Vegetable Section

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Vegetable Crops as Hosts of Thrips (Thysanoptera: Thripidae) Vectoring Tomato Chlorotic Spot Virus in Tomatoes

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Tomato chlorotic spot virus (TCSV) is a serious threat to the tomato industry in South Florida. Thrips are effective vectors of TCSV. The early larval instars are the most likely stage for thrips to acquire TCSV from infected plant parts and develop into adults, which can transmit this virus to a new healthy plant. Thrips infestation and incidence of disease start at the edge of tomato fields and spreads inward as the season progresses. Vegetable crops grown in South Florida show a high abundance of different species of thrips. Western flower thrips (*Frankliniella occidentalis*), common blossom thrips (*F. schultzei*) and melon thrips (*Thrips palmi*) are the most common thrips species found in South Florida. In this study we used six commonly grown vegetable crops as treatments in a tomato field to observe their impact on TCSV incidence and abundance of TCSV-vectoring thrips in tomatoes. We have found melon thrips to be the dominant thrips in all vegetables including tomatoes. Incidence of TCSV infected tomato plants was observed with pepper and bean treatments. The above information will be helpful to develop sustainable management practices against thrips and thrips transmitted tospovirus problems.

Thrips are members of the order Thysanoptera and family Thripidae. They are globally important economic pests, causing oviposition and feeding injury on cultivated crops. Among the more than 7000 species of thrips, only a few species of thrips have the ability to transmit plant viruses. Thrips are small insects with fringed wings and piercing-sucking mouth parts (Lewis 1973, Heming 1993). Because of their invasiveness, thrips biology, host range, natural control agents and disease transmission vary from one geographical area to another. Thrips as a potential virus vector is part of a complex relationship between the virus and a susceptible host. The early larval instars of thrips must acquire the virus from an infected plant during feeding for successful transmission. Viruliferous adults then transmit the virus to healthy plants during feeding for the rest of their lives (Persley 2006, Hogenhout et al. 2008, Riely et al. 2011).

In United States, thrips transmitted Tomato chlorotic spot virus (TCSV) was first identified in 2012 in Miami-Dade County, FL, with 50 to 70% of tomato fields infested by this plant pathogen (Hanssen 2010, Londoño et al., 2012; Zhang et al., 2015). The virus has been also identified from impatiens (*Impatiens walleriana*) and lettuce (*Lactuca sativa*). From its first appearance, this vector-borne viral disease has caused 20 to 30% yield loss in winter tomato production every year in Miami-Dade County. Fortunately, the disease has not spread beyond south Florida.

Miami-Dade County produces different types of fresh market vegetables during the growing season. Some of these vegetables are grown year-round which helps maintain pest populations. Western flower thrips (*Frankliniella occidentalis*), common blossom thrips (*F. schultzei*) and melon thrips (*Thrips palmi*) are three of the most abundant thrips species in this agro-ecosystem. Western flower thrips and common blossom thrips are two thrips known to transmit TCSV to tomatoes. The vector transmitting status of other local thrips species like melon thrips, onion thrips (*T. tabaci*), tobacco thrips (*F. fusca*), and Florida flower thrips (*F. bispinosa*) is still unknown (Webster et al. 2015). Alternative plants other than the known susceptible ones: native plants; weeds; ornamental plants and other cultivated plants must be considered when there is a need to manage a new plant pathogen because these plants can be a reservoir of the pathogen or a reservoir of the pathogen's vectors or both.

In the current study, we hypothesize that thrips which vector TCSV are abundant in some "adjacent" (nearby) vegetable crops and some are reservoirs of TCSV, perhaps asymptomatically. We also hypothesize that these adjacent vegetable crops may have some role in introducing vectoring thrips and TCSV into tomatoes. The objective of this study was to determine the effect of adjacent vegetable crops on the incidence of thrips and TCSV in tomatoes. We also evaluated different adjacent vegetable crops as potential reservoirs of TCSV.

Materials and Methods

Study period and area. The study was conducted from Dec. 2017 to Mar. 2018. Research plots at the University of Florida Institute of Institute of Food and Agricultural Sciences (UF/IFAS) Tropical Research and Education Center (TREC) were used for this study.

