

Evaluation of selected acaricides against twospotted spider mite (Acari: Tetranychidae) on greenhouse cotton using multispectral data

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Abstract Twospotted spider mite (TSSM), *Tetranychus urticae* Koch, is an early season pest of cotton in the mid-southern USA and causes reduction in yield, fiber quality and impaired seed germination. Objectives of this study were to investigate the efficacy of abamectin and spiromesifen with two divergent LC₅₀ values against TSSM in a computer-operated spray table which simulated aerial application parameters. Combined with a pressure of 276 kPa and a speed of 8 km/h, a 650033 nozzle delivered a spray rate of 18.7 L/ha. The active ingredient rates were 1/8, 1/4, 1/2 and the lowest label recommended rates for early season cotton. The intent was to study efficacy relative to deposition characteristics at active ingredient rates equal to and lower than those recommended by the label. Spectral reflectance values from a multispectral optical sensor were used to calculate the Normalized Difference Vegetation Index which numerically described the surface reflectance characteristics of cotton canopies concomitant to damage caused by *T. urticae* in the greenhouse. Water sensitive paper samplers described spray droplet spectra parameters ($D_{v0.1}$, $D_{v0.5}$ and $D_{v0.9}$, μm) and percent spray coverage. The volume median diameter ($D_{v0.5}$, μm) for abamectin and spiromesifen were respectively, 218 and 258 at one-half rate of the lowest label rate. These spray droplets were well above the driftable portions of the spray volume ($<141 \mu\text{m}$) for both abamectin and spiromesifen. Efficacy evaluations indicated that spiromesifen was more effective than abamectin in controlling *T. urticae* on early season cotton at one-half rate of the lowest label rate. Results reported herein demonstrate that the multispectral optical sensor in lieu of manually counting *T. urticae* appears to be a promising tool for efficacy evaluations against acaricides for early season plants grown in greenhouses.

Keywords Remote sensing · Spectral reflectance · Normalized difference vegetation index · Twospotted spider mite

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Introduction

Twospotted spider mite, *Tetranychus urticae* Koch, is a cosmopolitan pest of many field, horticultural and greenhouse crops (Hoy 2011; Jeppson et al. 1975). It lives inside complex three-dimensional webbings in a colonial microhabitat on the bottom surface of the plant, protecting its life stages from abiotic and biotic factors (Brandenburg and Kennedy 1987; Saito 1983). The pest status of *T. urticae* in the mid-southern United States has changed over the last decade from being a late season innocuous pest to an early season pest of damaging proportion (Gore et al. 2013). It has become the 6th most damaging pest of cotton in the mid-south region of the United States and caused an estimated loss of 57,441 bales of cotton in 2011 (Adamczyk et al. 2012; Williams 2012). Its short life cycle, high fecundity and haploid-diploid sex determination appear to contribute towards rapid development of resistance to acaricides (Van Leeuwen et al. 2010a). Furthermore, Grbic et al. (2011) reported that the genome of *T. urticae* is comprised of gene families that are implicated in the digestion and detoxification of xenobiotics, exacerbating the development of resistance to pesticides. Recently, Dermauw et al. (2013) reported that the response of the generalist herbivore, *T. urticae* to a diverse chemistry of plants was accompanied by rapid changes in the genome with altered expression of detoxifying family genes and new genes capable of transcriptional regulation in response to environmental changes. Cotton growers depend mostly on application of acaricides to suppress populations of *T. urticae*. Thus, evaluation of currently available and newly developed acaricides is a recurrent phenomenon, and will remain a challenge to field entomologists.

In large-scale field tests evaluating acaricidal efficacy against TSSM, Wilson et al. (1983) and Wilson and Morton (1993) proposed binomial sampling, where only the proportion of infested leaves was scored rather than the number of spider mites per leaf. Gore et al. (2013) estimated damage caused by TSSM on cotton based on a leaf reddening index scale, which varied from stippling and reddening to extensive reddening of vegetation canopy. However, in small-scale studies designed to assess efficacy of acaricides in a greenhouse environment, TSSM are usually counted before and after treatment of cotton plants. The spatial arrangement of *T. urticae* on cotton conformed to a clumped distribution pattern (Wilson et al. 1983; Wilson and Morton 1993). This distribution pattern coupled with its small size, mobility and prolific reproduction makes enumeration of TSSM difficult and alternative techniques are desired for assessment and evaluation of efficacy of acaricides against TSSM.

Objectives of this study were first to determine whether or not the optical multispectral sensor, GreenSeeker™, could effectively separate cotton plants infested with different density levels of TSSM from healthy plants in the greenhouse. Secondly, using this technology, the efficacy of abamectin and spiromesifen was assessed against *T. urticae* on cotton, relative to deposition characteristics via spray droplet spectra parameters, when applied in a computer-controlled spray table system. An important feature of this study was to assess the efficacy of rates lower than those recommended by the label to better identify coverage effects when control is marginal. Coverage of pest control materials is an essential component of improved application technology for TSSM which reside on the bottom surface of cotton foliage. As a corollary to this objective, we wanted to evaluate the efficacy of abamectin and spiromesifen against spider mite on cotton in a spray table at 1/8, 1/4, 1/2 and full label rates of the lowest spray rate recommended for early season cotton.

Abamectin and spiromesifen were chosen in the study because these two acaricides were found to be the most and least toxic chemicals, respectively, to *T. urticae*. Latheef

and Hoffmann (2014) reported that the contact toxicity (LC_{50}) of abamectin and spiromesifen were 0.014 and 14.086 μg per vial, respectively, for adult *T. urticae* collected from pinto beans raised in the greenhouse under similar test conditions reported in this study. The intent was to determine if the efficacy of these two chemicals with widely divergent LC_{50} values would vary at the lowest label rates relative to deposition characteristics.

Materials and methods

Mite sources

Pinto beans, *Phaseolus vulgaris* L. (Dwarf Horticulture, Bush), were grown in plastic trays (56 × 28 × 5 cm) in the greenhouse. In order to maintain a culture of spider mites, cotton leaves infested naturally with spider mite colonies on greenhouse-grown cotton plants were removed and were placed randomly on young pinto beans at the true leaf stage. Pinto beans were infested regularly with spider mites at the true leaf stage and continued after the trifoliate leaf stage until heavily infested trays of plants were obtained. Pinto beans were chosen for culturing *T. urticae* because they were inexpensive and easy to grow in the greenhouse. Indeed, Fellous et al. (2014) reported that *P. vulgaris* cv. Contender was readily accepted and consumed by mites collected from various host plants from widely different geographical areas.

