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Biological control of twospotted spider mite, *Tetranychus urticae*, with predatory mite, *Neoseiulus californicus*, in strawberries

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Abstract Greenhouse and field experiments were conducted from 2005 to 2007 to determine the effectiveness of different release times with the predatory mite, *Neoseiulus californicus* (McGregor), for control of the twospotted spider mite (TSSM), *Tetranychus urticae* Koch, in strawberries (*Fragaria x ananassa* Duchesne). The effect of *N. californicus* releases over time and on development of TSSM populations during a growing season were evaluated. Our hypothesis was that repeated applications of *N. californicus*, which is currently recommended by biological control companies, might be unnecessary to attain season-long control of TSSM. In greenhouse trials, three treatments consisting of releases of *N. californicus* at five-day intervals: day 0, day 5, and day 10, and an untreated control were evaluated. The treatment releases significantly reduced TSSM below the control within five days of each release. *Neoseiulus californicus* significantly reduced TSSM in treatments with high densities (leaflets with $\geq$ 40 TSSM) below that of treatments with lower densities (leaflets with $\leq$ 10 TSSM) demonstrating that if released at a predator: prey ratio of 1:10, timing of release does not alter the effectiveness of *N. californicus* in controlling TSSM. However, we found that if the ratio of predator: prey remains adequate, *N. californicus* is a more efficient predator at high TSSM densities. Field studies included three treatments consisting of releases of *N. californicus* at one-month intervals. All treatments significantly reduced TSSM compared with the control plots (no releases). Releases applied early in the season sustained TSSM significantly below those in the control plots for the whole season. Our results indicate that one release of *N. californicus* is able to sustained control of TSSM in strawberry throughout a growing season if released when TSSM populations are low early in the season in the southeastern United States.

Keywords Prey density · Predatory mites · Control costs · Timed releases · Southeastern United States

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Introduction

Twospotted spider mite (TSSM), *Tetranychus urticae* Koch, is a major pest of strawberries (*Fragaria* spp.) throughout the world (Huffakker et al. 1969; Sances et al. 1979; Wyman et al. 1979; Oatman et al. 1985; Stonneveld et al. 1996; Walsh et al. 2002; Sato et al. 2004; Cloyd et al. 2006). Twospotted spider mite has a high rate of fecundity and a short developmental time that can be as brief as one week at high temperatures of ~32°C. If growers fail to consistently manage TSSM populations by frequently rotating acaricides, long-term chemical control has shown to increase the incidence of TSSM and the development of resistant populations (Huffaker et al. 1969; Cross et al. 2001; Sato et al. 2004; Stumpf and Nauen 2001).

As an alternative to chemical tactics, strawberry growers have been moving towards biological control for TSSM management (Rhodes et al. 2006; Rhodes and Liburd 2005). *Phytoseiulus persimilis* is a predatory mite (Acari: Phytosiidae) that is commonly used to control TSSM in Florida strawberry fields. It is an extremely effective predator, but as a type I specialist, it is genus-specific with regard to prey preference (McMurtry and Croft 1997). *Phytoseiulus persimilis* consumes only *Tetranychus* spp. as its only viable food source and perishes or disperses as its prey is eliminated. *Phytoseiulus persimilis* is highly sensitive to acaricides and fungicides, and is unable to survive in temperate climates (Easterbrook 1992; Escudero and Ferragut 2005).

Neoseiulus californicus (McGregor) has shown to be more resilient than *P. persimilis.* As such, it is more common as a biological control agent in temperate Mediterranean climates and its use is increasing in the southeastern United States (Escudero and Ferragut 2005; Liburd et al. 2003; Rhodes and Liburd 2005). As a type II generalist, *N. californicus* can adapt to fluctuations in prey populations and temperature, providing consistent pest suppression (Croft et al. 1998; Greco et al. 2005; Escudero and Ferragut 2005). Easterbrook (1992), Croft et al. (1998), Escudero and Ferragut (2005), and Liburd et al. (2007) have demonstrated that *N. californicus* is tolerant to many insecticides and fungicides, and is able to remain viable at temperatures from 10°C to approximately 32°C (Hart et al. 2002). Rhodes et al. (2006) found that *N. californicus* is able to maintain more consistent control of TSSM populations compared with *P. persimilis* throughout the season in north Florida strawberry fields. In addition, Rhodes et al. (2006) observed that *N. californicus* displaced *P. persimilis* in both greenhouse and field experiments. Studies conducted in both California and Belgium evaluating two different rates and release times of *N. californicus* showed a significant reduction in TSSM populations when *N. californicus* was present early in the season and if experimental plots had low population densities of fewer than ~70 TSSM per trifoliate (Oatman et al. 1977). Greco et al. (2005) and Easterbrook et al. (2004) demonstrated that an initial predator: prey ratio of ~1:10 is important to attain high levels of control of phytophagous mites.