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Plant material. All studies were conducted using 'Sanibel' tomato (*Solanum lycopersicum)*. 'Sanibel' tomato is commonly grown as a commercial main season crop in south Florida. It has large, firm, smooth fruit with light green shoulders and tight blossom ends. It produces a large determinate bush. This crop is also resistant to Verticillium wilt, Fusarium wilt, root-knot nematodes, Alternaria stem canker and Gray leaf spot. 'Sanibel' tomato seeds were obtained from Seminis, St. Louis, MO. Seeds were placed individually in a 5.0 sq.cm. cell of a styrifoam seedling trays (Seedling, Inc., Sun City, FL) filled with Pro-Mix growing medium (Premium Horticultural Inc., Quakertown, PA). Plants were allowed to grow in a greenhouse for six to eight weeks before they were transplanted to the field.

Experimental design and treatments. In this study, we used six different adjacent vegetable crops as possible thrips and/or TCSV reservoirs: beans; squash; eggplant; cucumber; okra and pepper. Tomato plants were planted in the middle of 6 ft wide × 15 ft long white-on-black mulched beds. There were 5 ft unplanted buffers. The adjacent vegetables were planted 2 ft apart on both sides of the tomatoes on the same bed. Altogether we had seven treatments, tomato adjacent to bean, tomato adjacent to squash, tomato adjacent to eggplant, tomato adjacent to cucumber, tomato adjacent to okra, tomato adjacent to pepper and tomato without any adjacent vegetables. We used a randomized complete-block design with four replications.

Field preparation. Soil type of all fields was Krome gravelly loam (loamy-skeletal, carbonatic hyperthermic lithic Udorthents), consisting of about 67% limestone pebbles (> 2mm) and 33% finer particles (soil). The field was prepared by following standard commercial practices using a mold board plow (CASE International, WI) and disking (Athens Plow Co Inc. TN). Afterward, raised beds, each 3 ft wide × 6 in high with 6 ft spacing from center to center of two adjacent beds was prepared by machine (Kennco Manufacturing Inc., Ruskin, FL). Granular fertilizer (N–P–K: 8–16–16) was applied at 1200–1600 lb/acre in a furrow 20 cm from and parallel to both sides of the transplant row in the center of the bed which was incorporated within 15 cm of the soil surface. Halosulfuron methyl (0.5 oz/acre, Sandea®, Group#2, Gowan Company LLC, Yuma, AZ) was used as a pre-emergence herbicide. Two drip tapes (Ro-Drip, San Diego, CA) with 30 cm emitter spacing were placed 15 cm apart on each side parallel to the center of the bed. Each bed was then covered with white-on-black plastic mulch (IMAFLEX, Victoriaville, Quebec, Canada). Tomato seedlings were transplanted 45 cm apart in the center (transplant row) of each bed 21 d after the application of halosulfuron methyl.

Crop maintenance. Tomato and other crops were irrigated twice daily (9.30 am and 3.30 pm) one hour each time, using a drip irrigation system delivering 0.4 gallon per minute per 100 ft. Consequently, the total amount of water delivered each day was $0.4 \times 60 \times 2 \times 2 = 96$ gallon/300 ft^2. The fertilizer was applied at the rate of 5 lb/100 gal (22.8 g/gal) just after transplanting with the help of a back-pack sprayer (BIRCHMEIER Iris 15 L., GEMPLER'S, Janesville, WI, fan nozzle). Three weeks after planting, liquid fertilizer (N–P–K: 4–0–8) at 25.24 gallons/ac was injected through drip tubes to provide 2.14 lb N2/ac/day. Tomato seedlings were drenched with imidacloprid (Admire® Pro, Bayer CropScience LP, IRAC group 4A Insecticide, 10.5 fl oz/acre) at the time of planting seedlings to protect plants from whiteflies and other insects. Chlorothalonil (Bravo Weather Silk®, Syngenta Crop Protection LLC, IRAC group M5 Fungicide, 1.5 lb/acre), Mancozeb (Manzate® Pro-Stick™, United Phosphorus, Inc., Fungicide, Dispersible granules, 1.5 lb/acre), Famoxadone and Cymoxanil (DuPont™ Tanos®, DuPont Nemours and Co., Fungicide, Dry Flowable, 8 oz/acre), yeast extract hydrolysate from *Saccharomyces cerevisiae* (KeyPlex® 350, KeyPlex, 2 qt/acre), Mancozeb (DuPont™ ManKocide®, DuPont Nemours and Co., Fungicide/bactericide, Dry Flowable, 5 lb/acre), Pyrimethanil (Scala® brand SC, Bayer CropScience LLC, Group 9 Fungicide, 7 fl. oz./acre), Penthiopyrad (DuPont™ Fontelis®, SC, Group 7 Fungicide, 24fl. oz./acre) were used in weekly rotations to prevent fungal and bacterial diseases. There were some variations in the management programs of individual commercial growers, but all of them followed nearly similar pest management programs to grow tomato.