Non-Bt and Roundup Ready cotton plants (Deltapine 436 RR) were grown in similar plastic flats. Plants were infested with TSSM by placing bean leaves containing masses of mites over cotton plants at the 4–5 true leaf stage.

Infestation study

In order to determine whether or not the multispectral optical sensor could differentiate between different levels of mite-infested cotton from untreated control plants, two tests were conducted in which cotton plants were infested with spider mites at three levels: light, medium and heavy, where lightly infested plants received 3 masses of TSSM per tray, medially infested plants received 20 masses of TSSM per tray and heavily infested plants received 40 masses per tray. Each mass of TSSMs contained numerous mites and were difficult to count. Samples taken several days after infestation of test plants showed a mean density (\pm SE) of 10.9 ± 3.0 for lightly infested, 38.2 ± 5.5 for medially infested and 66.3 ± 4.1 *T. urticae* for highly infested trays as measured using a 4 cm^2 leaf plug.

Spray table evaluation

Using a flat fan nozzle, 650033 (Spraying Systems, Wheaton, IL, USA), the spray table was operated with a spray pressure of 276 kPa and a speed of 8 km/h, which provided a spray rate of 18.7 L/ha. Test plants were treated with abamectin (TempranoTM; Chemtura, Middlebury, CT, USA; EPA Reg. # 67760-71-400) at ingredient rates of 0.00X, 0.125X, 0.25X, 0.50X and 1.00X of the field application rate (47.9 ml/ha) for early season cotton. The zero rate (0.00X) represented untreated control. Prior to application, a 21.3-cm strip was marked on the spray chamber directly under the nozzle to identify the location that provided the best visually-assessed spray pattern. Each tray of plants, representing a

replication, was placed in the appropriate swath area, and was treated in a separate run of the spray chamber. There were 3 replications per treatment. Similar to abamectin, spray table tests were conducted with spiromesifen (Oberon[®] 4SC; Bayer CropScience, Research Triangle Park, NC, USA; EPA Reg. # 264-850). The operating conditions of the spray table were similar to that for abamectin. The effectiveness of the treatment was compared to an uninfested and untreated control for abamectin, while the spiromesifen test included an additional treatment designated as uninfested. These uninfested plants were maintained in a different greenhouse that did not have TSSM.

Abamectin is a macrocyclic lactone derived from the soil microorganism, *Streptomyces avermitilis* and acts on gamma-aminobutyric acid (GABA) and glutamate-gated chloride channels (Dekeyser 2005; Van Leeuwen et al. 2010b). Pitterna (2012) reported that abamectin potentiates the effect of neurotransmitters and increases the influx of chloride ions into nerve cells, disrupting nerve impulses and nerve functions. Spiromesifen is a tetrionic acid derivative and interferes with lipid biosynthesis (Bretschneider et al. 2003). Recently, Lümmer et al. (2014) reported that the cyclic keto-enol spirotetramat, a derivative of spiromesifen, inhibited lipogenesis in *T. urticae* by interacting with the carboxyltransferase domain.

A water sensitive paper (WSP) card (26 × 76 mm) (Spraying Systems) was placed axially to the direction of the spray in close proximity to the treated plants during each run to ensure adequate coverage of the vegetation canopies with spray solutions. Each strip was properly labeled and stored after drying. A control spray, comprised of water alone, was included for the abamectin test. No such treatment was included for the spiromesifen test. Spray droplet images captured on WSPs were analyzed by the DropletScan[™] scanner based-system (Whitney and Gardisser 2003). The image analysis system computed the $D_{v0.1}$, $D_{v0.5}$ and $D_{v0.9}$ statistics and percent area coverage. $D_{v0.1}$ is the droplet diameter where 10 % of the spray volume is comprised of droplets with diameters smaller than this value. Similarly, $D_{v0.5}$ and $D_{v0.9}$, respectively, contained 50 and 90 % of the spray volume comprised of droplets with diameters smaller than these values.

Spray droplets and mite mortality

Salyani and McCoy (1989) studied the efficacy of abamectin spray droplets against citrus rust mite, *Phyllocoptruta oleivora* (Ashmead), on field-collected Valencia oranges in Florida. Using a droplet generator, abamectin sprays were directed toward the fruit region divided into three test areas designated A, B and C. Area A faced the droplet spectrum, B was parallel to the spray stream and C was on the opposite side of A. Salyani and McCoy (1989) presented their data (Table 3) and reported that spray droplet size did not have a significant effect on control of *P. oleivora* for all post-treatment counts by areas. However, Salyani and McCoy (1989) concluded that the control of mites trended to decrease with increased droplet size.

Several workers have reported that spray droplet size of pesticides significantly influenced mortality of *T. urticae* (Alm et al. 1987; Fisher et al. 1974; Hall and Thacker 1993; Hall and Reichard 1978; Munthali 1984; Munthali and Wyatt 1986). A large body of research derived from computer modeling and field trials under different operational conditions indicated that spray droplets less than 200 µm are more prone to drift (Downer et al. 1995; Elsik et al. 2010; Hewitt et al. 2002; Hoffmann et al. 2010; Teske et al. 2000; Wolf 2000; Zabkiewicz 2000). The percentage of spray volume made up of droplets less than 141 µm was a consistent index of spray drift that could be used either in aerial or ground-based applications of pesticides (Hoffmann et al. 2012). It is, therefore, essential to determine the optimum spray droplet size to maximize control of the spider mites, while mitigating spray drift of air-borne particles during aerial application of acaricides. With

this intent in mind, we chose to revisit their data presented in Table 3 (Salyani and McCoy 1989) by regressing percentage mortality on droplet size during the 3 days of post-treatment assessment.

Feeding by eriophyoid mites causes callous formation in the epidermis, while tetranychid mites destroy plant tissues and reduce photosynthesis in the mesophyll (Lindquist et al. 1996). On citrus fruits, *P. oleivora* randomly probes at the fruit surface with a pair of protractile stylets attached to the chelicerae. The length of the stylets averages between 15 and 35 μm which restricts feeding on the epidermal cell layer. However, some rust mites with larger rostra can penetrate up to 50–60 μm (McCoy and Albrigo 1975; Royalty and Perring 1996). However, tetranychid mites possess much longer stylets and pierce the epidermis deeper to a depth of 70–120 μm and feed upon the contents of palisade and mesophyll cells (Baker and Connell 1963; Helle and Sabelis 1985). More importantly, pesticide labels are regulatory documents issued by the United States Environmental Protection Agency (EPA 2014). The pest control applicators are regulated by the rules specified in the labels for each pesticide, and are required to obey label regulations. Abamectin is labeled to control both of these species of mites (Specimen Label 2014 TempranoTM; EPA Reg. # 67760-71-400; Chemtura).