Biological control companies have different recommendations for release of *N. californicus*. For preventative measures, commercial distributors recommend releasing *N. californicus* at a rate of 1–2 mites/m² as often as once a month. Curative releases of 6-mites/ m² every two to four weeks are recommended for suppression of TSSM throughout the season. The cost of *N. californicus*, if repeated multiple times throughout the season as suggested is approximately five times the cost of a common acaricide. However, a one time preventative application of *N. californicus* at the recommended rate would cost one-third the price of a chemical alternative.

In this study, both greenhouse and field experiments were conducted to assess the effectiveness of different release times of *N. californicus* in strawberries grown in north Florida. Greenhouse trials were conducted to assess the effect of the predatory releases at low and

high TSSM densities over time. Field experiments were also conducted to determine the effectiveness of *N. californicus* in maintaining TSSM below damaging levels, determined by Greco et al. (2005) to be ~50 active TSSM per leaflet, throughout a season. The effect of *N. californicus* releases on TSSM populations over time was analyzed. We also examined the effect on TSSM populations each week throughout the season as we found a significant interaction between time and treatment. The objective of our study was to determine a cost effective way to control TSSM. Our hypothesis was that repeated applications of *N. californicus*, which is currently recommended, may be unnecessary to attain season-long control of TSSM. If applied and at low TSSM populations of less than 70 TSSM per trifoliate (average of 20–30 TSSM per leaflet) at the approximate predator: prey ratio of 1:10, one application of *N. californicus*is is able to sustain TSSM populations at tolerable levels throughout the season.

Materials and methods

Greenhouse trial

Colony

A TSSM colony was obtained from the rearing facility at the Entomology and Nematology Department at the University of Florida and maintained in the Small Fruit and Vegetable IPM Laboratory at the University of Florida, Gainesville, FL. The colony was maintained on strawberry transplants (var. Festival) contained in one-gallon polyethylene pots. Plants were located under two 60-watt incandescent bulbs with a 14:10 light: dark photoperiod. Temperatures ranged from 32°C (day) to 24°C (night) with 35% RH. Plants were provided with ~250 ml of water three times per week. New plants were introduced once per week.

Greenhouse trial

To assess the effectiveness of *N. californicus* at different TSSM population densities, TSSMs were placed on individual plants and populations were allowed to develop for a 25-day period. Four treatments were evaluated in an extended randomized complete block design with three blocks and five replicates in each block. Treatments included the release of *N. californicus* at 5-day intervals: (1) "early" release on day 0; (2) a "middle" release on day 5; (3) "late" release on day 10; and (4) "no releases". "Early", "Middle" and "Late" designations were determined by the day of release with respect to stage of TSSM population development.

The experiment was conducted in the Small Fruits and Vegetable IPM Laboratory greenhouse at the University of Florida, Gainesville, FL. Twenty strawberry plants (var. Festival) ~20 cm in height were removed from the greenhouse. Plants were cleaned with a 10% ethyl alcohol solution applied with a Kimwipe® (Kimberly Clark, Irving, TX) to ensure that there were no insects or mites on the leaves. Each plant was pruned to four trifoliates using hand sheers. Forty TSSM motiles (larva, nymphs, and adult stages) from the laboratory colony were distributed (10 mites/trifoliate) on each plant using a probe constructed from a 0.020 stainless steel morpho minutien insect pin (Bioquip, Rancho Dominquez, CA) attached to the stem of a medical cotton swab. Each plant was contained within a wire mesh cage to prevent cross-contamination. Each cage was constructed of 23 gauge 0.6 cm mesh galvanized hardware cloth (Garden Plus, North Wilkesboro, NC) formed into a cylinder (30.5 cm in height and 14.0 cm in diameter) and covered with no-thrips insect mesh screen, mesh size 81 μm × 81 μm (Bioquip, Rancho Dominguez, CA). The mesh

