prey density (Holling, 1959, 1961; Trexler et al., 1988; De Clercq et al., 2000; Timms et al., 2008). In terms of biological control, predators and parasitoids which exhibit the Type III functional response, by showing positive density-dependent prey consumption, are usually regarded as efficient biological control agents (Fernández-Arhex and Corley, 2003; Pervez and Omkar, 2005). Nevertheless, there are some examples of natural enemies with the Type II functional response model which have been successfully used as biological control agents (Hughes et al., 1992; Fernández-Arhex and Corley, 2003).

Trexler et al. (1988) compared different methods of analysis of functional response data and reported that the logistic regression analysis is better than other methods, such as linear least-square analysis and curve fitting with predator foraging models, in determining the true functional response of a predator to a prey. Other authors have also reported that the logistic regression analysis is particularly useful in differentiating between the Type II and Type III responses by estimation of linear coefficient (De Clercq et al., 2000; Timms et al., 2008).

The objectives of the study were to (1) determine the potential of three commercially available phytoseiid species, *P. persimilis*, *G. occidentalis* and *N. californicus*, as biological control agents of *P. citri* immatures by evaluating their functional responses; (2) investigate the prey-stage preferences of the three phytoseiid species, in order to determine what prey stage is suitable for their release; and (3) test the effect of starvation on the predation potential of the three phytoseiid species to determine their survival strategy during periods of food deprivation in the field.

2. Materials and methods

2.1. 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 ($25 \times 10 \times 7$ 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 $25 \pm 2\ °C$, $60 \pm 10\%$ RH and a photoperiod of 14:10 (L:D) h.

2.2. 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.

2.3. Experiment 1: functional response

The functional response of each of the three phytoseiid species on *P. citri* nymphs 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 × 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* nymphs killed and of the 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.

The type of functional response of each predacious mite species was determined by performing a binomial logistic regression analysis (Sigmaplot 8, Systat Software Inc., 2007) of proportion of prey consumed as a function of prey density as represented in Eq. (1). This analysis has been described in detail by several authors (Trexler et al., 1988; De Clercq et al., 2000; Juliano, 2001; Timms et al., 2008). In this equation ($Ne/N = a + bN + cN^2 + dN^3 + e$), Ne is the proportion of killed prey, N is the number of prey density offered, a is the intercept, b, c, and d are the linear, quadratic, and cubic coefficients, respectively.

Type I responses are described by an intercept and constant positive slope (b). In the Type II responses the linear coefficient (b) is <0 and proportion of prey consumed declines monotonically with the initial number of prey offered. Type III responses are characterized by the linear coefficient (b) >0 and the quadratic coefficient <0 (Trexler et al., 1988; De Clercq et al., 2000; Timms et al., 2008). Maximal models were fitted with linear, quadratic and cubic terms and simplified in stepwise manner removing higher order coefficients where they were not significant. A minimal adequate model was thus achieved, and the coefficients were inspected to determine the type of functional response (Timms et al., 2008).

After determining the correct shape of the functional response, a linearization of Holling's disc equation (Holling, 1959) was used to estimate parameters (De Clercq et al., 2000; Timms et al., 2008). In this equation ($Ne = aNT/(1 + aNT_h)$), Ne is the number of killed prey, N is the number of prey offered, T is the total time available for the predator, A is the attack rate, and T_h is the handling time.

This model provided a good fit to our data (as characterized by high R^2 values), especially since the model typically characterizes sampling with replacement (Fenlon and Faddy, 2006), as done in our experiment.

To determine differences among the predacious mite species, the functional responses of the three predacious mite species were compared by using a non-linear least squared regression where two predacious mite species were fitted with the same model to estimate differences in attack rate and handling time. A difference in the functional response of the two predacious mite species is inferred if the differences in parameter estimates are significantly different from 0 (Juliano, 2001; Pervez and Omkar, 2005; Timms et al., 2008).

