Biological Control of the Twospotted Spider Mite (Trombidiformes: Tetranychidae) with the Predatory Mite Neoseiulus californicus (Mesotigmata: Phytoseiidae) in Blackberries

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Biological control of the twospotted spider mite (Trombidiformes: Tetranychidae) with the predatory mite *Neoseiulus californicus* (Mesotigmata: Phytoseiidae) in blackberries

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Abstract

The twospotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is a key mite pest affecting blackberry production worldwide. *Tetranychus urticae* feeds on the underside of leaves, extracts chlorophyll, and reduces crop yield. *Amblyseius* (*Neoseiulus*) *californicus* (McGregor) (Acari: Phytoseiidae) has been identified as a potential predator of *T. urticae*. We conducted a greenhouse and a field experiment to evaluate the potential of *N. californicus* as a biological control agent for *T. urticae* on 'Arapaho,' 'Navaho,' and 'Quachita' blackberry varieties. Research on *N. californicus*-based biological control has not been conducted previously in blackberries because the crop matures during the summer when temperatures are high, and there are concerns whether *N. californicus* can control *T. urticae* populations during these high temperatures. The experimental design was a completely randomized block, and treatments included the following: (1) abamectin, (2) *N. californicus*, and (3) untreated blackberry plants. Abamectin was effective, but mite populations were cyclic and additional applications were needed. The study demonstrated that *N. californicus* provided the most effective and sustained control for *T. urticae* on blackberry plants under hot and humid conditions. A good assessment of local phytoseiids and other predators is needed before releasing *N. californicus* into blackberry plantings.

Key Words: *Rubus*; Tetranychidae; Phytoseiidae; abamectin; biological control; *Tetranychus urticae*

Resumen

La arañita, *Tetranychus urticae* Koch (Acari: Trombidiformes: Tetranychidae), es una plaga acarina clave que afecta la producción de mora en todo el mundo. *Tetranychus urticae* se alimenta del lado inferior de las hojas, extrae clorofila y reduce el rendimiento de los cultivos. *Amblyseius* (*Neoseiulus*) *californicus* (McGregor) (Acari: Mesotigmata: Phytoseiidae) ha sido identificado como un posible depredador de *T. urticae.* Se realizó un experimento del invernadero y de campo para evaluar el potencial de *N. californicus* como agente de control biológico para *T. urticae* en las variedades de mora 'Arapaho,' 'Navaho,' y 'Quachita.' No se han realizado investigaciones sobre el control biológico basado en *N. californicus* anteriormente en moras porque el cultivo madura durante el verano cuando las temperaturas son altas y existe la preocupación de si *N. californicus* puede controlar las poblaciones de *T. urticae* durante estas altas temperaturas. El diseño experimental fue un bloque completamente al azar y los tratamientos incluyeron lo siguiente: (1) abamectina, (2) *N. californicus*, y (3) plantas de mora sin tratamiento. La abamectina fue efectiva, pero las poblaciones de ácaros fueron cíclicas y se necesitaron aplicaciones adicionales. El estudio demostró que *N. californicus* proporcionó el control más efectivo y sostenido para *T. urticae* en plantas de mora en condiciones de calor y humedad. Se necesita una buena evaluación de los fitoseidos locales y otros depredadores antes de liberar *N. californicus* en las plantaciones de mora.

Palabras Clave: *Rubus*; Tetranychidae; Phytoseiidae; abamectina; control biológico; *Tetranychus urticae*

Several mite pests are known to feed aggressively on blackberry, *Rubus* spp. (Rosaceae), plants and can cause total crop loss (Vincent et al. 2010; Demchak 2015). In the USA, the principal mite pest that infests blackberry plants is the twospotted spider mite, *Tetranychus urticae* Koch (Trombidiformes: Tetranychidae) (Isaacs 2013). Larvae, nymphs, and adults of *T. urticae* attack plants and extract chlorophyll from leaves, reducing their photosynthetic ability, and ultimately resulting in yield loss and economic damage. The standard practice to manage phytophagous mites in blackberries is to use broad-spectrum miticides including bifenazate (Acramite®), etoxazole (Zeal®), and abamectin (Agri-Mek®) (Nicastro et al. 2010). However, only a few miticides are labeled for use in blackberries, and there is a high potential for resistance development (Sato et al. 2005). Furthermore, there is a growing intolerance among the general public towards the use of broad-spectrum pesticides on fruit crops; therefore, alternative strategies must be developed. One such strategy involves the use of biological control tactics including inoculative releases of predatory mites for management of *T. urticae* population.

There have been discussions regarding the potential for using *Neoseiulus californicus* (McGregor) (Mesostigmata: Phytoseiidae) for management of *T. urticae* in blackberries. One principal concern in the southeastern US is that blackberries mature later in the yr (compared with other small fruit crops) when temperatures are extremely high, so that *N. californicus* may not be able to pro-

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vide sustained control of pest mites under these high temperature conditions. *Neoseiulus californicus* has been used successfully for management of *T. urticae* in strawberry biological control programs (Easterbrook et al. 2001; Escudero & Ferragut 2005; Rhodes & Liburd 2006; Rhodes et al. 2006; Fraulo & Liburd 2007; Fraulo et al. 2008; Cakmak et al. 2009; Liu et al. 2016). The success of *N. californicus* is partially due to a number of factors, including its feeding habits and its ability to suppress *T. urticae* under varying weather conditions (Croft et al. 1998; Easterbrook et al. 2001). The morphological features of the plants also may affect the performance of *N. californicus* (Kazak et al. 2014). In addition, *N. californicus* has the ability to feed heavily on tetranychid mites, a characteristic of a type 2 specialist predator (Kustutan & Cakmak 2009), as well as feeding on other organisms when tetranychids are unavailable, which represents a type 3 generalist predator (Croft et al. 1998; Rhodes & Liburd 2005).

Rhodes & Liburd (2006) investigated *N. californicus*, *Phytoseiulus persimilis* Athias-Henriot (Mesostigmata: Phytoseiidae), and bifenazate (Acramite®) and found that *N. californicus* performed just as well as the reduced-risk miticide bifenazate in reducing population of *T. urticae*, and increasing yields in strawberry field studies. *Phytoseiulus persimilis* performed well early in the season, but later in the season the *T. urticae* population surged, resulting in significant yield reduction in *P. persimilis*-treated plots. Alzoubi & Çobanoğlu (2008) found that sublethal effects of selected pesticides can be used for managing *T. urticae* when combined with *P. persimilis* and *N. californicus*.

Agri-Mek® SC is a reduced-risk acaricide with the active ingredient abamectin. It was initially thought to be a neurotoxin, but evidence has accumulated that it affects a mitochondrial target site (Van Nieuwenhuyse et al. 2012). In the US, Agri-Mek® SC received a national label for *Polyphagotarsonemus latus* (Banks) (Trombidiformes: Tarsonemidae) in blackberry on 6 Jul 2016 (EPA Reg. No. 100-1351), and efforts are on the way to expand this label to include other key mite pests including *T. urticae*. This pesticide has been shown to be effective against tetranychid mites in many vegetable and fruit crops (Pochubay et al. 2017).