Remote sensing analysis

The GreenSeeker is an active sensor and uses light emitting diodes (LEDs) as a light source and detects reflection in the visible (VIS, ca. 400–700 nm) and near infrared (NIR, ca. 700–800 nm) spectral regions. Each tray containing the test plants was placed on a wheel push cart (61 cm W \times 122 cm L) and the cart was slowly pushed under a Red NDVI GreenSeekerTM handheld optical sensor (Model 505, Trimble Navigation, Sunnyvale, CA, USA) suspended from a greenhouse frame (Fig. 1). Each tray of plants was scanned 3 times by the sensor which provided the NDVI (Normalized Difference Vegetation Index)



Fig. 1 Multispectral scanning of cotton canopy using the GreenSeekerTM

values. NDVI is the most widely used statistic to describe the surface reflectance characteristics of vegetation canopy to assess plant stress and health consequent to spider mite infestations (Fitzgerald et al. 2004; Holden 2002; Lan et al. 2013; Luedeling et al. 2009; Piñuelas et al. 1995; Reisig and Godfrey 2006). It is obtained by averaging the surface reflectance over two specific wavelengths in VIS (660 nm) and NIR (770 nm) regions of the electromagnetic spectrum. NDVI was calculated from the following equation: $NDVI = NIR - RED / NIR + RED$, where NIR and RED are the spectral reflectance values (0–255) in the red and near-infrared spectrums at 660 and 770 nm, respectively. The maximum NDVI reading was used for each flat of plants for each treatment during each day of post-treatment evaluation to minimize background reflectance and provide a consistent, repeatable quantitative measurement. Post-treatment evaluation days varied with tests and ranged from 1 to 14 days after treatment (DAT).

Data analysis

WSP card data were analyzed using PROC GLM procedure using SAS version 9.4 (SAS 2012). When F -values were significant, means were separated at $\alpha = 5\%$ with Tukey's honestly significant difference (HSD) test. NDVI statistics data were analyzed using repeated measures PROC GLM procedure. Least square means for main effect interactions were separated at $\alpha = 0.05$ with Tukey's adjustment. Graphical illustrations of the data were conducted using Jump[®] software, Version 11 (SAS 2013).

Results and discussion

Infestation study

In Test 1, repeated measures ANOVA for between treatment effects showed that the percent reduction in NDVI in lightly, medially and heavily infested cotton plants with spider mites was highly significantly different compared to the control ($F_{3,32} = 430.28$; $P < 0.0001$). The univariate test for within post-treatment days showed that similarly highly significant differences in NDVI occurred between sampling days ($F_{9,288} = 1478.31$; $P < 0.0001$). Also, highly significant interactions in NDVI occurred between post-treatment sampling days and treatments ($F_{27,288} = 216.39$; $P < 0.0001$). Similarly, Wilk's λ statistic for MANOVA was highly significant, respectively, for DAT and DAT*treatment effects ($F_{9,24} = 360.28$; $P < 0.0001$; $F_{27,70.34} = 27.25$; $P < 0.0001$). Figure 2 shows percent change in NDVI values during each sample day for the infestation study: Test 1. A positive percent change in NDVI value indicates healthy growing plants, while a negative value indicates that the vigor of the plant has decreased since the first measurement (i.e. Day 0) was made. The non-infested control plants showed increased vegetative growth throughout the course of the study, while the TSSM-infested plants showed degradation in health over time. Mean separation of the treatments shown in Table 1 reveals that no definable difference in percent reduction in NDVI between treatment categories (light, medium and heavy) and the control was observed until Day 5 when infestation classes significantly deviated from the control and remained overwhelmingly so thereafter. Similar to Test 1, repeated measures ANOVA for treatment effects showed a highly significant difference in percent reduction in NDVI between infestation categories compared to control for Test 2 ($F_{3,32} = 232.31$; $P < 0.0001$). The univariate test showed that post-treatment day effects in NDVI reduction were highly

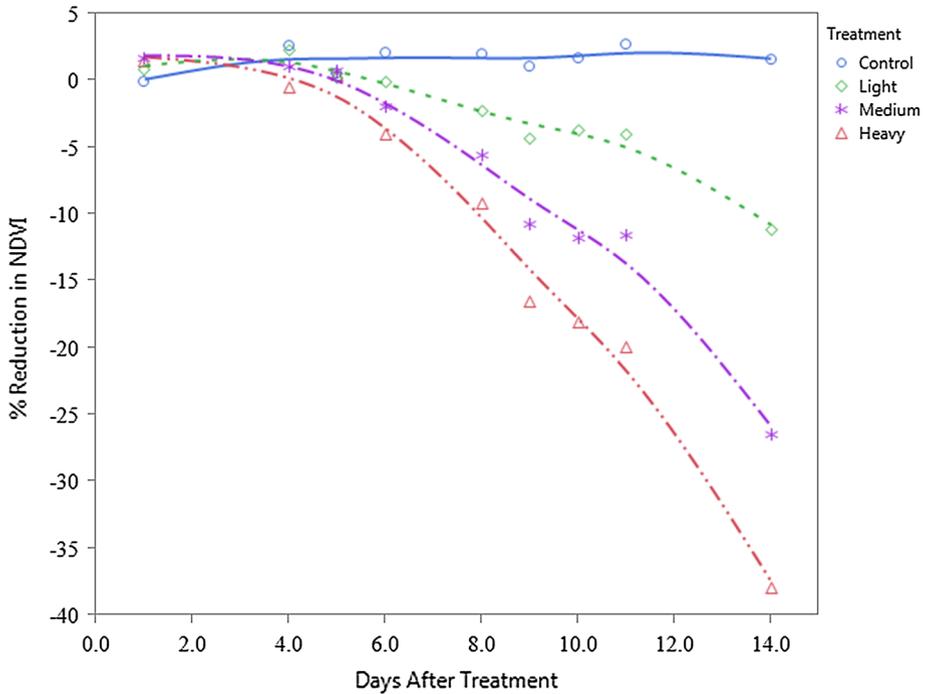


Fig. 2 Relationship between percent reduction in NDVI and post-treatment days in infestation study: Test 1. Cotton plants grown in trays (56 × 28 × 5 cm) in the greenhouse were infested with spider mites. Light received 3 masses or clusters of spider mites per tray, Medium received 20 masses per tray and Heavy received 40 masses per tray. Least square means between infestation categories were significantly different from control according to Tukey’s adjustment