Evaluations of adjacent vegetable treatments. Adjacent vegetable treatments were evaluated weekly. Evaluations of adjacent vegetable treatments on tomatoes were performed by collecting 5 full-grown leaves from the 4–5th node of five randomly selected tomato plants/plot (one leaf/plant) and 10 tomato flowers (one flower/plant). The same number of leaves and flowers were collected from each vegetable planted with tomatoes. Leaves or flowers were placed immediately in a zip-top bag to prevent escape of thrips. Zip-top bags were marked with the date and plot number. All sample bags were placed into a cooler and were transported to the vegetable IPM laboratory. Approximately 50–80 mL. of 70% ethanol was poured into the zip-top bag and shaken in a spiraling pattern to dislodge thrips larvae and adults from the leaves. The bags with leaves in alcohol were left undisturbed for 10 minutes when most of the adults and larvae had been dislodged from the leaves and dropped down to the bottom of the bags. The leaves were taken out individually by gently shaking to avoid any missing thrips on the leaves. The alcohol containing thrips was passed through a USA standard sieve, series no. 325 (opening 45 μm). All thrips in the sample were collected from the sieve by washing with a gentle jet of 70% ethanol and then stored in a glass bottle (100 mL) for identification and counting using a binocular microscope at 10×. Identification of thrips was conducted based on body color, size, antennal segments (number, size, and structure), and position of ocular setae. Prothoracic setae, lines on the metathoracic tergite, and the setal comb on the 8th abdominal segment were also checked to identify the thrips species.

At the time of sample collection, plants in each plot were checked thoroughly for TCSV symptoms, with data recorded by treatment, plot, and date.

Statistical analysis. Data were analyzed separately for larvae and adults. The data on the abundance of larvae and adults from each crop was averaged for all samples. Mean numbers of larvae and adults were compared using a two-way analysis of variance (ANOVA) (PROC GLM, SAS Institute Inc., version 9.4, location). Data were subjected to $\log^{10}(x + 1)$ transformation before statistical analyses in order to make it homogeneous if necessary, but only non-transformed means and standard errors were reported. Difference between means for larvae and adults were separated using the Tukey HSD (Honestly Significant Difference) procedure ($P < 0.05$).

Molecular analysis. Tomato leaves with TCSV symptoms were collected and preserveatd in –80 ºC. Leaves and flowers of other vegetables were also kept at –80 ºC. Purelink™ RNA Mini Kit (Ambion, Carlsbad, CA) according to the manufacturer's instructions was used to extract the total nucleic acid

from 50 mg plant tissue of those plant samples**.** Then the downstream reactions were done for the extracted total nucleic acid to detect TCSV by RT-PCR**.** Specific primers included TCSV-F: 5'-AGTATTATGCATCTATAGATTAGCACA-3' and TCSV-R: 5'-ACAAATCATCACATTGCCAGGA-3' were used to test the extracted plant nucleic acid samples to test for the presence of TCSV (targeting nucleocapsid N protein of S segment). The primers were designed based on the sequences available in GenBank utilizing the conserved region, targeting nucleocapsid N protein of S segment that are specific to the viruses. Purified nucleic acids (2.5 μL) were used in reverse transcription (RT) reactions containing 0.5 μL of 0.3 μg/μL random hexameric primers. Maxima™ reverse transcriptase (Fermentas, MA) (50 U) was used with 6 U of RiboLock RNase Inhibitor (Fermentas, MA), 0.4 mM DNTPs, 5μL 5× reverse transcriptase buffer (250 mM Tris-HCl, pH 8.3 at 25 °C, 375 mM KCl, 15 mM MgCl2, 50 mM DTT) and water to a final volume of 25 μL. The RT mix was incubated at room temperature for 10 min, followed by 50 °C for 75 min, and denatured for 5 min at 85 °C.