The objectives of this study were to: (1) evaluate the potential for biological control using the predatory mite *N. californicus* as an alternative to broad-spectrum pesticides for management of the phytophagous mite species, *T. urticae*, on blackberry plants under greenhouse and field conditions, and (2) compare the performance of *N. californicus* for reducing *T. urticae* density with a standard reduced-risk miticide, abamectin (Agri-Mek® SC), in blackberry.

Materials and Methods

COLONY

A *T. urticae* colony was established on pinto bean, *Phaseolus vulgaris* L., cv. 'Barbunia' (Fabaceae) plants and grown in 2.5 L polyethylene pots in the Small Fruit and Vegetable IPM greenhouse at the University of Florida in Gainesville, Florida, USA. The colony was initially obtained from a rearing facility at the University of Florida, Mid-Florida Research and Education Center in Apopka, Florida, USA, and kept at 27 ± 1.5 °C, 70 ± 1.5% RH, and a 14:10 h (L:D) photoperiod. To maintain the colony, damaged plants were replaced with new bean plants every 7 d. Plants were watered manually as needed.

Neoseiulus californicus mites (all mobile stages) were obtained from Koppert Biological Systems (Howell, Michigan, USA) in plastic bottles (250 mL) with 5 mm openings in the covers and were used within 48 h of arrival date. An initial test was performed in the laboratory to determine the viability of the predatory mites. Approximately 200 mites were placed in a Petri dish, and their activities were observed for approximately 10 min using a dissecting microscope (10×) (Leica Microsystems, Heerbrugg, Switzerland). This was done with each batch to ensure that mites were active and alive before they were released in the greenhouse and field experiments.

WEATHER PARAMETERS

Hobo® temperature and relative humidity data loggers (Tech Instrumentation Inc., Elizabeth, Colorado, USA) was used to record the temperature and humidity throughout the duration of the greenhouse and field experiments.

GREENHOUSE EXPERIMENT

Growing plants in large pots is a relatively new trend in small fruit production (blackberry and blueberry); subsequently, a greenhouse experiment was conducted at the University of Florida in Gainesville, Florida, USA, from 1 Sep 2016 to 21 Oct 2016. Mature blackberry potted plants were purchased from a commercial nursery in Alachua County, Florida. Blackberry (*Rubus* spp.) plants used in the study consisted of 2 varieties, 'Arapaho' and 'Navaho'. Blackberry plants were grown according to standard production practices for Florida (Andersen 2001) in professional mix soil (Jungle Growth, Statham, Georgia, USA) in 20 cm diam pots (Home Depot, Gainesville, Florida, USA). Briefly, plants were fertilized with N:P:K (10:10:10) and manually watered 3 to 4 times per wk. At the start of the experiment, blackberry plants were inspected with a 10× hand lens to make sure that plants were free of mites.

Experimental Design

The experimental design was a randomized complete block with 3 treatments and 5 replicates. Treatments were blocked by variety with individual plot size consisting of 4 potted blackberry plants. The 3 treatments were: (1) release of *N. californicus*, (2) application of abamectin, and (3) untreated control.

Release of *N. californicus* was made at approximately 1:10 ratio (1 *N. californicus* to 10 *T. urticae*). The total level of *N. californicus* was calculated using the following formula: (number of *T. urticae* per leaf) × (number of leaves per plant) × (number of plants in plot) / the ratio of release (Cakmak et al. 2005). To release *N. californicus*, we used plastic bottles (250 mL with 5-mm openings in the covers) by gently rotating and slightly shaking the bottle over blackberry plants (Liu et al. 2016). A 1:10 ratio was ensured by visually inspecting plants with a 10× hand lens after initial release. *Neoseiulus californicus* were released on 10 Sep 2016, which was the same d the abamectin treatment was applied.

Abamectin (Agri-Mek® SC) (Syngenta, Greensboro, North Carolina, USA) was applied at the manufacturer's labelled rate of 225 mL per hectare. Agri-Mek® SC was sprayed using a 15 L (4 gal) backpack sprayer fitted with XR Teejet nozzle (11004 VK) (spraysmarter.com; Mooresville, Indiana, USA). During the experiment, 2 separate applications were made on 10 Sep and 30 Sep 2016.

In the untreated control, the blackberry plants were left untreated. Each treatment group within blocks was separated by a 1.6 m × 1.8 m plastic barrier to prevent any movement of mites between treatments (Liu et al. 2016).

At the start of the experiment, blackberry plants were artificially infested by clipping an average of 5 *T. urticae* infested bean leaves (*P. vulgaris*), containing at least 5 *T. urticae* motiles, directly onto blackberry bushes that had at least 20 leaves. With the exception of the pesticide used in the Agri-Mek® treatment, no acaricides were used during experimentation. Blackberry plants were approximately 3 yr old with an average height of 1.5 m prior to the start of the experiment.

Sampling

A pretreatment sample was taken before treatments were applied to ensure that each plot had similar numbers of *T. urticae*. Leaf sampling started 7 d after initial treatments were applied. Once per wk for 6 wk, 20 leaves were randomly collected per treatment. Collected leaves from each plot were kept in separate Ziploc storage bags (Glad®, Oakland, California, USA) and brought back to the laboratory, and the number of *N. californicus*, *T. urticae* motiles (all stages except eggs), and eggs were visually inspected and counted within 24 h using a dissecting binocular microscope (10–20×) (Leica MZ12.5; McBain Instruments, Chatsworth, California, USA). The working hypothesis was that when *T. urticae* motile densities exceeded an average of 4 mites per leaf (grower-based threshold), *N. californicus* releases or Agri-Mek® SC would be used for mite management. Mite numbers in the *N. californicus* treatment never exceeded the grower threshold; therefore, a second application was made only in the Agri-Mek® SC treatment. Untreated control plots were never treated with predatory mites or miticides. We also collected data on other mite species and secondary pests.

FIELD EXPERIMENT

The field study was conducted from 1 Sep to 23 Oct 2016 outdoor at a temporary experimental site adjacent to the greenhouse at the University of Florida in Gainesville, Florida. Mature blackberry potted plants were purchased from a commercial nursery in Alachua County, Florida, and established next to the greenhouse approximately 2 mo prior to the start of the experiment. Three blackberry varieties, Arapaho, Navaho, and Quachita, were used in the field experiment. Plants were approximately 3 yr old with an average height of 1.5 m. Similar to the greenhouse experiments, plants were grown following standard horticultural practices for Florida, fertilized with N:P:K (10:10:10), and watered manually about 4 to 5 times per wk as needed (Andersen 2001). Approximately 2 wk prior to the start of the experiment, *T. urticae* reared in a colony on bean plants was introduced onto blackberry plants. This was done by following the same procedures as the greenhouse study.

Experimental Design

The experimental design was the same as the greenhouse study having a randomized complete block with 3 treatments and 5 replicates. Treatments were blocked according to variety, and a 20 m buffer zone was placed between blocks. Plot size consisted of 8 plants spaced 1 m between plants and 2 m between rows.

Tetranychus urticae were introduced onto blackberry plants using the same method as the greenhouse study. Three treatments were included: (1) release of *N. californicus* at the same 1:10 ratio as discussed under the greenhouse experiment; (2) treatment of abamectin (Agri-Mek® SC) applied using a 15 L (4 gal) backpack sprayer fitted with a XR Teejet nozzle (11004 VK) at the manufacturer's labeled rate of 225 mL per ha; (3) untreated plots, control plots are without treatment.