Table 1 Mean separation of percent reduction in Max NDVI after cotton plants were infested with clusters of spider mites: Test 1

| Infestation category | Days after treatment (DAT) | | | | | | | | | |
|----------------------|----------------------------|---|---|---|---|---|----|----|----|----|
| | 1 | 2 | 5 | 6 | 7 | 9 | 10 | 12 | 13 | 14 |
| Control | a | a | a | a | a | a | a | a | a | a |
| Light | ab | a | b | b | b | b | b | b | b | b |
| Medium | c | b | c | c | c | c | c | c | c | c |
| Heavy | bc | c | d | d | d | d | d | d | d | d |

Same lower case letter within a column was not significantly different from each other. Treatment*DAT interaction (univariate test for within subject effects: $F_{27,288} = 216.39$; $P < 0.0001$; MANOVA statistic Wilk’s λ : $F_{27,70.34} = 27.25$; $P < 0.0001$). Least square means were separated using adjust = Tukey option, $\alpha = 0.05$

significant as well ($F_{8,256} = 1071.75$; $P < 0.0001$). Also, interactions between post-treatment sample days and treatment categories relative to NDVI were highly significant ($F_{24,256} = 212.89$; $P < 0.0001$). Similarly, Wilk’s λ statistic for MANOVA was highly

significant, respectively, for DAT and DAT*treatment effects ($F_{8,25} = 404.97$; $P < 0.0001$; $F_{24,73.11} = 13.25$; $P < 0.0001$). Figure 3 shows the relationship between percent reduction in NDVI and post-treatment sample days for Test 2 and the results were identical to those presented earlier in Fig. 2 for Test 1. The control plants remained healthy with little reduction in plant vigor except during day 1, while the treated plants continued to degrade in plant health. Table 2 shows that well-defined mean separation in percent reduction in NDVI between density levels and the control did not occur until Day 8. This was because the initial infestation of the plants did not produce definable differences between the density categories during the 1st week of the study and the test plants were, therefore, reinfested at the same density levels on Day 7. Data, thus, demonstrate that the GreenSeeker can be effectively used in lieu of labor-intensive manual sampling to assess treatment efficacy against acaricides for early season cotton.

Droplet deposition of abamectin and spiromesifen

Table 3 shows mean spray droplet characteristics of abamectin on WSP cards. Overall, all of the three spray droplet spectra parameters ($D_{v0.1}$, $D_{v0.5}$ and $D_{v0.9}$) significantly varied between active ingredient rates ($F_{4,65} = 3.99$; $P = 0.006$ for $D_{v0.1}$; $F_{4,65} = 5.01$; $P = 0.001$ for $D_{v0.5}$; and $F_{4,65} = 2.67$; $P = 0.04$ for $D_{v0.9}$). However, percent spray coverage was not significantly different between active ingredient rates and the control

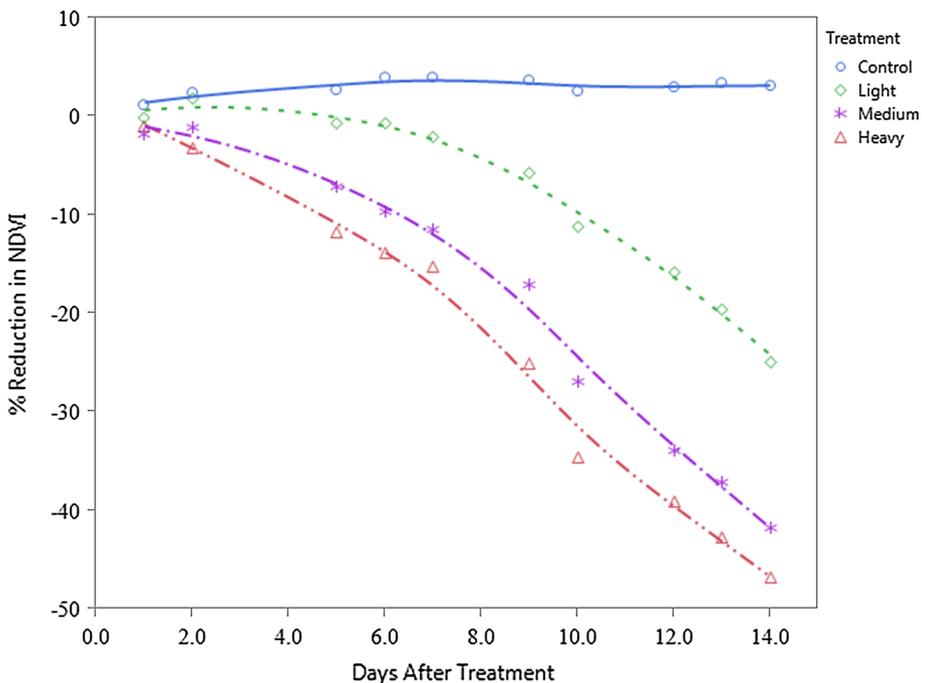


Fig. 3 Relationship between percent reduction in NDVI and post-treatment days in infestation study: Test 2. Cotton plants grown in trays ($56 \times 28 \times 5$ cm) in the greenhouse were infested with spider mites. Light received 3 masses or clusters of spider mites per tray, Medium received 20 masses per tray and Heavy received 40 masses per tray. Least square means between infestation categories were significantly different from control according to Tukey's adjustment

Table 2 Mean separation of percent reduction in Max NDVI after cotton plants were infested with clusters of spider mites: Test 2

| Infestation category | Days after treatment (DAT) | | | | | | | | |
|----------------------|----------------------------|----|---|---|---|---|----|----|----|
| | 1 | 4 | 5 | 6 | 8 | 9 | 10 | 11 | 14 |
| Control | b | a | a | a | a | a | a | a | a |
| Light | ab | ab | a | b | b | b | b | b | b |
| Medium | a | b | a | c | c | c | c | c | c |
| Heavy | a | c | a | c | d | d | d | d | d |

Same lower case letter within a column was not significantly different from each other. Treatment*DAT interaction (univariate test for within subject effects: $F_{24,256} = 212.89$; $P < 0.0001$; MANOVA statistic Wilk's λ : $F_{24,73.11} = 13.25$; $P < 0.0001$). Least square means were separated using adjust = Tukey option, $\alpha = 0.05$