Later PCR was done for the RT product to detect the presence of the viruses. For a 25 μL PCR reaction, 2 μL cDNA was used and the reaction consisted of 2.5 μL of 10× PCR reaction buffer (500 mM KCl, 100 mM Tris-HCl, pH 9.0, 1% Triton X-100), 2 mM MgCl2, 0.4 mM respective primers, 0.2 mM dNTPs, 1.25 units of Taq Polymerase (Genescript, Piscataway, NJ) and water. The PCR program consisted of initial denaturation at 94 °C for 2 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 15 s, and extension at 72 °C for 30 s, and a final extension of 72 °C for 10 m. The samples were visualized in 1.5% TBE agarose gel stained with CYBRSAFE (Invitrogen, Carlsbad, CA). We sent the positive plant samples for PCR purification and DNA sequencing at the Molecular Cloning Lab (MCLAB, South San Francisco, CA) using TCSV specific forward and reverse primers as described earlier.

Results and Discussion

Abundance of thrips in adjacent vegetables. Higher thrips populations, mostly larvae, were observed in squash, eggplant and cucumber leaf samples. We found western flower thrips, common blossom thrips, and melon thrips, though we could not identify the larvae to species level. Western flower thrips and common blossom thrips populations were very low in all adjacent vegetable leaf samples (western flower thrips, F = 0.36; df = 6, 161; $P > 0.9017$, common blossom thrips, F = *3.22*; df = 6,161; $P > 0.0052$). Melon thrips appeared as the dominant thrips species among the thrips species complex (melon thrips, F= 8.05; df = 6, 161*;* $P < 0.0001$). Higher numbers of larval thrips were observed in bean, squash, eggplant and cucumber leaf samples (larva, F = 9.20; df = 6,161; $P < 0.000$) (Table 1). We found a similar trend for thrips population in flower samples from all vegetables. The melon thrips populations were higher in squash, eggplant and cucumber flowers (melon thrips, F = 8.05; df = 6, 161; $P < 0.0001$). The larval population was highest in eggplant flowers followed by squash and cucumber flowers (larva, F = 9.20; df = 6,161; $P < 0.0001$). Both western flower thrips and common blossom thrips population were low in all vegetable flowers (western flower thrips, F = 0.36; df=6, 161; $P < 0.9017$ / common blossom thrips, F = 3.22; df = 6,161; $P < 0.0052$) (Table 2).

Effect of adjacent vegetable crops on thrips abundance in tomatoes. Thrips population was low (0–1.4 adults/5leaves) in tomato leaves irrespective of the vegetable crop treatments including the untreated control, with no significant differences. Western flower thrips and common blossom thrips populations were very low in tomato leaves (0–0.1 adults/5 leaves) (western flower thrips, $F = 0.89$; df = 6, 357; $P > 0.5018$ / common blossom thrips, F = 0.90; df = 6, 357; $P > 0.4981$). The melon thrips population was comparatively higher in tomato leaves (melon thrips, F = 2.35; df = 6, 357*;* $P > 0.0309$). The melon thrips larval population was lower than that of the adults (larva, F = 0.07; df = 6, 357; $P > 0.9987$) (Table 3)**.** Thrips populations were higher in tomato flowers compared to tomato leaves. Western flower thrips and common blossom thrips populations were low in all tomato flowers regardless of adjacent vegetable treatment (western flower thrips F = 1.36; df = 6, 160; P > 0.2333 / common blossom thrips F = 1.93; df = 6, 160; P > 0.0792). Melon thrips population was higher in tomato flowers in all treatments compared to the other two thrips species (melon thrips, F = 1.70; df = 6, 160; $P > 0.1250$). Larval thrips population was low in all treated and untreated tomato flowers (larva, F = 1.11; df = 6, 160; $P > 0.3574$ (Table 4).