was attached to the cylinder with heated glue (Surebonder glue gun, FPC Corp., Wauconda, IL). The greenhouse received natural light during the course of the treatment, with no artificial light source or climate controls. The temperature ranged from 28°C (day) to 15°C (night). Plants were hand-watered with ~500 ml of water every five days. Predatory mites were purchased from Koppert Biological Systems (Romulus, MI). Viability was tested by observing 20–30 predatory mites in a Petri dish for 15 min to ensure that the mites were vigorously active. The predators were used within 2 h of observation. A predator: prey ratio of 1:10 was calculated for each release. The ratio was determined by calculating the mean number of TSSM motiles from the sample leaflets in each treatment. The number of motiles found on the leaflets of each treatment was averaged, multiplied by the total number of leaflets on the plant and divided by 10. The laboratory trials were conducted three times; March 2006, December 2006, and January 2007. The trials were conducted during months in which climates were favorable for TSSM development. Each trial was considered a replicate and lasted approximately 25 days.

Sampling

Plants were sampled every five days. Five individual trifoliate leaflets from each treatment were detached at the base of the leaflet by hand pinching and the number of TSSM and *N. californicus* motiles and eggs on each leaflet were visually assessed and counted under a dissecting microscope (10–20×) (Leica MZ12.5, McBain Instruments, Chatsworth, CA) to quantify the effect of *N. californicus* releases on TSSM populations.

Statistical analysis

Data were subjected to statistical analysis with a repeated measures analysis using the PROC MIXED procedure of the SAS statistical software package (SAS Institute 9.0 2002).

Due to the significant interaction between time and treatment, we conducted an ANOVA at five-day intervals to determine the effect of *N. californicus* releases on TSSM populations at specific stages of TSSM population development. The General Linear Model (GLM) procedure of the SAS statistical software package and the Least squares difference (LSD) means separation ($P < 0.05$) were used for these analyses (SAS Institute 9.0 2002).

Field experiment

The field was located at the University of Florida Plant Science Research and Education Unit in Citra, Florida (82.17°W, 29.41°N). The soil in the field was Chandler Sand, and had not been previously cultivated. Granulated fertilizer N:P:K (10–8.3–4.4) was applied to the soil prior to planting at a rate of 653.3 kg/ha. Beds with black polyethylene mulch (1.6 mil) were laid using a Kennco™ power bedder (Ruskin, FL) and the soil was injected with a mixture of methyl bromide:chloropicrin (80:20) (Hendrix and Dail, Palmetto, FL) at a rate of 326.4 kg/ha. Devrinol (napropamide) (United Phosphorus Inc., Trenton, NJ) was applied between rows at the rate of 4.32 kg/ha as a pre-emergent herbicide. Six drip irrigation lines were placed under the polyethylene mulch in each bed. One-centimeter slits located every 20.3 cm served as emitters for the drip irrigation lines. Strawberry transplants, variety 'Festival', were planted the first week of October 2005 and 2006 in raised beds. There were six 7.3 m long rows per plot, each raised bed contained two rows of transplants positioned 0.35 m apart within row and 0.35 m apart between the rows (24 plants per row). For the first two weeks overhead irrigation was run 2 h per day between 10:00 AM and 2:00 PM.

After the plants were established, they were watered using automated drip irrigation 3 times a day for 30 min at a rate of 8.7 l per 100 m (0.65 gal/100 ft). Strawberry plants were fertilized through the drip irrigation once per week with 18.5 kg of ammonium nitrate (Southern States Cooperative, Inc., Richmond, VA) and 32.7 kg of muriate of potash (Southern States Cooperative, Inc., Richmond, VA) per ha. In February, the nitrogen was increased to 27.1 kg/ha of ammonia nitrate to accommodate increased nutrient demand as fruit development increased. Fungicides were applied to all experimental plots throughout the season three times per week to combat Botrytis fruit rot (*Botrytis cinerea*) and anthracnose fruit rot (*Colletotrichum acutatum*). Several different products were used in rotation: Abound® (azoxystrobin) (Syngenta Crop Protection, Greensboro, NC), Topsin® (thiophanate) (Cerexagri Inc., King of Prussia, PA), Aliette® (aluminum tris) (Bayer Crop Science, Research Triangle Park, NC), and Serenade® (*Bacillus subtilis*) (Agraquest, Davis, CA. No insecticides or acaricides were applied to the research plots. Weeds were removed during the season by hoeing between rows and using an s-tine around the plot border.