Table 3 Spray droplet characteristics of abamectin as deposited on water sensitive papers^a

| Treatment | $D_{v0.1}$ (μm) | $D_{v0.5}$ (μm) | $D_{v0.9}$ (μm) | Coverage (%) |
|------------|------------------------------|------------------------------|------------------------------|-------------------|
| Control | 157.4 ^a | 242.1 ^a | 344.8 ^a | 11.3 ^a |
| 0.125 | 136.3 ^b | 208.4 ^b | 305.4 ^a | 8.9 ^a |
| 0.25 | 142.2 ^{ab} | 222.6 ^{ab} | 324.9 ^a | 10.4 ^a |
| 0.50 | 141.4 ^b | 217.6 ^b | 312.4 ^a | 8.8 ^a |
| 1.00 | 141.1 ^b | 225.6 ^{ab} | 343.8 ^a | 10.2 ^a |
| $F_{4,65}$ | 3.99 | 5.01 | 2.67 | 1.12 |
| P | 0.01 | 0.001 | 0.04 | 0.35 |

^a Means within a column followed by the same lower case letter were not significantly different ($\alpha = 0.05$) according to Tukey's HSD test. Treatment rates were fractions of the lowest labeled rate. Control represents water spray only

($F_{4,65} = 1.12$; $P = 0.35$). There were significant difference in $D_{v0.1}$ and $D_{v0.5}$ between active ingredient sprays and water only (control) treatment. $D_{v0.1}$ was the highest for the control solution and was the lowest for the 0.125X spray rate. Differences between active ingredient rates for $D_{v0.5}$ followed a pattern similar to those for $D_{v0.1}$. It is well established that adding an active ingredient to water, generally, reduces the surface tension of the spray mixture; thereby, creating smaller droplets. Regardless of active ingredient rates, a predominant volume of the sprays ($D_{v0.9}$) contained droplets $< 300 \mu\text{m}$.

Spray droplet characteristics for spiromesifen are presented in Table 4. There was no significant difference between active ingredient rates for any of the droplet spectra parameters ($F_{3,20} = 1.27$; $P = 0.31$ for $D_{v0.1}$; $F_{3,20} = 1.07$; $P = 0.38$ for $D_{v0.5}$; and $F_{3,20} = 1.06$; $P = 0.39$ for $D_{v0.9}$). Neither did spray coverage vary significantly between active ingredient rates ($F_{3,20} = 1.22$; $P = 0.32$). Regardless of active ingredient rates, a preponderance of the spray volume for spiromesifen ($D_{v0.9}$) was comprised of droplets well beyond the driftable portions of the spray spectrum similar to that for abamectin. Indeed, Hoffmann et al. (2012) reported that the percentage of spray volume made up of droplets less than $141 \mu\text{m}$ possesses a greater potential for spray drift for ground and aerial application of pesticides alike.

Significant differences which occurred between active ingredient rates and the control water spray indicated that the pesticide formulations probably contributed to the differences observed in this study. In fact, Chapple et al. (1993) reported that swath pattern was significantly altered for most adjuvants and that droplet spectra were shifted to smaller and larger frequency distributions relative to water. Kirk et al. (1993) reported that significant differences occurred between active ingredient rates for amitraz, thiodicarb and profenofos, relative to droplet density and spray coverage. Bouse et al. (1992) found that the concentrations of the surfactant (X-77) not only significantly reduced surface tension and viscosity of the spray mixture, but that spray droplet characteristics were significantly altered as well.

Spray droplets and mite mortality

The results of the analysis of the Salyani and McCoy (1989) data are presented in Table 5. There was no significant relationship between mortality of *P. oleivora* and droplet size of abamectin for all test areas of Valencia oranges, 1 and 2 days post-treatment. However, at 6 days post-treatment, the correlation between mortality and droplet size was strong. For Day 1, the regression of mite mortality on droplet size in the test Area A revealed that droplet size accounted for only 31 % of the variation in the model with the slope coefficient ($b = -0.024$) being not significantly different from zero ($t = -1.63$; $P = 0.15$). In the test Area B, droplet size accounted for 46 % of the variation in the model with the slope coefficient ($b = -0.054$) being not significantly different from zero ($t = -2.29$; $P = 0.06$). In the test Area C, droplet size accounted for 30 % of the variance in the model

Table 4 Spray droplet characteristics of spiromesifen on water sensitive papers^a

| Treatment | D _{v0.1} (µm) | D _{v0.5} (µm) | D _{v0.9} (µm) | Coverage (%) |
|------------|------------------------|------------------------|------------------------|-------------------|
| 0.125 | 167.2 ^a | 260.1 ^a | 381.0 ^a | 8.9 ^a |
| 0.25 | 171.4 ^a | 247.2 ^a | 353.1 ^a | 10.4 ^a |
| 0.50 | 157.7 ^a | 258.3 ^a | 379.3 ^a | 8.8 ^a |
| 1.00 | 166.3 ^a | 244.1 ^a | 355.0 ^a | 10.2 ^a |
| $F_{3,20}$ | 1.27 | 1.07 | 1.06 | 1.22 |
| P | 0.31 | 0.38 | 0.39 | 0.32 |

^a Means within a column followed by the same lower case letter were not significantly different according to PROC GLM. Treatment rates were fractions of the lowest labeled rate

Table 5 Regression statistics for the relationship between mortality of citrus rust mite and spray droplet size of abamectin in three test areas (A, B and C) of Valencia oranges, 1, 2 and 6 days post-treatment (DAT)

| Test area | DAT = 1 | | | | DAT = 2 | | | | DAT = 6 | | | |
|-----------|----------------|--------|-------|------|----------------|--------|-------|------|----------------|--------|-------|-------|
| | R ² | b | t | P | R ² | b | t | P | R ² | b | t | P |
| A | 0.31 | -0.024 | -1.63 | 0.15 | 0.19 | -0.006 | -1.20 | 0.27 | 0.72 | -0.023 | -3.92 | 0.01 |
| B | 0.46 | -0.054 | -2.29 | 0.06 | 0.11 | -0.009 | -0.87 | 0.42 | 0.81 | -0.062 | -5.12 | 0.002 |
| C | 0.30 | -0.018 | -1.62 | 0.16 | 0.15 | -0.019 | -1.04 | 0.34 | 0.61 | -0.046 | -3.07 | 0.02 |

Data from Salyani and McCoy (1989)

with the slope coefficient ($b = -0.018$) being not significantly different from zero ($t = -1.62$; $P = 0.16$). For Day 2, the regression model in test Area A explained 19 % of the variation in the model with the slope coefficient ($b = -0.006$) being not significantly different from zero ($t = -1.20$; $P = 0.27$). In test Area B, droplet size explained 11 % of the variation in the model with the slope coefficient ($b = -0.009$) being not significantly different from zero ($t = 0.42$). In test Area C, droplet size accounted for 15 % of the variance in the model with the slope coefficient ($b = -0.019$) being not significantly different from zero ($t = -1.04$; $P = 0.34$).