Table 1. Mean number of thrips in 5 leaves/vegetable.

Vegetables	Western flower thrips	Common blossom thrips	Melon thrips	Larva
Bean	0.208 a[z]	0.292 a	13.675 c	13.71 cd
Squash	0.333 a	0.5 a	31.417 a	36.96 a
Eggplant	0.292 a	2.75 b	37.667 b	66.88 bc
Pepper	0.5 a	0.167 a	19.584 e	8.83 d
Cucumber	0.333 a	0.083 a	37.583 a	53.5 ab
Okra	0.375 a	0.042 a	9.584 c	5.42 d

[z]Means followed by the same letter are not significantly different at 0.05%.

Table 2: Mean number of thrips in 10 flowers/vegetable.

Vegetables	Western flower thrips	Common blossom thrips	Melon thrips	Larva
Bean	0.208 a[z]	0.292 bc	13.675 c	13.71 c
Squash	0.333 a	0.5 bc	31.417 ab	36.96 b
Eggplant	0.292 a	2.75 a	37.667 ab	66.88 a
Pepper	0.5 a	0.167 bc	19.584 b	8.83 c
Cucumber	0.333 a	0.083 ab	37.583 a	53.5 b
Okra	0.375 a	0.042 c	9.584 c	5.42 c

[z]Means followed by the same letter are not significantly different at 0.05%.

Table 3. Mean number of thrips/5 leaves of tomatoes treated with different vegetables.

Treatments	Western flower thrips	Common blossom thrips	Melon thrips	Larva
Tomato (Bean)	0.0192 a[z]	0.096 a	1.21 a	0.4038 a
Tomato (Squash)	0.0192 a	0 a	1.365 a	0.4038 a
Tomato (Eggplant)	0.03846 a	0.058 a	1.038 ab	0.3654 a
Tomato (Pepper)	0.01923 a	0.058 a	0.653 b	0.3654 a
Tomato (Cucumber)	0 a	0.038 a	1.269 a	0.4231 a
Tomato (Okra)	0.03846 a	0.038 a	1.44 a	0.4038 a
Tomato	0.077 a	0.077 a	1.19 a	0.4615 a

[z]Means followed by the same letter are not significantly different at 0.05%.

Table 4. Mean number of thrips/10 flowers of tomatoes treated with different vegetables.

Treatments	Western flower thrips	Common blossom thrips	Melon thrips	Larva
Tomato (Bean)	0.333 ab[z]	0.999 a	5.083 a	0.333 a
Tomato (Squash)	0.0417 a	0.7083 ab	2.4684 b	0.0833 a
Tomato (Eggplant)	0.1634 a	1.1267 a	3.9166 ab	0.1739 a
Tomato (Pepper)	0.3912 a	1.217 a	5 a	0.4348 a
Tomato (Cucumber)	0.4166 a	0.6667 ab	4.333 ab	0.083 a
Tomato (Okra)	0.1634 a	0.4167 b	4.0417 ab	0.083 a
Tomato	0.25 a	0.7916 ab	4.083 ab	0.333 a

[z]Means followed by the same letter are not significantly different at 0.05%.

Table 5. Mean number of TCSV plants/vegetable treatment and mean number of marketable yield for 4 tomato plants/ vegetable treatment.

Treatments	TCSV infected plants	Marketable yield
Tomato + Bean	0.25 a[z]	14.25 ab
Tomato + Squash	0.25 a	12.63 bc
Tomato + Eggplant	0.25 a	10.5 c
Tomato + Pepper	0.5 a	13.63 abc
Tomato + Cucumber	0 a	10.63 c
Tomato +Okra	0.25 a	14 abc
Tomato	0 a	18 a

[z]Means followed by the same letter are not significantly different at 0.05%.