Data on maximum number of prey killed (number of prey killed at the density at which the highest prey consumption was recorded for each species: 75 *P. citri*/arena for *P. persimilis* and *G. occidentalis*, and 100 *P. citri*/arena for *N. californicus*) were first normalized by using the square-root ($\sqrt{x + 0.5}$) transformation, and then analyzed with analysis of variance (ANOVA) followed by Tukey–Kramer honestly significant difference (HSD) test to determine significant differences in maximum number of prey consumed among the three species ($P < 0.05$, JMP Version 7.0.1, SAS Institute Inc., 2007).

2.4. Experiment 2: prey-stage preference

Experiments were conducted to determine which prey stage (egg or nymph) is preferred by each of the three 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.

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

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

N (eggs) and N' (nymphs) are the numbers of each prey type provided, and Nc (eggs) and Nc' (nymphs) 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 β value was calculated for each replicate and averaged to determine the mean β value for each treatment.

Data on prey-stage preference were first normalized by using square-root transformation ($\sqrt{x + 0.5}$) and then analyzed with ANOVA. For each species at a given ratio, differences between the mean numbers of each prey stage (eggs versus nymphs) killed were established at ratios 1:1, 1:2, and 2:1 eggs:nymphs by using the Student's *t*-test ($P < 0.05$, JMP Version 7.0.1, SAS Institute Inc., 2007). Significant differences among the three species in the mean numbers of each prey stage killed and the combined (eggs + nymphs) numbers of prey killed were also established (1:1, 1:2, and 2:1 ratio of eggs to nymphs) by using the Tukey–Kramer HSD test or Student's *t*-test ($P < 0.05$, JMP Version 7.0.1, SAS Institute Inc., 2007).

2.5. 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 be-

fore 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. Starvation data were first normalized by using square-root transformation ($\sqrt{x + 0.5}$) and then analyzed with ANOVA followed by the Tukey–Kramer HSD ($P < 0.05$) test to determine significant differences among starvation treatments (0, 24, 48 h) in the mean numbers of each prey stage killed and the combined numbers of prey killed.

3. Results

3.1. Experiment 1: functional response

All three phytoseiid species exhibited the Type II functional response on *P. citri* nymphs (Fig. 1). For *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. For *G. occidentalis*, the number of *P. citri* killed per day increased from ∼1 at a prey density of 2 to a maximum of 12 (∼17% predation) at a prey density of 75 per arena. For *N. californicus*, 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 per arena. For all three species, the percentage of prey killed decreased as a function of prey density offered, and linear coefficient (b) was <0, resulting in the Type II (convex) functional response (Table 1).

Comparison of functional response curves revealed no significant differences among the three species. There was no significant difference between the functional response of *P. persimilis* and *G. occidentalis* ($F = 0.58$; df = 1, 16; $P = 0.45$), between *P. persimilis* and *N. californicus* ($F = 3.26$; df = 1, 16; $P = 0.09$), and between *G. occidentalis* and *N. californicus* ($F = 1.70$; df = 1, 16; $P = 0.21$). However, the maximum number of prey killed was significantly higher for *P. persimilis* than for *N. californicus* ($F = 7.05$; df = 2, 83; $P = 0.0015$) (Table 2). Estimates of functional response parameters for the three species showed that *G. occidental* had slightly higher attack rate than the remaining two species, whereas *P. persimilis* had the shortest handling time (Table 2). Very few eggs were laid by each of the three phytoseiid species on *P. citri* nymphs, irrespective of prey density (Table 3).

In general, *P. persimilis* consumed the highest number of prey (*P. citri* nymphs), while *N. californicus* consumed the lowest (Fig. 1).
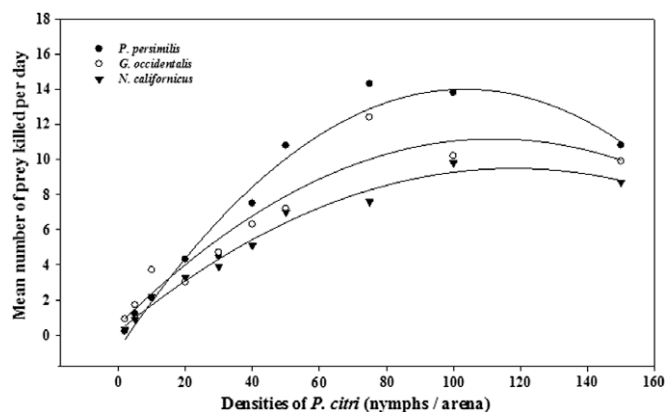

Fig. 1. Relationship between numbers of *Panonychus citri* preyed on by a female of *Phytoseiulus persimilis*, *Galendromus occidentalis*, or *Neoseiulus californicus* and the density of *P. citri* (nymphs) provided per day. For all three species, the data followed the Type II (convex) functional response model in which the number of prey consumed increased with prey availability but began to decrease when a maximum point was reached.