SamplingtA pretreatment sample was taken before treatments were applied to ensure that each treatment plot had similar numbers of *T. urticae*. Regular leaf sampling started 7 d later and was conducted once per wk until 17 Oct 2016. Each wk, 20 leaves were randomly collected per treatment. The total number of eggs and motiles (all stages except eggs) of *N. californicus* and *T. urticae* were counted under a 10× dissecting microscope and recorded.

Additionally, all detected phytoseiids in the greenhouse and field studies were mounted and identified to ensure that these mites were either *N. californicus* or other species.

DATA ANALYSIS

Egg and motile data were analyzed using repeated measures ANOVA with mean separation by Tukey's HSD test using PROC GLM (SAS Institute 2012). Data were considered significant when the P value ≤ 0.05.

A '*t*' test was used to compare the susceptibility of varieties Arapaho and Navaho to *T. urticae* in the greenhouse experiment. An ANOVA was used to compare the susceptibility of the 3 blackberry varieties, Arapaho, Navaho, and Quachita, to *T. urticae* in the field experiment. All motile and egg data from the various mite species were square root transformed to normalize the distribution and homogenize the variances. Untransformed means are presented in the tables and figures.

Results

THE EFFECT OF *NEOSEIULUS CALIFORNICUS* AND AGRI-MEK® SC ON *TETRANYCHUS URTICAE* POPULATIONS ON DIFFERENT BLACKBERRY VARIETIES

Greenhouse Experiment

There were no significant differences in the numbers of *T. urticae* motiles and eggs (t = 2.1; df = 18; P = 0.15) on the blackberry varieties Arapaho and Navaho.

Twospotted spider mite (motiles). During the pre-treatment sample period (9 Sep 2016), there were no differences among the treatments. In the second wk (16 Sep 2016) of sampling, we observed significant differences among all treatments (Table 1). The control plants had the highest number of *T. urticae* motiles, averaging 40.27 ± 3.0 motiles per leaf, and was significantly higher than blackberry plants treated with the *N. californicus* treatment (8.35 ± 1.42 motiles per leaf) and Agri-Mek® SC (1.06 ± 0.33 motiles per leaf). Agri-Mek® SC had significantly fewer motiles than *N. californicus*. Untreated blackberry plants (control) continued to maintain the highest number of motiles (30.90 ± 2.06 motiles per leaf) during the third wk of sampling (23 Sep 2016), and was significantly higher than the other treatments (Table 1). The *Neoseiulus californicus* treatment performed better than Agri-Mek® SC and had significantly fewer *T. urticae* motiles. A second application of Agri-Mek® SC was made on 30 Sep 2016. During wks 4 to 6 of sampling, *N. californicus* had the fewest *T. urticae*, barely exceeding 0.2 motile mites per leaf, significantly fewer than in the Agri-Mek® SC and control treatments. The control maintained the highest population of motiles, averaging between 17.31 ± 0.72 and 32.85 ± 0.81 motile mites per leaf (Table 1).

On varieties Arapaho and Navaho, after the first release of *N. californicus*, the highest population peaks of *T. urticae* never reached the threshold under greenhouse conditions. However, the population level of *T. urticae* was above the threshold (about 5 *T. urticae* per leaf) on 9 and 30 Sep in the Agri-Mek® SC plots on both varieties. After spraying with Agri-Mek® SC on these dates, the population remained below damaging levels for 2 wk (Figs. 1, 2).

Eggs. During the pre-treatment sampling, there were no differences among the treatments. During the second (16 Sept 2016) and third wk (23 Sep 2016) of sampling, the control plants had significantly high-

Table 1. Mean ± SEM number of *T. urticae* motiles and eggs per leaf in different treatments on blackberry plants in a greenhouse experiment.

	Treatment					
	(Mean ± SEM* motiles per leaf)			(Mean ± SEM* eggs per leaf)		
Dates	Agri-Mek® SC	*N. californicus*	Control	Agri-Mek® SC	*N. californicus*	Control
9 Sep 2016**	67.96 ± 4.01 a	62.04 ± 4.00 a	52.21 ± 3.99 a	2.40 ± 0.33 a	2.31 ± 0.75 a	2.83 ± 0.32 a
16 Sep 2016	1.06 ± 0.33 c	8.35 ± 1.42 b	40.27 ± 3.00 a	0.50 ± 0.19 b	0.42 ± 0.22 b	1.77 ± 0.45 a
23 Sep 2016	3.65 ± 0.25 b	0.27 ± 0.17 c	30.90 ± 2.06 a	0.00 ± 0.00 b	0.00 ± 0.00 b	1.37 ± 0.14 a
30 Sep 2016***	9.65 ± 0.35 b	0.21 ± 0.17 c	32.85 ± 0.81 a	0.98 ± 0.19 a	0.00 ± 0.00 b	1.35 ± 0.37 a
07 Oct 2016	0.21 ± 0.09 b	0.08 ± 0.05 c	29.67 ± 0.83 a	0.00 ± 0.00 b	0.13 ± 0.09 b	1.67 ± 0.22 a
14 Oct 2016	1.21 ± 0.24 b	0.13 ± 0.05 c	17.31 ± 0.72 a	0.40 ± 0.20 ab	0.06 ± 0.03 b	0.81 ± 0.30 a
Overall	13.96 ± 3.61 b	11.85 ± 3.37 c	33.87 ± 1.78 a	0.71 ± 0.14 b	0.49 ± 0.17 c	1.47 ± 0.17 a

The data presented in the table are the actual means; however, the letters are the result of square root transformations. Means within the row followed by a different letter are significantly different at $P < 0.05$ based on Tukey's Studentized Range (HSD) test.

*Standard Error of Mean. In 2016, the Degrees of Freedom (df) for each wk is 2, 14; and overall 2, 119. For motiles (all life stages except eggs) and eggs 10 Sep $F = 4.27$; $P = 0.827$ (motiles); $F = 0.64$; $P = 0.5429$ (eggs); 16 Sep $F = 138.86$; $P < 0.0001$ (motiles); $F = 8.64$; $P = 0.0036$ (eggs); 23 Sep $F = 489.04$; $P < 0.0001$ (motiles); $F = 10.21$; $P = 0.0018$ (eggs); 30 Sep $F = 625.42$; $P < 0.0001$ (motiles), $F = 14.04$; $P = 0.0005$ (eggs); 07 Oct $F = 850.79$; $P < 0.0001$(motiles), $F = 68.28$; $P < 0.0001$ (eggs); 14 Oct $F = 408.10$; $P < 0.0001$ (motiles); $F = 3.51$; $P = 0.0581$ (eggs); Overall $F = 876.51$; $P < 0.0001$ (motiles); $F = 47.62$; $P < 0.0001$ (eggs).