The treatment assessment of the data on Day 6 revealed a significantly negative linear relationship between mortality of citrus rust mite and droplet size in all test areas of the citrus fruit. In test Area A, the regression model produced an R^2 of 0.72 with the slope coefficient ($b = -0.023$) being significantly different from zero ($t = -3.92$; $P = 0.01$). Similarly, in test Area B, droplet size explained 81 % of the variation in mortality with the slope coefficient ($b = -0.062$) diverging highly significantly from zero ($t = -5.12$; $P = 0.002$). In test Area C, droplet size accounted for 61 % of the variation in the model with the regression coefficient ($b = -0.046$) being significantly different from zero ($t = -3.07$; $P > 0.02$). When overall mean mortality of the mite averaged across all three test areas of the citrus fruit during 3 days of post-treatment was regressed against spray droplet size, a highly significant but a negatively linear relationship evolved between the two variables (Fig. 4); $R^2 = 0.74$; $b = -0.029$; $t = -4.13$; $P = 0.0006$). Figure 4 shows that spray droplets $< 200 \mu\text{m}$ caused greater mortality of eriophyoid mites compared to larger droplets. Spray droplets of $50 \mu\text{m}$ or less resulted in 75 % or greater mite mortality. Similarly, Munthali (1984) found an inverse relationship for droplets between 20 and $100 \mu\text{m}$ relative to efficacy of dicofol against eggs, larvae and protonymphs of *T. urticae* on *Phaseolus* leaf discs. Alm et al. (1987) found that in a laboratory study on lima beans, droplets of $120 \mu\text{m}$ were more efficient than $200 \mu\text{m}$ droplets in decreasing egg production in *T. urticae*.

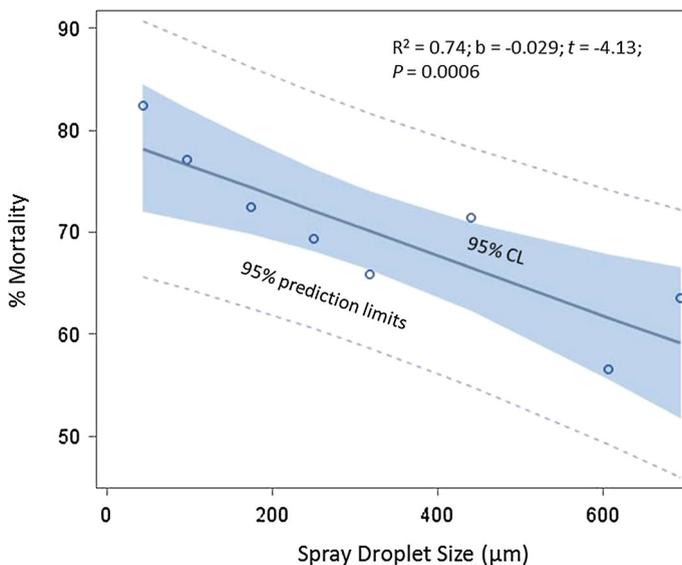


Fig. 4 Relationship between percent mortality of citrus rust mite, *Phyllocoptruta oleivora* and abamectin spray droplets (Salyani and McCoy 1989)

Damage assessment for abamectin

Repeated measures ANOVA for treatment effects were highly significant ($F_{4,25} = 5.28$; $P = 0.003$) and indicated that variations in percent reduction in NDVI between active ingredient rates predominated. The univariate test for post-treatment day effects were highly significant ($F_{3,75} = 213.22$; $P < 0.0001$) and indicated that percent reduction in NDVI varied between days of sampling. Also, post-treatment day effects*treatment interactions were highly significant ($F_{12,75} = 2.46$; $P = 0.009$). Similarly, Wilk's λ statistic for MANOVA was highly significant, respectively, for DAT and DAT*treatment effects ($F_{3,23} = 321.62$; $P < 0.0001$; $F_{12,61.14} = 4.29$; $P < 0.0001$). Figure 5 shows the relationship between percent reduction in NDVI and days after treatment for abamectin and mean separation of the treatments are shown in Table 6. On Day 1, there was no significant difference in damage assessment between active ingredient rates of abamectin and untreated cotton. By the 8th day after treatment, separation of damage between treatments began to emerge with the 1/8 rate being heavily damaged, and was significantly different from the control. However, all other treatments did not significantly vary from the control. A similar trend persisted on the 9th day after treatment with little change in reduction in NDVI between treatments. On the 12th day after treatment, there was no consistent pattern between treatments, although the 1/8 rate showed persistently the highest percentage change in NDVI. Overall, the data indicates that abamectin did not control *T. urticae* at the active ingredient rates lower than the label rates used in this study.

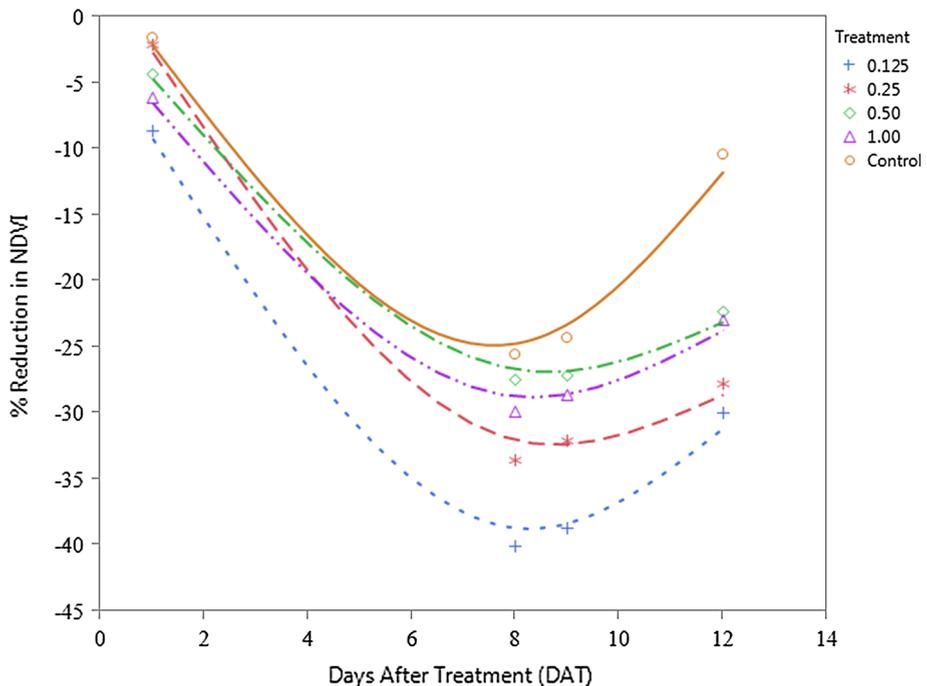


Fig. 5 Relationship between percent NDVI reduction and days after treatment with abamectin on cotton in a spray table. Treatments were 1/8, 1/4, 1/2 and full label rates of 47.9 ml/ha for early season field cotton. Control was uninfested and untreated