Table 1
Estimates of coefficients in a binomial logistic regression of the proportion of prey eaten on total prey for three predatory mites against *Panonychus citri* under laboratory conditions.

Predacious mite species	Type of functional response	Estimate	SE	t	P
P. persimilis	Intercept	0.5418	0.0745	7.268	0.0003
	Linear (b)	−0.0429	0.0453	−0.9456	0.0380
	Quadratic (c)	0.0078	0.0076	1.0288	0.3433
	Cubic (d)	−0.0005	0.0004	−1.3544	0.2244
G. occidentalis	Intercept	1.0246	0.1176	8.7103	0.0001
	Linear (b)	−0.2202	0.0715	−3.0794	0.0217
	Quadratic (c)	0.0261	0.012	2.1764	0.0724
	Cubic (d)	−0.0011	0.0006	−1.8554	0.1129
N. californicus	Intercept	0.4238	0.0314	13.505	<0.0001
	Linear (b)	−0.0006	0.0023	−0.2506	0.0809
	Quadratic (c)	−0.00001	0.00004	−0.3209	0.7591
	Cubic (d)	0.0000006	0.0000002	0.3325	0.7509

Table 2
Estimates of functional response parameters from linearization of Holling's Type II model.

Predacious mite species	Maximum no. prey killed[a]	Attack rate (A)	Handling time (T_h)	R^2
P. persimilis	14.28 ± 0.95a	0.0691	3.02	0.9867
G. occidentalis	12.36 ± 0.78ab	0.124	3.36	0.9048
N. californicus	9.75 ± 0.81b	0.0454	3.69	0.9892

[a] Means having no letters in common are significantly different ($P < 0.05$, ANOVA, Tukey HSD).

Table 3
Reproduction of three species of predacious mites at different densities of the prey, *Panonychus citri* under laboratory conditions.

Densities/arena	Mean (±SE) number of eggs laid per day		
	P. persimilis	*G. occidentalis*	*N. californicus*
2	0 ± 0	0 ± 0	0 ± 0
5	0 ± 0	0 ± 0	0 ± 0
10	0 ± 0	0 ± 0	0 ± 0
20	0.1 ± 0.1	0 ± 0	0 ± 0
30	0.6 ± 0.3	0 ± 0	0.1 ± 0.1
40	0.1 ± 0.1	0.3 ± 0.2	0.1 ± 0.1
50	0.4 ± 0.3	0 ± 0	0.2 ± 0.1
75	0.7 ± 0.3	0.4 ± 0.3	0.1 ± 0.1
100	0.7 ± 0.3	0.3 ± 0.2	0.3 ± 0.2
150	0.4 ± 0.3	0.6 ± 0.3	0.3 ± 0.3

Natural mortality of prey observed in the control was minimal: 0–4.6%, 0–9.3%, and 0–6.7% for *P. persimilis*, *G. occidentalis*, and *N. californicus*, respectively. This suggests that predation by the predacious mites was the dominant source of *P. citri* nymphs mortality recorded in this experiment.

3.2. Experiment 2: prey-stage preference

When eggs and nymphs of *P. citri* were simultaneously presented, all three species fed on both stages but highly preferred nymphs, irrespective of prey ratio (Table 4). This result was confirmed by the very high (>0.65) preference index (β) recorded for all three species (Fig. 3): β 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). *P. persimilis* also consumed more nymphs than the other two species, when only this prey stage was provided (Table 4). Similar results were recorded when the number

of each prey-stage consumed was pooled across the 1:1, 1:2, and 2:1 ratios and compared among the three species. *P. 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 β. 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: both increased preference for nymphs in response to an increase in the relative abundance of eggs (Fig. 3).

