Tomato spotted wilt virus Can Infect Resistant Tomato when Western Flower Thrips Inoculate Blossoms

J. L. Houle and G. G. Kennedy†, Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, 27695-7630

Abstract

Tomato spotted wilt is a major disease of crops worldwide. Resistant cultivars carrying the Sw-5 allele for resistance to tomato spotted wilt disease (TSW) provide the most effective control method in tomato (Solanum lycopersicum). However, infections of fruit on Sw-5 tomato plants suggest the virus resistance may not be fully expressed in blossoms or developing fruit. The objective of this study was to determine if the thrips vector, the western flower thrips (Frankliniella occidentalis), can transmit non-resistance breaking Tomato spotted wilt virus (TSWV) isolates when confined to blossoms on plants with and without the Sw-5 resistance allele. Twenty-one percent of 33 Sw-5+ plants inoculated by adult thrips feeding on blossom clusters or small fruit developed infections in the reproductive tissue, whereas 68% of 25 Sw-5– plants developed infections. Systemic infections also occurred following inoculation of blossoms in host genotypes with and without Sw-5. These results were further supported by field experiments that showed high proportions of infected fruit as well as a limited infection of foliage on the same stem as the infected fruit in Sw-5+ plants when F. occidentalis were abundant in blossoms. These findings help to explain observations of abundant late season infections of Sw-5 cultivars in commercial plantings and suggest that management of F. occidentalis infestations during the bloom period may be important for effective management of TSWV in susceptible tomato cultivars as well as cultivars expressing the Sw-5 allele for TSW resistance.

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foliar symptoms surrounding infected fruit test positive for TSWV using DAS ELISA (J. L. Houle, personal observation). Frequent observations of Sw-5+ plants expressing only symptomatic fruit late in the season led to the hypothesis that direct feeding on the fruit by viruliferous thrips can cause localized virus infections in plants carrying Sw-5 (Aramburu and Rodriguez 1999). Aramburu et al. (2000) tested this hypothesis by placing viruliferous thrips on harvested, green fruit. The appearance of ringspots on several of the inoculated fruit confirmed this phenomenon. In the field, they observed Sw-5+ plants with systemic infections at low titers, but attributed them to RB isolates or incomplete penetration of Sw-5. Their study was limited to harvested fruit and did not consider the potential for disease progression within the plant if the fruits were infected while still on the plant. Furthermore, their study did not evaluate whether the Sw-5 resistance allele increases protection against infection by TSWV when fruit are fed upon by viruliferous thrips as compared with susceptible Sw-5– plants. Due to the frequent occurrence of infected fruit in Sw-5+ tomato plants, a better understanding is needed of the types of transmission events that result in symptomatic, infected fruit and associated yield losses, so that they can be more effectively managed.

Our study examines the question of whether feeding by viruliferous F. occidentalis on blossom clusters or very young fruit of Sw-5+ tomato plants can lead to symptomatic TSWV-infected fruit. Herein, we test the hypothesis that inoculation of NRB isolates of TSWV by F. occidentalis feeding on blossoms or very young fruit results in infection of the developing fruit in both Sw-5+ and Sw-5– plants. We also test the hypothesis that systemic infections develop in host genotypes with and without the Sw-5 resistance allele following inoculation of blossom or young fruit tissues by F. occidentalis.

Materials and Methods

Plants. Greenhouse and field experiments used Solanum lycopersicum cultivars Mountain Glory and Mountain Spring (Clifton Seeds, Faison, NC). Mountain Glory (Sw-5+) contains the Sw-5 allele for resistance and Mountain Spring (Sw-5–) lacks the Sw-5 allele for resistance against TSWV.

Thrips. A virus-free lab colony of F. occidentalis was maintained on Phaseolus vulgaris bean pods at ca. 26°C, 55% RH, and continuous light. To obtain viruliferous adults used for TSWV transmission, neonate (<3 h post-eclosion) F. occidentalis were placed on excised symptomatic Emilia sonchifolia leaves infected with one of the three field-collected NRB TSWV isolates (see below). The leaves and thrips were maintained inside a 50 mm diameter × 9 mm Parafilm-sealed Petri dish (Fisher Scientific, Pittsburgh, PA) containing moist filter paper and placed in an incubator for a 48 to 72 h acquisition-access period at 30°C. The thrips were then transferred onto P. vulgaris bean pods in plastic containers with thrips-proof mesh tops and bottoms, reared to adult, and used in the thrips inoculation experiments.