**Pretreatment sample, first application of abamectin (Agri-Mek® SC), and *Neoseiulus californicus*

***Second application of Agri-Mek® SC

er number of *T. urticae* eggs compared with plants in the *N. californicus* and Agri-Mek® SC treatments (Table 1). There were no differences between plants treated with *N. californicus* and Agri-Mek® SC. During the fourth wk of sampling (30 Sep 2016), there was no significant difference between *T. urticae* population on plants treated with Agri-Mek® SC and the control. However, *T. urticae* population on plants treated with *N. californicus* remained significantly lower than both Agri-Mek® SC and the control (Table 1). One wk after the second application of Agri-Mek® (7 Oct 2016), there were no differences between *N. californicus* and Agri-Mek® SC, and these blackberry plants had significantly fewer *T. urticae* eggs than the control. Similarly, during the sixth wk (14 Oct 2016) of sampling, there was no difference between *N. californicus* and Agri-Mek® SC; however, the *N. californicus* treatment had significantly fewer *T. urticae* eggs than the control (Table 1). The egg population of *T. urticae* in all plots showed similar trends as the motile stages on varieties Arapaho and Navaho blackberries (Figs. 3, 4).

Other mites and insects recorded on blackberry plants. A small number of mites in the families Phytoseiidae, Tenuipalpidae, and Tydeidae were recorded in the untreated pots in the control treatment. None of these mites were recorded in the Agri-Mek® SC or *N. californicus* treatments. Other arthropods recorded in the greenhouse during the course of the experiment were aphids, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), and whiteflies, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). None of these arthropods were significantly dif-

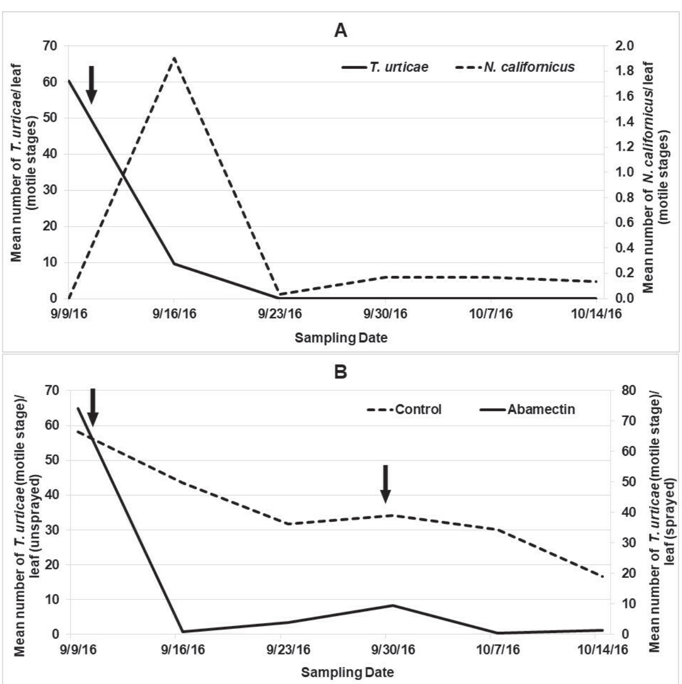

Fig. 1. Population of *T. urticae* (TU) and *N. californicus* (NC) motiles in treatments of *N. californicus* (A), Abamectin, and unsprayed (control) plots (B), on Arapaho variety in a greenhouse experiment. (The arrows on the graph indicate the time of treatment and mite density at that time.)

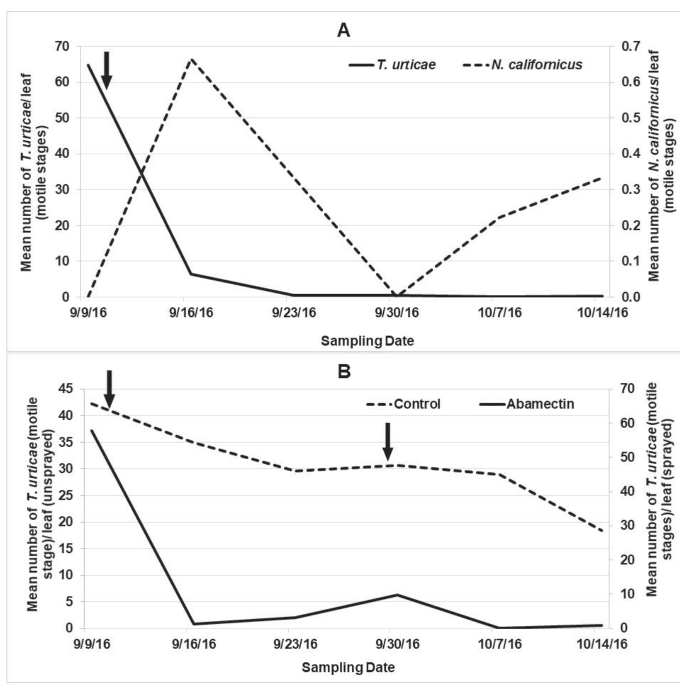

Fig. 2. Population of *T. urticae* (TU) and *N. californicus* (NC) motiles in treatments of *N. californicus* (A), Abamectin, and unsprayed (control) (B), on Navaho variety in a greenhouse experiment. (The arrows on the graph indicate the time of treatment and mite density at that time.)

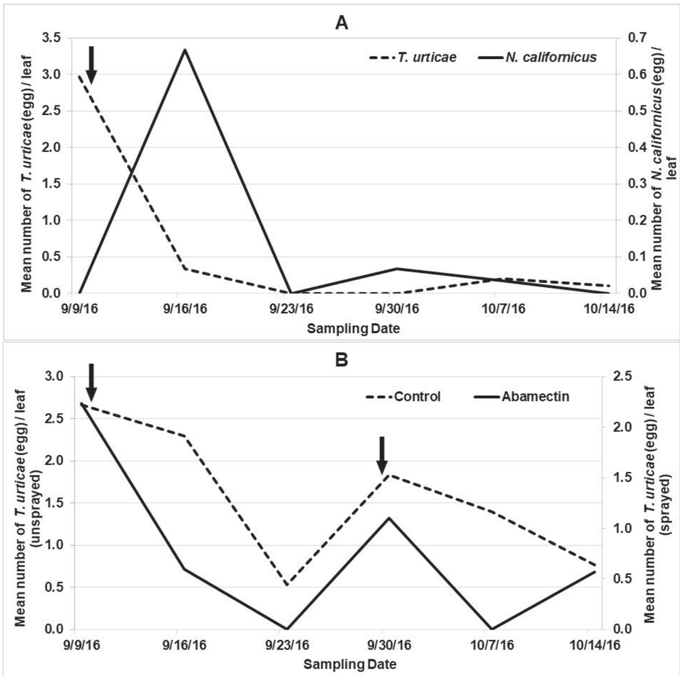

Fig. 3. Population of *T. urticae* (TU) and *N. californicus* (NC) eggs stages in *N. californicus* (A), Abamectin, and unsprayed (control) (B), plots on Arapaho variety in a greenhouse experiment.