3.3. 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 5). In other words, similar numbers of eggs and nymphs were consumed in the different starvation treatments. Furthermore, starvation had no significant effect of the prey-stage preferences of the phytoseiids: all three species showed similar preference for nymphs, irrespective of starvation duration. These results were also confirmed by the lack of a significant change in β with starvation (*P. persimilis*: $F = 0.211$; $P = 0.8$, *G. occidentalis*: $F = 0.965$; $P = 0.39$, *N. californicus*: $F = 0.764$; $P = 0.48$, df = 2, 27) (Fig. 4).

4. Discussion

The results of this study demonstrated the predation potential of the three phytoseiids, *P. persimilis*, *G. occidentalis*, and *N. californicus*, on *P. citri* nymphs. The functional responses of all three

Table 4
Prey-stage preference of three species of predacious mites when offered eggs and nymphs of *Panonychus citri* at different ratios for 24 h under laboratory conditions.

Predacious mite species	Ratio (eggs:nymphs)	Mean (±SE) number of *P. citri* killed		t-test
		Eggs	Nymphs	t, P
P. persimilis	1:0	3.4 ± 0.4	0 ± 0	–
	0:1	0 ± 0	6.7 ± 0.8	–
	1:1	0.9 ± 0.3b	7.3 ± 0.5a	10.2, 0.0001
	1:2	1.2 ± 0.5b	8.1 ± 0.4a	9.07, 0.0001
	2:1	1.0 ± 0.3b	7.6 ± 0a	9.31, 0.0001
G. occidentalis	1:0	2.0 ± 0.5	0 ± 0	–
	0:1	0 ± 0	4.7 ± 0.6	–
	1:1	0.6 ± 0.3b	4.4 ± 0.5a	7.08, 0.0001
	1:2	0.5 ± 0.2b	3.7 ± 0.3a	8.01, 0.0001
	2:1	0.5 ± 0.2b	5.1 ± 0.6a	8.74, 0.0001
N. californicus	1:0	0.4 ± 0.3	0 ± 0	–
	0:1	0 ± 0	4.0 ± 0.6	–
	1:1	0.0 ± 0.0b	1.6 ± 0.4a	9.01, 0.0001
	1:2	0.3 ± 0.2b	3.0 ± 0.7a	3.68, 0.0001
	2:1	0.3 ± 0.2b	4.6 ± 0.6a	8.78, 0.0001