Verification that TSWV isolates were non-resistance-breaking. Three TSWV isolates (designated Cart10, John10a, and John11) were collected from TSWV-susceptible cultivars in commercial tomato plantings in Moore and Montgomery counties in North Carolina. These isolates were mechanically inoculated into E. sonchifolia and maintained in thrips-proof cages in a greenhouse. The isolates were initially tested for the ability to break Sw-5 resistance by mechanically inoculating into the foliage of tomato seedlings (two true leaves) of both Mountain Glory and Mountain Spring. Inoculum was prepared by grinding infected leaf tissue from E. sonchifolia in a chilled buffer containing 10 mM Tris-HCl (pH 7.8), 10 mM Na2SO3, and 0.1% L-cysteine. All fully expanded leaves were coated with 600-mesh carborundum and gently rubbed using a cotton swab saturated with inoculum. Sw-5+ and Sw-5– tomato seedlings were inoculated with each TSWV isolate prior to the experiment to test for RB. None of the 5+ plants became systemically infected by any of the isolates (no. infected/no. plants inoculated: Cart10, 0/18; John10a, 0/22; John11, 0/24). The same inoculum from each isolate readily, systemically infected Sw-5– plants (Cart10, 12/20; John10a, 8/21; John11, 13/24). To further confirm that isolates were NRB, thrips inoculations were conducted on Sw-5+ and Sw-5– seedlings using viruliferous adult F. occidentalis.

A subsample of 30 putatively viruliferous F. occidentalis adults were released onto 2- to 3-week-old Sw-5+ and Sw-5– tomato seedlings (one to two true leaves) growing in thrips-proof cages within a greenhouse in four separate experiments for a total of 45, 48, 49, and 48 Sw-5+ plants and 48, 46, 48, and 49 Sw-5– plants for isolates Cart10, John10a, John 11, and John11, respectively. Thrips were allowed to feed for 1 week on seedlings before plants were sprayed with spinosyn insecticide to kill the thrips. Samples of newly emerged young leaves were collected 3 weeks after the thrips were released and tested for TSWV using double sandwich (DAS)-ELISA for the nucleocapsid protein following the manufacturer’s protocol (AGDIA, Elkhart, IN).

Transmission to reproductive tissue. One blossom cluster containing blossoms and/or blossoms with small developing fruit (ca. 2 mm diameter) per plant was enclosed in a thrips-proof cage constructed from 100 micron screen (Midwest Filter Corp, Lake Forest, IL). There was one cage per plant. A total of 46 plants had cages containing only blossoms at the time of thrips release and 12 plants had cages containing blossoms with small developing fruit when the thrips were released. Each cage was sealed around the peduncle of individual blossom clusters prior to blooming using putty (Oatley, Cleveland, OH) or poster putty (Duck, Avon, OH). All uncaged blossom clusters were regularly removed from the experimental plants before the flowers opened. Putatively viruliferous adult F. occidentalis (2 to 4 days post-eclosion) were released into each cage containing freshly opened flowers or newly set fruit per plant. Five to 10 thrips per individual blossom or fruit were released into an inflorescence cage containing 2 to 4 blossoms or fruit. A total of 33 Sw-5+ plants and 25 Sw-5– plants were included in the experiment. TSWV isolate John10a was used to inoculate five Sw-5+ and five Sw-5– plants, isolate Cart10 to inoculate six Sw-5+ and five Sw-5– plants, and John11 to inoculate 22 Sw-5+ and 15 Sw-5– plants, respectively.

To minimize the risk of thrips escaping from the blossom cages and moving to other plants, each experimental plant was placed inside large thrips-proof enclosures (0.76 × 0.76 × 1.22 m) along with a yellow sticky trap to detect the presence of thrips outside of the blossom/fruit cages. To detect unintended virus spread, an indicator plant (E. sonchifolia) was also placed inside each enclosure and replaced every 2 weeks. No indicator plants developed infections and no F. occidentalis were caught on the yellow sticky traps or observed outside of the blossom cages. In addition, five TSWV- and thrips-free tomato plants of each genotype were placed randomly throughout the greenhouse as sentinels to detect virus spread within the greenhouse; none became infected by TSWV.

To determine if TSWV infections spread systemically from inoculated blossom clusters to noninoculated fruit, additional blossom clusters were enclosed in thrips-proof cages prior to the blossoms opening to exclude thrips. At least one blossom cluster was caged to exclude thrips prior to the thrips release into the other caged blossom cluster on the same plant. Additional blossom clusters that were initiated by the plant during weeks three and six following the thrips release were also caged to exclude thrips prior to the blossoms opening. All other blossoms were removed prior to opening. A systemic insecticide was added to the soil 5 to 7 days after the thrips release to kill all thrips for the remainder of the experiment.