Table 6 Mean separation of percent reduction in Max NDVI after cotton plants were treated with abamectin

| Treatment | Days after treatment (DAT) | | | |
|-----------|----------------------------|----|----|----|
| | 1 | 8 | 9 | 12 |
| Control | a | a | a | a |
| 0.125 | a | b | b | b |
| 0.25 | a | ab | ab | b |
| 0.5 | a | a | ab | ab |
| 1 | a | ab | ab | ab |

Treatment rates were fractions of the lowest labeled rate. Control was uninfested and untreated. Same lower case letter within a column was not significantly different from each other. Treatment*DAT interaction (univariate test for within subject effects: $F_{12,75} = 2.46$; $P = 0.0092$; MANOVA statistic Wilk's λ : $F_{12,61.14} = 4.29$; $P < 0.0001$). Least square means were separated using adjust = Tukey option, $\alpha = 0.05$

Damage assessment for spiromesifen

Repeated measures ANOVA showed that active ingredient rates of spiromesifen were highly significant ($F_{5,48} = 135.35$; $P < 0.0001$). The univariate test also showed that post-treatment day effects were highly significant ($F_{6,288} = 1001.77$; $P < 0.0001$) and post-treatment day effects*treatment interactions were highly significant ($F_{30,288} = 117.05$; $P < 0.0001$). Similarly, Wilk's λ statistic for MANOVA was highly significant, respectively, for DAT and DAT*treatment effects ($F_{6,43} = 404.08$; $P < 0.0001$; $F_{30,174} = 18.74$; $P < 0.0001$). Figure 6 presents the relationship between percent change in NDVI and days after treatment for spiromesifen. Mean separation of percent reduction in NDVI for different treatments of spiromesifen is presented in Table 7. Significant differences between treatments occurred throughout post-treatment assessments. However, there was no consistent trend favoring one treatment over the other until the 11th day when definable differences between treatments emerged. The 1/8 rate, with significantly higher percent reduction in NDVI, began to deviate significantly from the 1/4, 1/2 and full rates. However, there was no significant difference in percent reduction in NDVI values between the 1/4, 1/2 and full rates on the 11th day after treatment. On the 14th day after treatment, the 1/8 rate sustained significantly higher damage than the 1/4 rate. There was, however, no significant difference in percent reduction in NDVI values between the 1/2 and the full rate on the 14th day post-treatment. The 1/2 rate appears to suppress *T. urticae* effectively but as equivalently well as the full rate.

Data reported here shows that cotton canopy treated with abamectin at the 1/2 rate showed greater decline in vegetative growth ($\Delta\text{Max NDVI} = -22.32\%$) at the 12th day compared to cotton canopy treated with spiromesifen ($\Delta\text{Max NDVI} = -9.42\%$) at the 14th day. Spiromesifen is generally considered to be effective against juvenile and egg stages of *T. urticae* (Dekeyser 2005; Sato et al. 2011), while its activity on adults is slower with reduction in fecundity and egg hatching (Bretschneider et al. 2007; Marcic et al. 2011). A greater effectiveness of spiromesifen against the instar stages and the egg populations of *T. urticae* compared to abamectin could have contributed to improved canopy health with significantly lower NDVI values. It has been reported that the acaricidal activity of spiromesifen is long-lasting and stable (Marcic et al. 2010; Nauen et al. 2003) with additional translaminar activity. Cloyd et al. (2009) reported that spiromesifen was not only effective against *T. urticae* nymphs with 89–99 % mortality on greenhouse grown

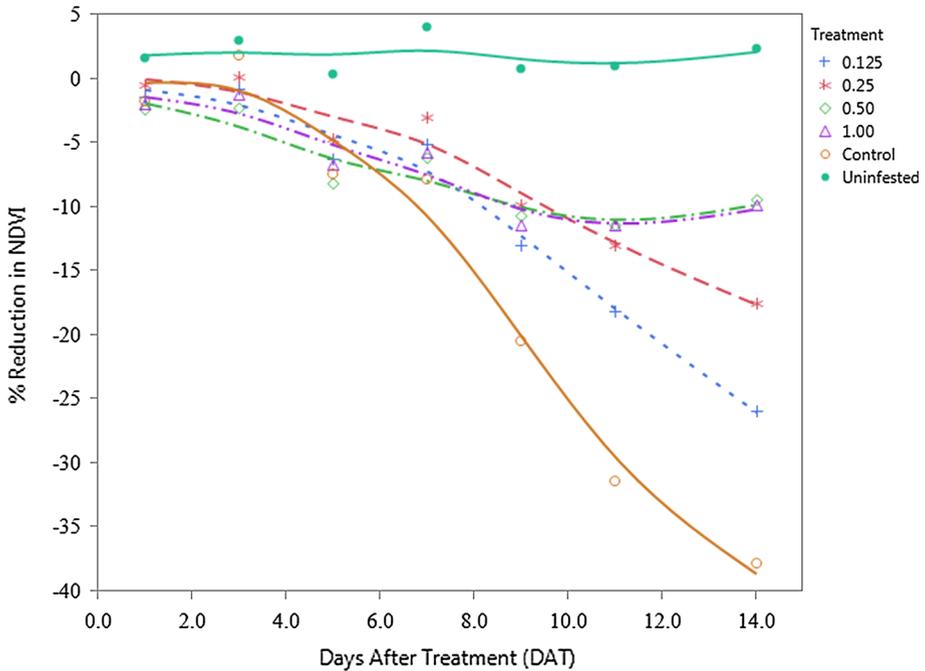


Fig. 6 Relationship between percent NDVI reduction and days after treatment with spiromesifen on cotton in a spray table. Treatments were 1/8, 1/4, 1/2 and full label rates of 47.9 ml/ha for early season field cotton. Control was uninfested and untreated. Uninfested cotton was kept in a different greenhouse with no exposure to *Tetranychus urticae*

Table 7 Mean separation of percent reduction in NDVI following application of spiromesifen on cotton

| Treatment | Days after treatment (DAT) | | | | | | |
|------------|----------------------------|----|----|----|---|----|----|
| | 1 | 3 | 5 | 7 | 9 | 11 | 14 |
| Uninfested | a | a | a | a | a | a | a |
| Control | c | cd | cd | d | c | d | e |
| 0.125 | bc | bc | bc | bc | b | c | d |
| 0.25 | b | b | b | b | b | b | c |
| 0.5 | c | d | d | cd | b | b | b |
| 1 | c | cd | cd | cd | b | b | b |