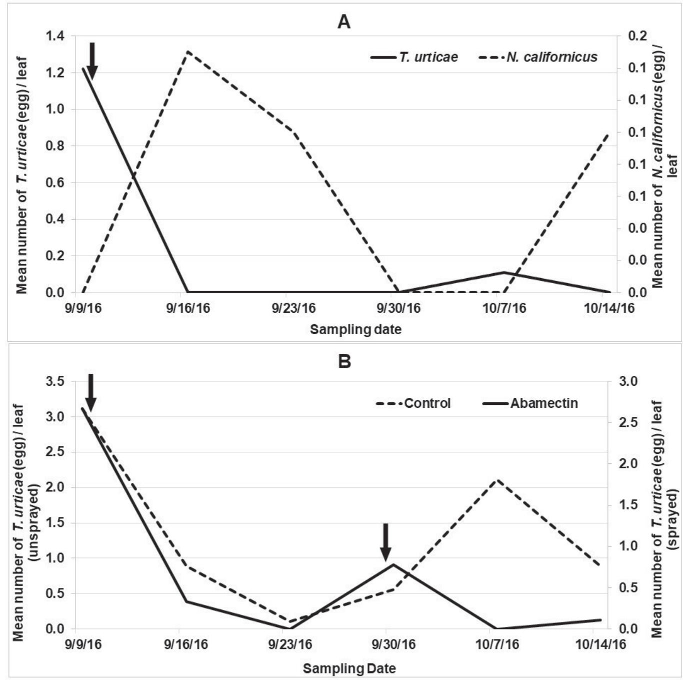

Fig. 4. Population of *T. urticae* (TU) and *N. californicus* (NC) eggs in treatments of *N. californicus* (A), Abamectin, and unsprayed (control) (B), plots on Navaho variety in a greenhouse experiment.

ferent among treatments. The only beneficial insect that was recorded in the greenhouse was the larvae of the flower fly, *Allograpta obliqua* (Say) (Diptera: Syrphidae).

Field Experiment

There were no significant differences in the numbers of *T. urticae* motiles and eggs on blackberry varieties Navaho, Quachita, and Arapaho (F = 2.39; df = 2, 350; P = 0.093).

Motiles. During the pre-treatment sample (5 Sep 2016), there was no difference in *T. urticae* population levels among the treatments. In the second wk (12 Sep 2016) of sampling, the control had significantly higher *T. urticae* levels than Agri-Mek® SC and *N. californicus*, but there was no difference in the number of *T. urticae* between Agri-Mek® SC and *N. californicus* (Table 2). During the third wk of sampling (19 Sep 2016), we recorded significant differences in *T. urticae* population among all treatments. The control had significantly higher numbers of *T. urticae* per leaf (10.47 ± 0.37) compared with the other treatments, Agri-Mek® SC and *N. californicus*, which had only 0.97 ± 0.16 motiles per leaf and 0.00 ± 0.00 motiles per leaf, respectively (Table 2). This trend continued for the rest of the sampling period, wherein blackberry plants treated with *N. californicus* had significantly fewer *T. urticae* mites than plants treated with Agri-Mek® SC and the control.

Table 2. Mean ± SEM number of *T. urticae* motiles and eggs per leaf in different treatments on blackberry plants in a field experiment.

	Treatment					
	(Mean ± SEM* motiles per leaf)			(Mean ± SEM* eggs per leaf)		
Dates	Agri-Mek® SC	*N. californicus*	Control	Agri-Mek® SC	*N. californicus*	Control
05 Sep 2016**	66.80 ± 5.76 a	57.75 ± 4.04 a	64.50 ± 6.90 a	8.75 ± 3.24 a	1.10 ± 0.06 a	5.20 ± 2.89 a
12 Sep 2016	1.83 ± 0.60 b	3.47 ± 0.54 b	26.70 ± 1.14 a	1.07 ± 0.65 ab	0.60 ± 0.30 b	2.80 ± 2.47 a
19 Sep 2016	0.97 ± 0.27 b	0.00 ± 0.00 c	10.47 ± 0.37 a	0.00 ± 0.00 a	0.10 ± 0.10 a	0.00 ± 0.00 a
26 Sep 2016	0.77 ± 0.16 b	0.00 ± 0.00 c	10.57 ± 0.72 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
03 Oct 2016	0.47 ± 0.03 b	0.00 ± 0.00 c	8.54 ± 0.48 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
10 Oct 2016	0.60 ± 0.24 b	0.00 ± 0.00 c	5.83 ± 0.17 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
17 Oct 2016	0.13 ± 0.061 b	0.00 ± 0.00 c	3.63 ± 0.12 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.17 ± 0.13 a
Overall	10.22 ± 4.03 b	8.75 ± 3.48 c	18.61 ± 3.54 a	1.40 ± 0.67 b	0.31 ± 0.11 b	1.17 ± 0.59 ab

The data presented in the table are the actual means; however, the letters are the result of square root transformations. Means within the row followed by a different letter are significantly different at $P \leq 0.05$ based on Tukey's Studentized Range (HSD*)* test.

*Standard Error of Mean. In 2016, the Degrees of Freedom (df) for each wk is 2, 8; and overall 2, 80. For motiles (all life stages except eggs) and eggs 7 Sep F = 0.76; P =0.4966 (motiles); F = 2.38; P = 0.155 (eggs); 12 Sep F = 121.3; $P \leq 0.0001$ (motiles); F =10.40; P = 0.054 (eggs); 19 Sep F 325.12; $P \leq 0.0001$ (motiles); F =1.0; P = 0.4096 (eggs); 26 Sep F = 545.55; $P \leq 0.0001$ (motiles); 03 Oct F = 985.64; $P \leq 0.0001$ (motiles); 10 Oct F = 1090.29; $P \leq 0.0001$ (motiles); 17 Oct F = 196.18; $P \leq 0.0001$ (motiles) F = 2.26; P = 0.16 (eggs); Overall F = 514.00; $P \leq 0.0001$ (motiles), F = 2.26; P = 0.028 (eggs).

**Pretreatment sample, first application of abamectin (Agri-Mek® SC), and *Neoseiulus californicus*

Plants receiving *N. californicus* remained without *T. urticae* mites from the third wk of sampling until the end of the experiment. After applying the first Agri-Mek® SC spray, the population of *T. urticae* was below the threshold for the rest of the sampling period (Table 2). An interesting observation is that spider mite densities also were reduced slightly in the control treatment, suggesting other factors also could have played a role in the reduction of *T. urticae* mites (Figs. 5–7).

Eggs. During the pre-treatment sample (7 Sep 2016), there were no significant differences in *T. urticae* populations among any of the treatments. One wk later (12 Sep 2016) experimental plots treated with *N. californicus* had significantly fewer *T. urticae* eggs than the control (untreated). However, the population of *T. urticae* on *N. californicus*-treated plants was not different from plots treated with Agri-Mek® SC (Table 2). For the remainder of the sampling period there were no *T. urticae* eggs found on the treatment plants with the exception of the control, which had 0.17 ± 0.13 *T. urticae* eggs on 17 Oct 2016 (Table 2).

Overall, blackberry plants treated with *N. californicus* had significantly fewer *T. urticae* egg density than the control. The abundance of *T. urticae* eggs in all treatments followed almost the same fluctuations as the populations of the motile stages for Arapaho, Navaho, and Quachita varieties during the entire length of the field experiment (Table 2) (Figs. 8–10).

Other mites and insects recorded on blackberry plants. A small number of phytoseiid, tydeid, acarid, oribatid, eriophyoid, and tarsonemid mites were recorded. These mite numbers were sporadic throughout the season. Other arthropods recorded in the field experiment were aphids, *M. persicae*, whiteflies, *B. tabaci,* and thrips, *Scirtothrips* sp. (Thysanoptera: Thripidae). The populations in the field were higher than those recorded in the greenhouse, with the highest populations of aphids averaging 7.71 ± 3.41 (in the control), whiteflies averaging 0.43 ± 0.20 (in the *N. californicus* treatment), and thrips 0.14 ± 0.14 (in the control treatment). None of these arthropods populations were significantly different among treatments.