The experimental design for the 2005–2006 trial was a randomized complete block with four treatments and six replications. In the 2006–2007 trial, there were four treatments and four replications. *Neoseiulus californicus* was released in all treatments at the recommended rate of 1–2 predators per square meter. Each plot was 7.3 m² with an 11 m buffer zone between plots. The treatments included releases of *N. californicus* at approximately one month intervals throughout the growing season: (1) an "early" release of *N. californicus* 4 weeks after planting (WAP); (2) a "middle" release at 8 WAP; (3) a "late" release at 12–16 WAP; and (4) "no release". The late-release was performed four weeks earlier for the 2006–2007 trial due to high temperatures and faster accumulation of degree days (DD). In both seasons, the late release was applied at approximately 1380 DD at a 10°C threshold.

Sampling

A pretreatment sample was taken one week prior to treatment application to establish a baseline for TSSM populations in the field and to ensure that TSSM populations are similar among treatments. Systematic random samples were taken once per week throughout the season. Each week (including the pretreatment sample), eight trifoliate leaves were randomly taken from the three inner rows of each plot; four old and four young leaves (48 trifoliates per treatment in the 2005–2006 trial, and 32 trifoliates for the 2006–2007 trial). The number of replicates was reduced in the 2006–2007 trial due the limited availability of land. Young leaves were taken from the upper strata of the crown and the old leaves were taken from the lower strata. We analyzed the old and young leaves separately since previous research by Croft and Coop (1998) and Sances et al. (1981) indicated that TSSM distribution differs with leaf age. The trifoliates were collected in Zipper Seal Storage Bags© (American Value, Goodlettsville, TN) and transported back to the laboratory. *Neoseiulus californicus* and TSSM motiles and eggs were counted within 24 h using a dissecting binocular microscope (10–20×) (Leica MZ12.5, McBain Instruments, Chatsworth, CA) and numbers of motiles and eggs were recorded.

Statistical analysis

The data for the number of TSSM motiles and eggs were analyzed using the PROC MIXED procedure for repeated measures to evaluate the interaction of the release treatments with time. The data were also analyzed with an analysis of variance (ANOVA) to determine the effect of treatment releases on TSSM populations at specific points in time since the repeated

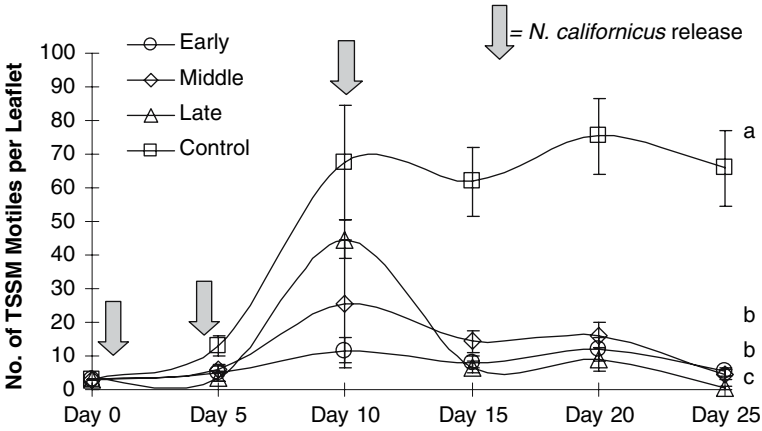

Fig. 1 Means (±SE) of TSSM motiles per leaflet collected at five day intervals during laboratory trial. Gray arrows indicate the days of *N. californicus* releases. Markers with different letters are significantly different ($P < 0.05$) using LSD test by the end of the trial

measures indicated that the time by treatment interaction was significant. To comply with the equal variance assumption of the ANOVA, the data were transformed using log base 10. Least significant differences (LSD) test was performed to separate means among treatments ($P < 0.05$). Differences in TSSM motiles and eggs observed on the old and young leaves were analyzed using ANOVA and Student's *t*-test was performed to separate means. All statistical analyses were performed using SAS 9.0 (SAS Institute Inc. 2002).