For each species, means in the same row having no letters in common are significantly different ($P < 0.05$, Student's t-test). Note that numbers of eggs versus nymphs consumed were not compared at the first two ratios (0:1 and 1:0).
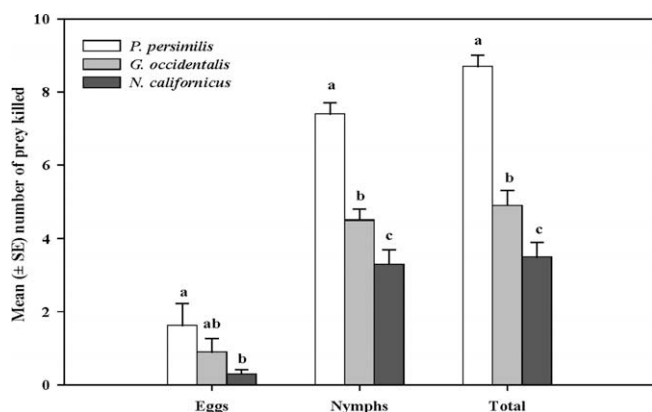
Fig. 2. Predation potential of three phytoseiid species on *Panonychus citri*. Figure shows mean (±SE) number of eggs, nymphs, and total number of prey killed by *Phytoseiulus persimilis*, *Galendromus occidentalis*, and *Neoseiulus californicus* in 24 h. Note that figure was generated using pooled data for the 1:1, 2:1, and 1:2 eggs:nymphs ratios.
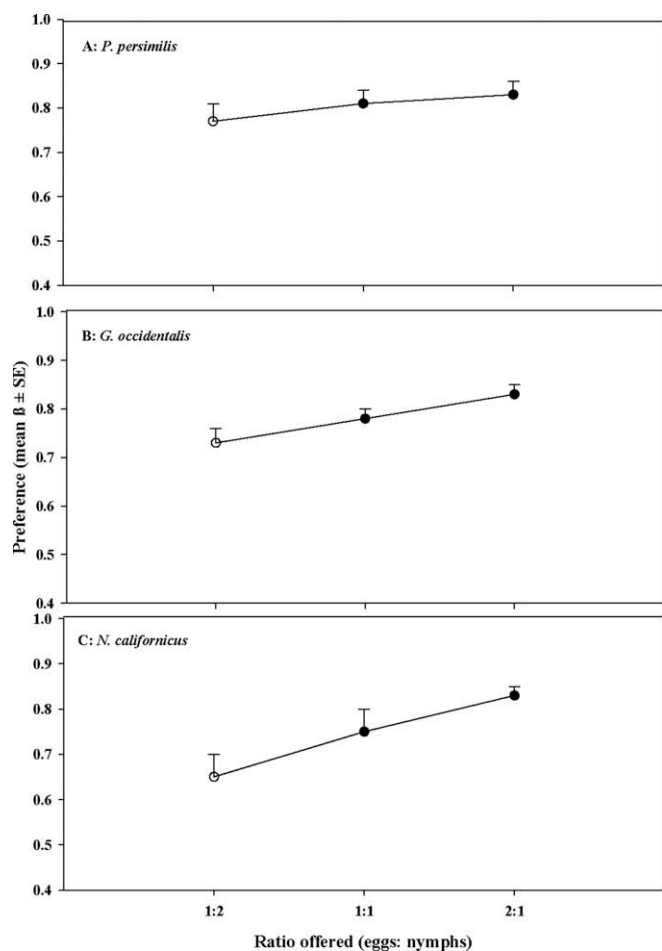
Table 5
Effect of starvation on the predation capability of three species of predacious mites when offered a 1:1 ratio of *Panonychus citri* eggs and nymphs.

Predacious mite species	Starvation duration (h)	Mean (±SE) number of *P. citri* killed		
		Eggs	Nymphs	Total
P. persimilis	0	1.4 ± 0.3	5.2 ± 0.4	6.6 ± 0.5
	24	1.5 ± 0.3	6.5 ± 0.7	8.0 ± 0.5
	48	1.4 ± 0.5	5.8 ± 0.6	7.2 ± 0.8
G. occidentalis	0	1.0 ± 0.3	6.0 ± 0.4	7.0 ± 0.6
	24	0.3 ± 0.2	5.2 ± 0.6	5.5 ± 0.7
	48	0.3 ± 0.2	5.8 ± 0.8	6.1 ± 0.8
N. californicus	0	0.5 ± 0.3	4.5 ± 0.6	5.0 ± 0.7
	24	0.7 ± 0.3	6.3 ± 0.5	7.0 ± 0.5
	48	0.3 ± 0.2	5.7 ± 0.6	6.0 ± 0.5

No significant effect of starvation was recorded for any of the species ($P > 0.05$).