Similar as in the greenhouse study, the only beneficial insect that was recorded was larvae of the flower fly, *A. obliqua*.

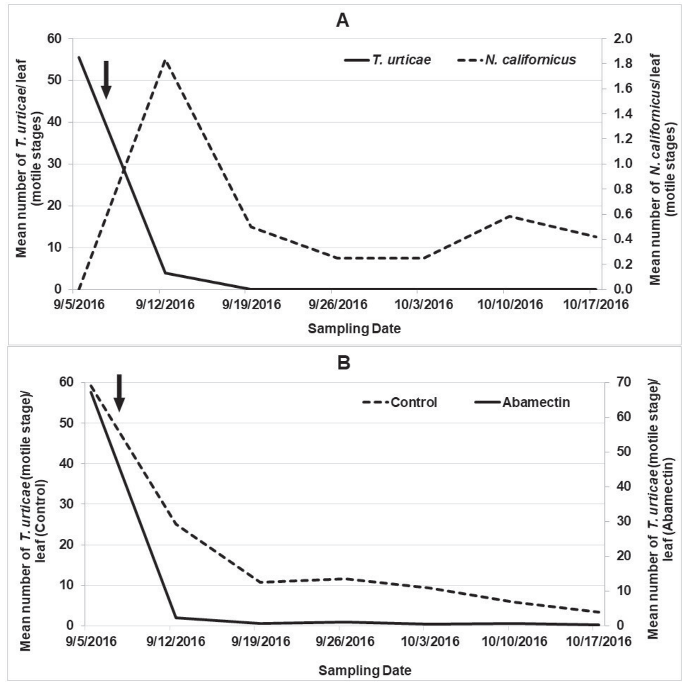

Fig. 6. Population of *T. urticae* (TU) and *N. californicus* (NC) motiles in treatments of *N. californicus* (A), Abamectin, and unsprayed (control) (B), plots on Navaho variety in a field experiment. (The arrows on the graph indicate the time of treatment and mite density at that time.)

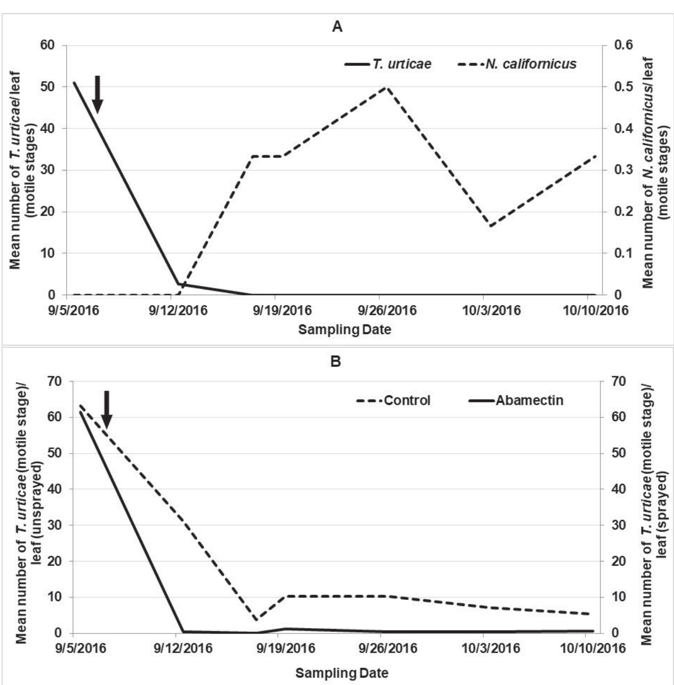

Fig. 5. Population of *T. urticae* (TU) and *N. californicus* (NC) motiles in *N. californicus* (A), Abamectin, and unsprayed (control) (B), plots on Arapaho variety in a field experiment. (The arrows on the graph indicate the time of treatment and mite density at that time.)

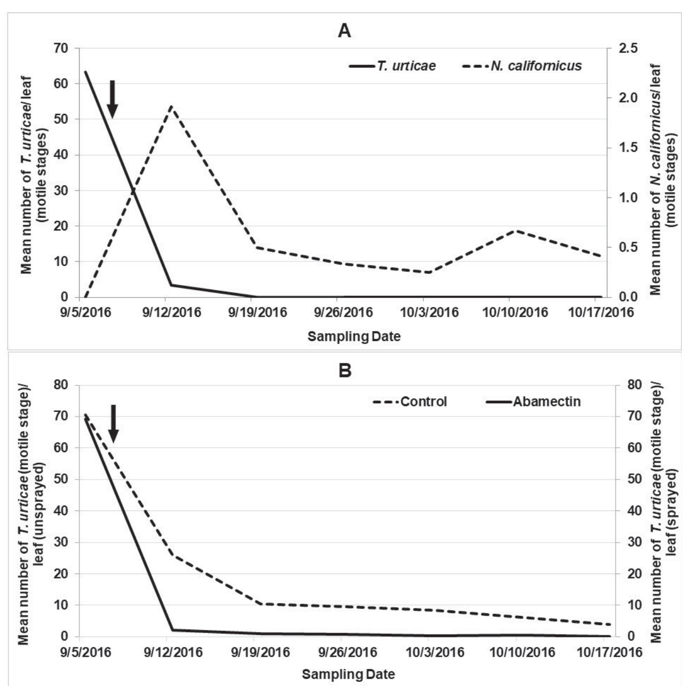

Fig. 7. Population of *T. urticae* (TU) and *N. californicus* (NC) motiles in treatments of *N. californicus* (A), Abamectin, and unsprayed (control) (B), plots on Ouachita variety in a field experiment. (The arrows on the graph indicate the time of treatment and mite density at that time.)

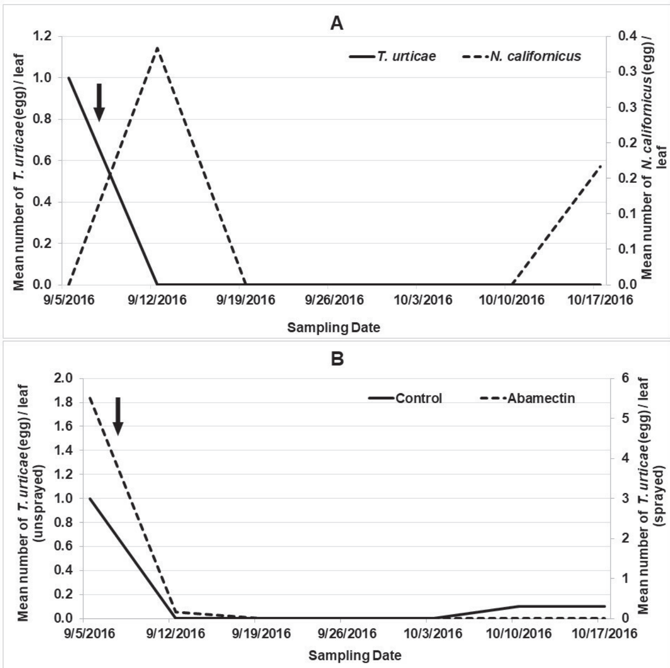

Fig. 8. Population of *T. urticae* (TU) and *N. californicus* (NC) eggs in treatments of *N. californicus* (A), Abamectin, and unsprayed (control) (B), plots on Arapaho variety in a field experiment.