Results

Greenhouse experiment

Repeated measures analysis indicate that the early, middle, and late releases of *N. californicus* resulted in significantly lower numbers of TSSM motiles compared with the "no release" treatment ($F = 24.70$; df = 3, 176; $P < 0.0001$) and significantly fewer eggs ($F = 19.79$; df = 3,179; $P < 0.0001$). ANOVAs performed at five day intervals demonstrated that on day 25 the late treatment (release at day 10) (1.0 ± 0.5 TSSM per leaflet) resulted in significantly fewer TSSM motiles and eggs compared with the early (5.0 ± 2.0 mites per leaflet) and middle releases (7.0 ± 2.0 mites per leaflet) (motiles: $F = 29.83$; df = 4, 55; $P < 0.0001$, eggs: $F = 6.82$; df = 4, 55; $P = 0.0005$) (Fig. 1).

Field experiment

2005–2006 field trial

During the first 15 weeks of the season (October–February), TSSM populations were extremely low. Fewer than five TSSM were observed per trifoliate among all treatments. Therefore, data were not analyzed. Twospotted spider mite numbers began to increase in late February. Data were collected during the remaining six weeks of the season. The release of *N. californicus* resulted in significantly fewer TSSM motiles and eggs on the old

Table 1 Mean number of TSSM motiles per treatment plot counted from the field samples collected in the 2005–2006 field season on "old" trifoliates

Week	Treatment			
	Early	Middle	Late	No release
8-Mar	50.8 ± 50.0	0 ± 0	28.8 ± 21.2	30.5 ± 0.0
15-Mar	10.3 ± 9.7 (b)	3.3 ± 1.5 (b)	76.8 ± 36.1 (a)	59.5 ± 38.5 (a)
22-Mar	72.3 ± 71.4	17.2 ± 13.1	251.8 ± 7.5	70.0 ± 26.2
29-Mar	0.7 ± 0.3 (b)	2.8 ± 2.6 (b)	153.3 ± 49.7 (a)	184.5 ± 73.5 (a)
5-Apr	0.5 ± 0.3 (b)	4.7 ± 4.5 (b)	169.0 ± 50.5 (a)	111.7 ± 26.5 (a)
12-Apr	0.2 ± 0.2 (b)	0 ± 0 (b)	97.0 ± 31.2 (a)	144.5 ± 42.1 (a)

Means with the same letter within rows are not significantly different ($P < 0.05$) using LSD test. Datas with no letters represent datas with no significant differences. All data collected before 8 March are 0 ± 0

Table 2 Mean number of TSSM motiles per treatment plot counted from the field samples collected in the 2005–2006 field season on "young" trifoliates

Week	Treatment			
	Early	Middle	Late	No release
8-Mar	7.0 ± 6.0	0 ± 0	3.8 ± 3.1	0 ± 0
15-Mar	0.7 ± 0.5 (b)	1.5 ± 1.1 (b)	19.3 ± 9.1 (a)	32.7 ± 14.8 (a)
22-Mar	1.7 ± 1.0	24.3 ± 20.4	12.8 ± 7.5	23.8 ± 7.8
29-Mar	17.7 ± 11.7 (b)	10.2 ± 10.0 (b)	175.8 ± 41.7 (a)	235.3 ± 72.7 (a)
5-Apr	1.5 ± 1.0 (b)	9.2 ± 8.8 (b)	192.3 ± 65.5 (a)	212.8 ± 38.1 (a)
12-Apr	0.2 ± 0.2 (b)	0 ± 0 (b)	148.2 ± 88.8 (a)	152.7 ± 50.7 (a)

Means with the same letter within rows are not significantly different ($P < 0.05$) using LSD test. Datas without letters represent datas with no significant differences. All data collected before 8 March are 0 ± 0

trifoliates in the early (16 November) and middle (14 December) release treatments compared with the late (22 March) release and "no release" treatments (motiles: $F = 6.95$; df = 3, 120; $P = 0.0002$, eggs: $F = 8.18$; df = 3,120; $P < 0.0001$) (Table 1). The young trifoliates also contained significantly fewer TSSM motiles and eggs in the early and middle releases of *N. californicus* compared with the "no release" (motiles: $F = 17.90$, df = 3, 120, $P < 0.0001$; eggs: $F = 16.81$; df = 3, 120; $P < 0.0001$) (Table 2). Leaf age did not affect distribution of TSSM and subsequently the number of motiles or eggs were not significantly different between young and old age classes ($t = 1.30$; df = 1,280; $P = 0.3$).