Treatment rates were fractions of the lowest labeled rate. Control was uninfested and untreated. Uninfested was kept in a greenhouse with no exposure to *Tetranychus urticae*. Same lower case letter within a column was not significantly different from each other. Treatment*DAT interaction (univariate test for within subject effects: $F_{30,288} = 117.05$; $P < 0.0001$; MANOVA statistic Wilk's λ : $F_{30,174} = 18.74$; $P < 0.0001$). Least square means were separated using adjust = Tukey option, $\alpha = 0.05$

plants, butterfly bush, *Buddleia davidii* F. and marigold, *Tagetes erecta* L., but also was effective against adults with 37.3–87.9 % mortality. Conversely, although abamectin possesses higher contact toxicity to TSSM adults compared to spiromesifen (Latheef and Hoffmann 2014), the data reported herein further reinforces that the test plants probably

contained more immature stages than adults. Data, therefore, demonstrate that remote sensing of spider mite damage should coincide with an assessment of immature stages of the pest in order to obtain better insight on factors impacting TSSM mortality on cotton. Such an assessment becomes relevant especially to studies comparing the efficacy of acaricides with divergent toxicities to the life stages of spider mite.

Several researchers have reported abamectin does possess some translaminar activity as well (Abro et al. 1989; Dybas 1989; Horowitz et al. 1997; Jansson and Dybas 1998). Earlier, Wright et al. (1985) reported that the translaminar movement of abamectin in the foliage of greenhouse grown beans, cotton and Chrysanthemum controlled mites, but failed to control aphids. They proposed that abamectin reservoirs were probably more abundant in the parenchyma where mites feed than in the phloem where aphids feed. Schuster and Taylor (1987) found that increased mortality of leaf miner, *Liriomyza trifolii* (Burgess) on tomatoes occurred only on the day of treatment because the surface residues of abamectin dissipated rapidly. Beers et al. (1997) reported that abamectin showed little translaminar activity on apple regardless of the leaf surface it was applied. Also, MacConnell et al. (1989) demonstrated that the degradation of abamectin in Petri dishes kept in a lighted test condition was negatively linear compared to that kept in the dark and possessed a half-life of 10 h. Yet, the prolonged stability of abamectin in the dark resulted in greater penetrability into leaves and improved efficacy against mites (MacConnell et al. 1989). Abro et al. (1989) reported that translaminar activity of abamectin was greater in Chinese cabbage compared to that in cabbage or Brussels sprouts probably because of its lower wax content. Beers et al. (1990) reported that penetration and absorption of abamectin varied with apple and pear foliage with mortality of mites declining more rapidly in apple than in pear. Furthermore, Walsh et al. (1996) reported that abamectin improved control of *T. urticae* on reproductive strawberry plants compared to semidormant plants because of increased translaminar activity on physiologically active strawberry plants. They (Walsh et al. 1996) proposed that the translaminar activity of abamectin in semidormant strawberry plants was lower than in sexual strawberry plants, probably due to increased leaf rigidity and thickness of the cuticle. Reports cited here indicate that the better performance of spiromesifen compared to abamectin appears to involve a complement of factors that we did not investigate in this study.

Using a spectroradiometer, Lan et al. (2013) studied the efficacy of abamectin against TSSM in a similar spray table environment and found that the spectral signatures of cotton canopy from 1/2 rates had reflectance values comparable to full rates, and that the 1/2 rate was as effective as the full rate in combating *T. urticae* on cotton. Fitzgerald et al. (2004) reported that multispectral remote sensing has limited spectral coverage, while hyperspectral remote sensing provides numerous spectral signatures to identify and detect crop stresses and canopy characteristics. In this study, however, multispectral reflectance values using just two spectral bands were able to detect arthropod-damaged cotton after application of abamectin and spiromesifen. Furthermore, infestation studies reported earlier demonstrate that multispectral data effectively differentiated between lightly, medially and heavily infested cotton compared to uninfested cotton. Such situations are more likely to be encountered under field conditions where the spatial distribution pattern of *T. urticae* remains patchy and contagious (Wilson et al. 1983; Wilson and Morton 1993).

In this study, both abamectin and spiromesifen were applied to the top canopy using a computer-controlled spray table which simulated a conventional aerial application system. It is noteworthy that the air turbulence produced by aerial sprays was absent in these test conditions and that field tests with improved application hardware designed to increase spray deposition in the bottom canopy are essential to determine whether or not lower label

rates will adequately suppress TSSM on cotton. However, the development of an improved application technology for controlling TSSM on cotton is complicated by the behavior of TSSM which seeks protection from spray droplets reaching its feeding site. For instance, Rudd (1997) reported that TSSM spins down in webbing threads to avoid contact with certain acaricides and either moves to a less hostile environment or disperses to another feeding site after detachment of threads from the webbings. Furthermore, TSSM causes leaf puckering which likely makes pesticide sprays run-off from the top canopy. These biological characteristics of *T. urticae* should be considered as factors while developing improved application technology for the suppression of TSSM on cotton.

Conclusions

The efficacy of abamectin and spiromesifen against *T. urticae* on early season cotton was evaluated at 1/8, 1/4, 1/2 and the lowest recommended label rate in a computer-controlled spray table which simulated a conventional aerial application system. Treatment efficacy was assessed with a multispectral optical sensor which provided NDVI (Normalized Difference Vegetation Index) values, describing quantitatively the surface reflectance characteristics of cotton canopies infested with TSSM. Water sensitive paper samplers described spray droplet spectra parameters ($D_{v0.1}$, $D_{v0.9}$, $D_{v0.5}$ μm) and percent coverage. Highly significant differences in percent reduction in NDVI occurred between lightly, medially and heavily infested cotton canopies compared to the control. The percent change in NDVI statistic indicates that spiromesifen at one-half rate of the lowest label rate significantly improved plant health and consequently increased suppression of *T. urticae* on cotton compared to abamectin. The volume median diameter ($D_{v0.5}$, μm) for abamectin and spiromesifen were respectively, 218 and 258 when applied at one-half rate. These spray droplets were well above the driftable portions of the spray volume ($< 141 \mu\text{m}$) for both abamectin and spiromesifen. Results reported herein demonstrate that the use of remote sensing technology in lieu of manual enumeration of *T. urticae* to assess acaricidal efficacy appears to be applicable for early season cotton as well as for horticultural and floricultural plants grown in greenhouses.

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