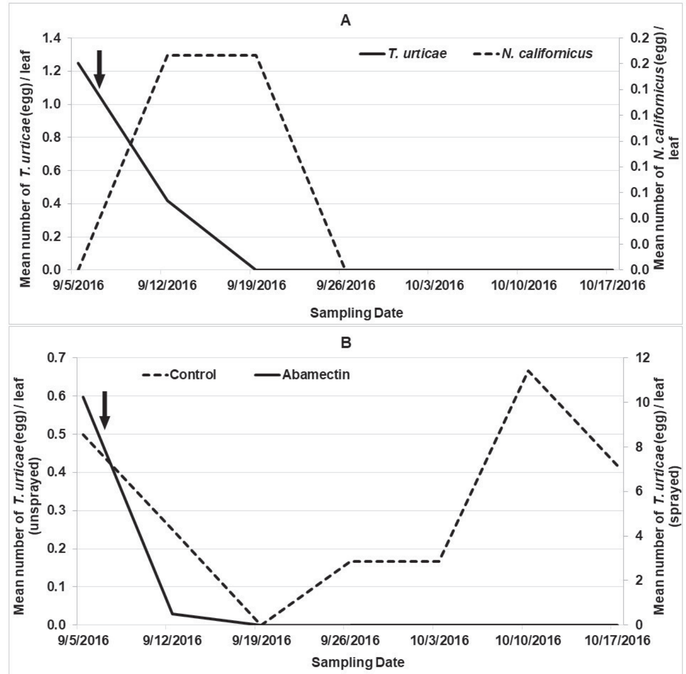

Fig. 10. Population of *T. urticae* (TU) and *N. californicus* (NC) eggs in treatments of *N. californicus* (A), Abamectin, and unsprayed (control) (B), plots on Ouachita variety in a field experiment. (The arrows on the graph indicate the time of treatment and mite density at that time.)

Temperature and relative humidity. The average temperature in the greenhouse was fairly constant and ranged from 27 to 33 °C. Similarly, relative humidity was constant and ranged from 66 to 71% (Fig. 11 A).

The initial temperatures in the field were higher than the greenhouse, averaging 33 °C on the first sampling date (Fig. 11 B). This was the highest temperature recorded throughout the study. By the end of the sampling period on 17 Oct 2016, the temperature reached a high of only 23 °C. The relative humidity (RH) in the field was lower than in the greenhouse. The highest RH recorded was 59%, and was recorded on the sixth sample date (10 Oct 2016). The lowest RH recorded was 30%, and this occurred on the warmest day during our first sampling period (Fig. 11B).

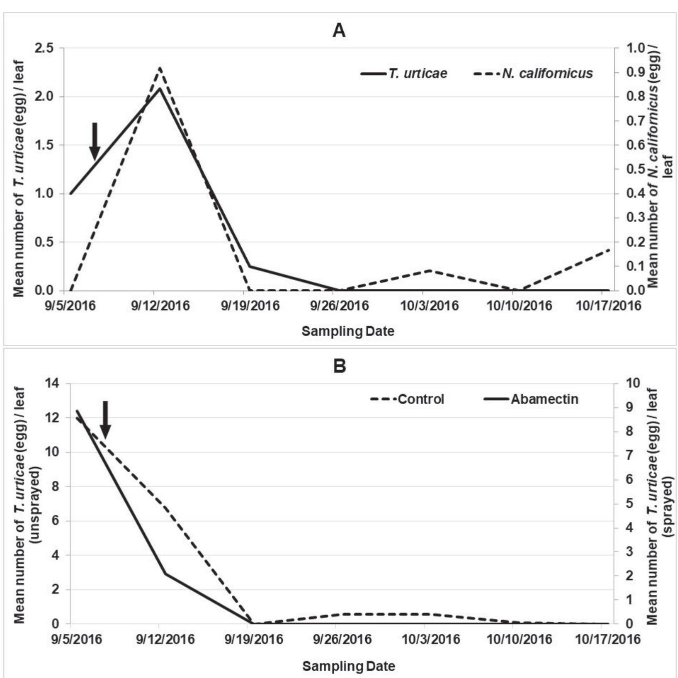

Fig. 9. Population of *T. urticae* (TU) and *N. californicus* (NC) eggs in treatments of *N. californicus* (A), Abamectin, and unsprayed (control) (B), plots on Navaho variety in a field experiment.

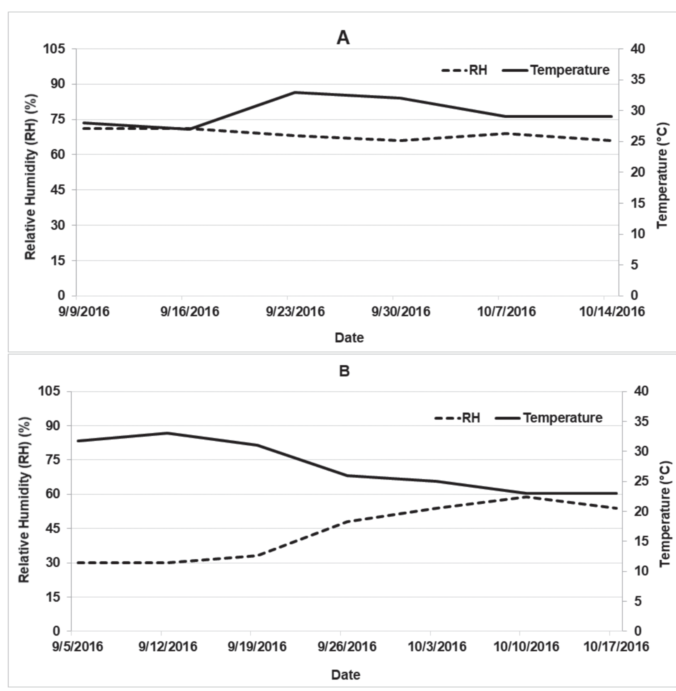

Fig. 11. Average temperature and humidity recorded in greenhouse (A) and field (B) during experiment.

Discussion

Overall, our greenhouse and field experiments showed that *N. californicus* provided the most sustained control for *T. urticae* populations under relatively high temperatures. These findings are significant because there have been several discussions whether *N. californicus* can compete with a pesticide to manage *T. urticae* populations in blackberries, because the crop is grown under hot, humid conditions in the southeastern USA. *Neoseilus californicus* has been shown to be a voracious feeder of *T. urticae* mites in several studies (Croft et al. 1998; Gotoh et al. 2004; Rhodes & Liburd 2006), and an early release of *N. californicus* can result in effective and sustained control of *T. urticae* populations (Fraulo & Liburd 2007).