2006–2007 field trial

During the pretreatment period, there were no significant differences in TSSM populations among any of the treatments in the old trifoliates (motiles: $F = 1.53$; df = 3,12; $P = 0.3$ eggs: $F = 1.05$; df = 3,12; $P = 0.4$) or in the young trifoliates (motiles: $F = 0.41$; df = 3,12; $P = 0.7$ eggs: $F = 1.58$; df = 3,12; $P = 0.2$). Significant differences in TSSM populations among old trifoliates began on 26 December.

Repeated measures analysis indicated that the old trifoliates contained significantly fewer TSSM motiles and eggs over time among the early, middle and late treatments compared with the "no release" treatment (motiles; $F = 42.36$; df = 3, 12; $P < 0.0001$, eggs:

Table 3 Mean number of TSSM motiles per treatment plot counted from the field samples collected in the 2006–2007 field season on "old" trifoliates

Week	Treatment			
	Early	Middle	Late	No release
14-Nov	115 ± 29	124 ± 31	196 ± 2	102 ± 31
21-Nov	249 ± 35	190 ± 33	210 ± 36	205 ± 36
28-Nov	447 ± 255	112 ± 17	292 ± 94	163 ± 60
5-Dec	150 ± 8	98 ± 26	90 ± 23	175 ± 36
12-Dec	175 ± 32	247 ± 64	244 ± 78	135 ± 58
19-Dec	105 ± 52	195 ± 43	118 ± 34	87 ± 29
26-Dec	240 ± 24	99 ± 42	590 ± 140	497 ± 117
2-Jan	158 ± 28 (b)	359 ± 203 (b)	571 ± 162 (a, b)	912 ± 87 (a)
9-Jan	243 ± 45 (b)	139 ± 45 (b)	723 ± 118 (a)	529 ± 223 (a)
16-Jan	124 ± 7 (b)	128 ± 35 (b)	572 ± 118 (a)	730 ± 191 (a)
23-Jan	357 ± 29 (c)	304 ± 74 (c)	657 ± 130 (b)	1958 ± 262 (a)
30-Jan	379 ± 30 (b)	310 ± 34 (b)	435 ± 45 (b)	1283 ± 260 (a)
6-Feb	776 ± 275 (b)	397 ± 58 (c)	535 ± 126 (b)	1202 ± 95 (a)
13-Feb	203 ± 27 (b)	217 ± 22 (b)	244 ± 23 (b)	1524 ± 206 (a)
20-Feb	335 ± 46 (b)	330 ± 58 (b)	398 ± 87 (b)	1568 ± 244 (a)
27-Feb	82 ± 9 (b)	124 ± 25 (b)	158 ± 47 (b)	342 ± 48 (a)
6-Mar	200 ± 44 (b)	353 ± 64 (b)	235 ± 66 (b)	857 ± 146 (a)

Means with the same letter within rows are not significantly different ($P < 0.05$) using LSD test. Dates without letters represent dates with no significant differences. All data collected before 8 March are 0 ± 0

$F = 11.54$; df = 3, 12; $P = 0.0008$) (Table 3). No treatment differences were observed on the young leaves (motiles: $F = 2.9$; df = 3, 12; $P = 0.08$, eggs: $F = 1.21$; df = 3,12; $P = 0.35$).

Discussion

Greenhouse trial

The greenhouse experiment demonstrated that releases of *N. californicus* at a 1:10 predator: prey ratio maintained low TSSM populations for the duration (25 days) of the experiment. Within five days of each release, *N. californicus* maintained numbers of TSSM in the treated plants compared with increasing numbers in the untreated plants. Early and middle releases had TSSM populations of less than 15 TSSM per leaflet at the time of *N. californicus* release. These populations were sustained at the initial low numbers after the introduction of *N. californicus*. McMurtry and Croft (1997) also observed that *N. californicus* is able to maintain TSSM populations at low densities. Twospotted spider mite populations naturally increased in the untreated plants to higher densities of greater than ~70 TSSM per leaflet. Within five days of releasing *N. californicus*, the TSSM populations in the late release fell sharply and were significantly ($P < 0.05$) lower than those in the early and middle releases with lower TSSM densities (Fig. 1). The sharp decline in the late releases to levels significantly below the earlier releases at lower prey densities demonstrates that *N. californicus* is an effective and voracious predator at high prey density if the 1:10 predator: prey ratio stays unmodified (Hassel et al. 1976).