Effect on adjacent vegetable treatments on the incidence of TCSV in tomatoes. We found TCSV-infected tomato plants in all treatments, including the untreated control. There was no significant difference among the treatments (TCSV, $F = 0.42$; df = 6, 21; $P > 0.85$). Though numerically we found a higher number of infected plant in pepper treated tomato plants (Table 5), thrips abundance was low in pepper plants both from leaf and flower samples (Tables 1 and 2) but it might be possible that some of the pepper plants were the reservoir of TCSV or their thrips vector. The marketable yield from all treated tomato plants showed no significant difference among the treatments. The highest yield was in the untreated control tomatoes (marketable yield, $F = 3.93$; df = 6,21; $P > 0.0087$)

Results from molecular analysis. In the gel electrophoresis, we found that the TCSV symptomatic tomato plant samples were positive for TCSV. We could not find any positive results for any other vegetable samples (Fig. 1). There is a strong possibility of these vegetables may be a reservoir of the TCSV pathogen. In this study we found more TCSV infected tomato plants planted adjacent to pepper. The TCSV virus has been identified from pepper plants. So, there is a possibility of this vegetable may be a reservoir of the pathogen and at the same time a reservoir of pathogenic thrips.

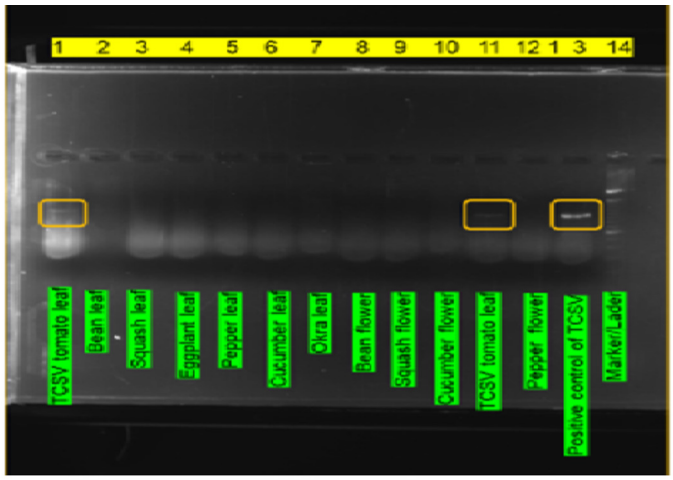

Fig. 1 Gel electrophoresis of vegetables leaf and flower samples showing positive or negative results for TCSV, which was used as a positive control. The last column (Column no. 14) was used as the ladder which was 100kb.

Literature Cited

Hanssen, I.M., M. Lapidot, and B.P. Thomma. 2010. Emerging viral diseases of tomato crops. Mol. Plant Microbe Interact. 23(5):539–548.

Hogenhout, S.A., E.D. Ammar, A.E. Whitfield, and M.G.Redinbaugh. 2008. Insect vector interactions with persistently transmitted viruses. Annu. Rev. Phytopathol., 46:327–359.

Heming, B.S., 1993. Structure, function, ontogeny, and evolution of feeding in thrips (Thysanoptera). Functional Morphology of Insect Feeding, p. 3–41.

Lewis, T., 1973. Thrips, their biology, ecology and economic importance. Thrips, their biology, ecology and economic importance.

Londoño, A., H. Capobianco, S. Zhang, and J.E. Polston. 2012. First record of Tomato chlorotic spot virus in the USA. Trop. Plant Pathol. 37(5):333–338.

Persley, D.M., J. E. Thomas, and M. Sharman. 2006. Tospoviruses—An Australian perspective. Australas. Plant Pathol. 35(2):161–180.

Riley, D.G., S.V. Joseph, R. Srinivasan, and S. Diffie. 2011. Thrips vectors of tospoviruses. J. Integr. Pest Mgt., 2(1):I1–I10.

Webster, C.G., G. Frantz, S.R. Reitz, J.E. Funderburk, H. C. Mellinger, E. McAvoy, W.W. Turechek, S.H. Marshall, Y. Tantiwanich, M.T. McGrath, and M.L. Daughtrey. 2015. Emergence of groundnut ringspot virus and tomato chlorotic spot virus in vegetables in Florida and the southeastern United States. Phytopathology 105(3):388–398.

Zhang, S., D. Seal, Q. Wang, and E. McAvoy. 2015. Evaluation of tomato cultivars and insecticides for management of tomato chlorotic spot virus (TCSV) and thrips species recorded in virus-infected tomato fields. Florida Tomato Inst. Proc. p. 28–30.