Fig. 3. Prey-stage preferences of female *Phytoseiulus persimilis* (A), *Galendromus occidentalis* (B), and *Neoseiulus californicus* (C) when provided varying ratios (1:1, 1:2 or 2:1) of eggs and nymphs of *Panonychus citri*. Figure shows mean (±SE) preference index (β).
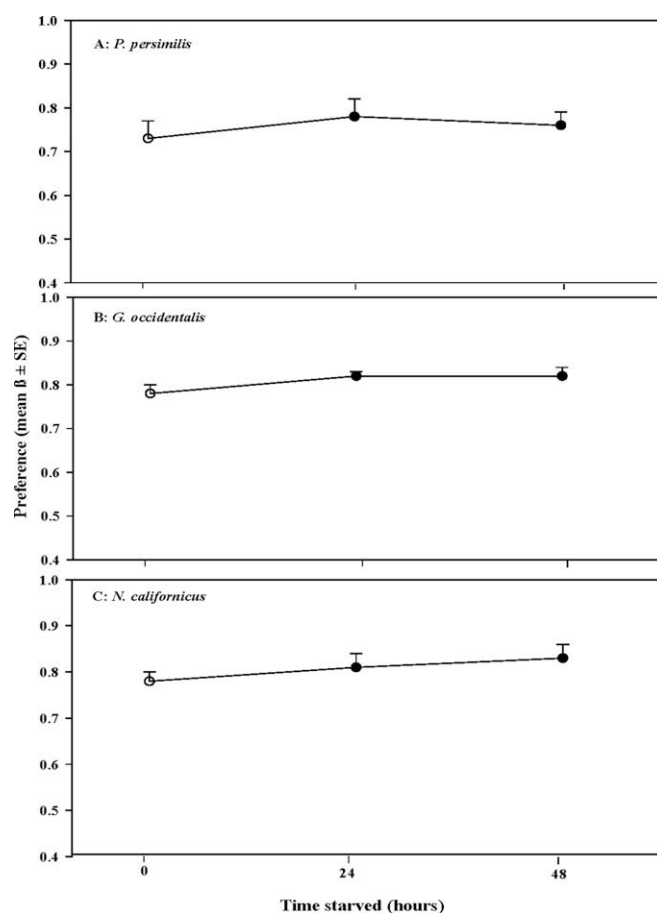

Fig. 4. Effect of starvation on the prey-stage preferences of three species of female *Phytoseiulus persimilis* (A), *Galendromus occidentalis* (B), and *Neoseiulus californicus* (C) when provided a ratio (1:1) of eggs and nymphs of *Panonychus citri*. Figure shows mean (±SE) preference index (β) at 0, 24, and 48 h starvation without food.

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. Although predators which exhibit the Type III functional response are commonly regarded as efficient biological control agents (Fernández-Arhex and Corley, 2003; Pervez and Omkar, 2005), many of the predators that have been successfully released as biological control agents have been shown to exhibit the Type II functional response on their prey (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; De Clercq et al., 2000; Timms et al., 2008). 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*.

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 phytoseiid (*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* nymphs as prey. However, previous studies in which the above or related phytoseiid species were evaluated on *T. urticae* have produced contrasting 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 larvae, 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 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) suggests 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 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 suggests that eggs may be a more profitable prey stage for some predacious mites (Burnett, 1971; Croft and McMurtry, 1972; McMurtry and Rodriguez, 1987). For instance, Croft and McMurtry (1972) showed that *G. occidentalis* females fed on eggs or nymphs of *Tetranychus pacificus* 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 (McMurtry 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 fed 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 eggs versus larvae of *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 significant potential in regulating *P. citri* nymphs. In general, *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 greater number of prey than the other two species in particular at high prey densities and had the shortest handling time. However, very few eggs were laid by the phytoseiids, which may suggest that *P. citri* nymph is not an optimal prey for their reproduction. Our results on the predation potential of the three phytoseiids on *P. citri* nymphs may be explained by the differences in size and activity among the tested phytoseiids. *Phytoseiulus* species are relatively large, and both immatures 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. The results may also be related to the 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 on *P. citri* nymphs than *G. occidentalis*, and *N. californicus*, which are less specialized Type II predators. It is plausible that a specialized predacious mite species such as *P. persimilis*, may have evolved a more efficient prey location and attack strategy than generalist predacious mites.

In conclusion, our results demonstrated for the first time the ability of the commercially available phytoseiids, *P. persimilis*, *G. occidentalis*, and *N. californicus*, to regulate populations of *P. citri* nymphs. Ongoing field studies should confirm the potential of the phytoseiids as biological control agents of *P. citri* in Alabama satsuma orchards. Further studies are necessary to evaluate the predation potential of the phytoseiids on *P. citri* adults and to determine the basis of our results which showed minimal reproductive ability of the predacious mites on *P. citri* nymphs. Also important is the determination and rectification of the factors

limiting the population densities of local predacious mites in Alabama Satsuma orchards (Fadamiro et al., 2009), since these factors may also impact the establishment and performance of introduced predacious mites.

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