Field trials

2005–2006 trial

The 2005–2006 trial had initial TSSM populations of less than five TSSM motiles per trifoliate in all plots. However, in late February, the population in the late releases plots and "no release" plots increased throughout the rest of the season. *Neoseiulus californicus* in both the early and middle release plots were able to establish in strawberry plots in the absence of TSSM and when TSSM populations developed, populations remained at significantly lower levels compared with the late release and "no release" treatments demonstrating that *N. californicus* is able to sustain itself in the absence of its preferred prey and maintain season-long control of TSSM when populations develop. We found no significant differences in TSSM numbers between the old and young leaf age classes, most likely due to the low TSSM populations on all trifoliates, which never exceeded 200 TSSM motiles per trifoliate in the untreated plots. The number of TSSM in the early and middle release treated plots of both old and young trifoliates never exceeded the initial population density of five motiles per trifoliate. At the time of the late release, TSSM populations were too high to achieve the 1:10 ratio at the recommended rate of 1000 predatory mites per plot. Therefore, when *N. californicus* was applied, it never achieved control in the late release plots.

The 2006–2007 trial

Despite higher TSSM population during the 2006–2007 trial, *N. californicus* maintained TSSM populations to low levels ($\leq$80 TSSM per trifoliate) in all releases throughout the season with the early and middle releases being significantly lower than the late releases on older trifoliates. There were no significant differences on young trifoliates in the 2006–2007 season, which may be due to the overall low numbers of TSSM present. Twospotted spider mite populations never exceeded 60 motiles per trifoliate in any treatment on young trifoliates. Twospotted spider mites were observed to aggregate on the older trifoliates (mean number of TSSM motiles was 160.0 ± 18.0 per trifoliate on untreated trifoliates). *Neoseiulus californicus* may have dispersed to areas of high prey density within the plant, possibly due to olfactory response to kairmones emitted from TSSM infested leaves (Dicke and Sabelis 1988; Liburd et al. 2007).

In both field trials, *N. californicus* demonstrated that timing is not the key factor in successful season long control of TSSM. However, the ratio of *N. californicus* to TSSM is critical. *Neoseiulus californicus* cannot devour and suppress high TSSM populations, but as we observed in field trials, TSSM population remained constant from approximately two weeks of the date from which *N. californicus* was released and remained so for the duration of the season. *Neoseiulus californicus* population corresponds to the increases and decreases of TSSM (Oatman et al. 1985). *Neoseiulus californicus* has a slower metabolism and lower searching efficiency compared with other phytoseiids, but it has a high rate of spatial coincidence with TSSM and can tolerate starvation (Greco et al. 2004). High rates of *N. californicus* development and prey consumption will enable this predator to achieve and maintain control over TSSM populations (Sabelis and Janssen 1994). Due to its life strategies, releases of *N. californicus* attained consistent control of TSSM throughout both seasons. Although we did observe that *N. californicus* is a more effective predator in treatments with high prey densities, it effectively maintained TSSM populations below damaging levels ($\leq$70 TSSM per trifoliate) and more cost efficiently in early release treatments of lower TSSM densities.

Our results indicate that when released at the appropriate ratio of between 1:5 and 1:10 predator:prey and when TSSM population are "low", or less than ~70 TSSM per trifoliate, regardless of calendar date, *N. californicus* is able to maintain populations of TSSM below damaging levels throughout a growing season (using the recommended dosage of 1–2 *N. californicus*/ m^2). Our recommendation for growers is to maintain a comprehensive TSSM monitoring program and when TSSM are detected at low densities ($\leq$ 70–80 TSSM per trifoliate) one release of *N. californicus* at the recommended "preventative" rate should be applied. A one time strategic application of *N. californicus* could attain season-long control of TSSM with substantial economic saving for growers compared with current recommendations of repeated predatory releases or multiple applications of acaricides.

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