The motile population in the greenhouse, which includes all immature and adult stages of *T. urticae*, is a direct reflection of the potential crop damage that can be inflicted, because these stages feed on blackberry leaves, causing indirect injury and resulting in economic damage (Pedigo & Rice 2009). When treatments were applied in the greenhouse (10 Sep 2016), there was a quick knockdown of *T. urticae* population in the predatory mite and insecticide treatments. Agri-Mek® SC was the best treatment in our first sample (not including pre-treatment sample), killing 98% of the motiles. However, Agri-Mek® SC did not maintain that level of efficacy as the population of *T. urticae* rebounded (above grower threshold) by the fourth sample, which necessitated a second application (30 Sep 2016). This was not the case in our *N. californicus* treatment, where the population of *T. urticae* decreased and remained lower than in the Agri-Mek® SC treatment for the remainder of the season.

Our greenhouse findings were similar to those of Sato et al. (2007), who investigated the performance of *N. californicus* for control of *T. urticae* in strawberries in greenhouses, with and without selected insecticides. They found that the *T. urticae* populations decreased rapidly from an average of 87 to about 3 mites per leaflet in the first 3 wk of sampling. After that time, the population of *T. urticae* was maintained at about 1.5 mites per leaflet until the end of the season. Similarly, Fraulo & Liburd (2007) demonstrated that the introduction of *N. californicus* in strawberries early in the season was able to reduce the motile population for the rest of the season. In the absence of tetranychids, *N. californicus* is known to feed on other species (Escudero & Ferragut 2005; Fraulo et al. 2008). We recorded low populations of mites in other families, including Phytoseiidae, Tenuipalpidae, and Tydeidae. However, these mites were found only in the control treatment group, possibly indicating that *N. californicus* may have preyed on other mites in the *N. californicus* treatment group. The lack of phytoseiids, tenuipalpids, and tydeids in the Agri-Mek® SC treatment may be related to the toxic effects of this acaricide on those species (Van Nieuwenhuyse et al. 2012).

Tetranychus urticae egg numbers remained fairly low, with the highest counts occurring in the control throughout the 7 wk of sampling. In contrast to the Agri-Mek® SC treatment, where *T. urticae* egg numbers increased by the fourth sample and required a second application (30 Sep 2016), the reduction in the egg population in the *N. californicus* treatment was sustained (without reapplication) and no eggs were recovered from these pots until the fifth sample was taken. This demonstrates the effectiveness of *N. californicus* in controlling the *T. urticae* egg population, as well as its potential as a biological control agent for *T. urticae* in blackberry, despite operating in temperatures > 27 °C. Ahn et al. (2010) subjected *T. urticae* eggs, larvae, and nymphs to various temperatures, and investigated the effect of trichrome densities on abaxial leaf surfaces of strawberry. They found *N. californicus* to be a highly effective predator on all life stages of *T. urticae*, potentially increasing its attack rate as well as decreasing its prey handling time during higher temperatures. Furthermore, trichrome densities had no significant effect on *N. californicus* performance, demonstrating the versatility of this predator. By the end of Sep, *N. californicus* was the only treatment in the greenhouse that had a lower number of *T. urticae* eggs than the control.

The motile counts from the field plots were similar to those from the greenhouse. *Neoseilus californicus* consistently proved to be the most effective and sustainable treatment throughout the field trial. The population of *T. urticae* motiles in the *N. californicus* plots remained lower than Agri-Mek® SC plots from the third wk of sampling until the end of the experiment. In fact, after the second wk of sampling, we did not recover any *T. urticae* motiles from the *N. californicus* treatment plots, eliminating the need for a second application of this treatment. During the same period, the Agri-Mek® SC treatment had relatively higher numbers of *T. urticae* motiles compared with *N californicus*, but a second application was not made since the population did not reach the grower threshold of 4 mites per leaf. Previous research has shown that there is often no need to make a second application with *N californicus* during the strawberry growing season, because the predator is able to become established and regulate *T. urticae* populations for the remainder of the growing season (Easterbrook et al. 2001; Fraulo & Liburd 2007). The population of *T. urticae* motiles in the control was higher than the other treatments throughout the study. The lack of pest management actions in the untreated plots (control) allowed *T. urticae* motiles to reproduce rapidly.

Only a few *T. urticae* eggs were found in our field study. *Neoseiulus californicus* was the only treatment that had lower numbers of *T. urticae* eggs than the control during the second wk of sampling. During the remainder of the sampling period, we did not find any *T. urticae* eggs in the treatment plots except in the control during our last sample. The lack of *T. urticae* eggs in treatment plots was initially surprising, but a number of factors could have been responsible for the absence of eggs after the second sample. For instance, when plants are exposed to the natural environment, there is the potential for increased control from abiotic (temperature and humidity) and biotic (natural predation-syrphid fly larvae) factors (Pedigo & Rice 2009). Similar to our findings, Oatman & McMurtry (1966) found that natural predation, including a syrphid fly larva, completely eliminated *T. urticae* population (eggs and motiles) in several treatments, including the control, in strawberry. In addition to tetranychids, we recorded low populations of phytoseiid, tydeid, eriophyoid, and tarsonemid mites. Tydeids live in diverse habitats; some are predatory in nature, whereas others are scavengers, and a few species are fungivorous. Eriophyoids and tarsonemids are plant feeding, and generally are pests of blackberry. For instance, *P. latus* has become a major pest of blackberry (Vincent et al. 2010), and management tactics are being developed to address this problem (Demchak 2015). It was not surprising that these tarsonemids were not found in *N. californicus* and Agri-Mek® SC treatments because *N. californicus* has been known to prey on these species (Easterbrook et al. 2001), and Agri-Mek® SC received a national label (EPA Reg. No. 100-1351) in the US for *P. latus* due to its efficacy on this pest (Van Nieuwenhuyse et al. 2012; Pochubay et al. 2017).

The initial temperature in the greenhouse during the first 2 wk of sampling was about 5 °C lower than the initial temperature in the field. This may have accounted for the slightly slower establishment of *T. urticae* numbers in the greenhouse, as seen in the pre-treatment sample data. However, starting with the second wk of regular sampling, more *T. urticae* mites were recorded in the control in the greenhouse compared with the control in the field. The higher temperatures in the greenhouse may have been more conducive for *T. urticae* population growth. Similarly, Nyoike & Liburd (2013) found that *T. urticae* population density per leaf increased as temperature increased and more degree-days were accumulated.

Overall, our study demonstrated that *N. californicus* can provide effective and sustained control for *T. urticae* and other secondary mite pests in blackberry, despite the fact that the crop matures later in the season when temperatures in the southeastern US are much warmer. *Neoseiulus californicus* is a generalist predator and will feed on other predators in the absence tetranychids (Croft et al. 1998; Easterbrook et al. 2001). Therefore, before releasing *N. californicus*, a good assessment of local phytoseiids and other predators, including syrphid larvae, should be noted because in the absence of *T. urticae*, these natural enemies can be eliminated. The findings are significant because it was not clear whether *N. californicus* could provide sustained control of this key mite pest, *T. urticae*, as well as other secondary pests, including tarsonemid mites, because these species usually are present later in the season when temperatures in the southeastern US are much higher. Agri-Mek® SC also is an effective pesticide, although it appears that repeated applications will be necessary to provide sustained control for *T. urticae* and other phytophagous mite species.

Our study involved the potential of *N. californicus* to control *T. urticae* during the high temperature conditions that are typical during the summer in northern Florida. Future research should examine other factors, including rainfall, wind speed, and humidity over several field seasons to determine if *N. californicus* can control *T. urticae* population under these adverse conditions.

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