To determine TSWV infection, tissue samples were collected from ripened fruit inside the blossom/fruit cages into which the thrips were released as well as from control cages that did not receive thrips. In addition, to determine if systemic spread of the infection had occurred, samples were taken of young leaf tissue produced on the same branch at the node immediately above and outside the blossom/fruit cage. Samples of young leaf tissue growing on other arbitrarily selected branches were also collected 3 weeks and 6 weeks after thrips were released into the blossom/fruit cages. These samples were tested for TSWV infection using DAS-ELISA. Samples were classified as TSWV-positive when the optical density exceeded the mean plus four standard deviations of the negative controls (three noninoculated samples from same tissue type). Measurements were taken at 405 nm using a THERMOMax microtiter plate reader (Molecular Devices Corp., Menlo Park, CA).
Analysis. The experiment was analyzed as a completely randomized design using logistic regression (Logistic Procedure, SAS v9.3, Cary, NC). Because an initial analysis revealed no significant effect of isolate on number of plants infected, isolate was not included in the analysis to test for significant effects of inoculation site (blossom only or blossom with small fruit at the time of thrips release), and genotype (Sw-5+ and Sw-5–) on the frequency of infections.

Field experiments. Small plot field experiments were conducted in 2011 and 2012 to monitor the prevalence of fruit-limited and systemic infections of TSWV in Mountain Glory (Sw-5+) and Mountain Spring (Sw-5–) plants. In both years, the experimental plantings were located in Candor, NC, adjacent to a commercial planting of the TSW-resistant tomato cultivar Redline, which expresses the Sw-5 gene. Transplants were set in the field on 2 May 2011 and 16 May 2012. The Sw-5+ and Sw-5– treatments were included along with other tomato varieties in a larger randomized complete block experiment to evaluate varietal performance. Detailed data on TSW incidence were not collected from the other varieties; hence those varieties were not included in the analysis of results reported here. The 2011 experiments consisted of four replicates, each consisting of three plants of Mountain Spring (Sw-5–) or Mountain Glory (Sw-5+) transplanted on 2 May. The 2012 experiment consisted of five replicates, each consisting of five Sw-5+ plants and five Sw-5– plants transplanted on 16 May.

During both years, all plots were inspected at ca. 2-week intervals to identify TSWV-infected plants. To determine the presence of systemic infections, samples of young emerging leaf tissue were taken from each plant and subjected to DAS-ELISA. In 2011, samples were taken on 5 July and 25 August. In 2012, foliage samples were collected from plants with foliar or fruit symptoms every 2 weeks from 30 May through 31 July, at which time foliage from all plants not previously classified as infected was tested. In addition, during both years, symptomatic fruit were collected at the time that they were first observed and the plants from which they were collected were classified as either expressing or not expressing foliar symptoms. On 25 August 2011, all remaining fruit present on the surviving 10 Sw-5+ plants were removed and tested for infection using DAS-ELISA. In 2012, all fruit present on 30 July were collected from each plot and classified as TSWV-infected or noninfected based on presence or absence of symptoms and then subjected to DAS-ELISA to verify infection status and detect asymptomatic infections.

The abundance of TSWV vectors in tomato blossoms was assessed during both years of the field trial. On each sample date, four to five samples each consisting of 10 blossoms selected arbitrarily across all cultivars were collected and placed in 70% ethanol. Samples were taken to the laboratory where the blossoms were dissected and all thrips counted. Adult thrips were slide-mounted and identified to species. In samples containing more than 25 thrips, a subsample of 25 were identified to species. In 2011, samples were collected on 14, 22, and 30 June, and 22 July; in 2012, they were collected on 28 June and 17 and 30 July. Results are presented with standard errors.

Results

Verification that TSWV isolates were non-resistance breaking. In addition to the mechanical inoculation tests for RB, thrips inoculation tests were conducted to test if each isolate could infect systematically 2- to 3-week-old seedlings of Sw-5+ and Sw-5– plants. No Sw-5+ seedlings became infected (no. infected/no. inoculated: 0/45, 0/48, 0/49, 0/48) by any of the isolates, but each isolate was able to infect Sw-5– seedlings (28/48, 23/46, 32/48, 6/49) for experiments 1 (isolate John10a), 2 (isolate Cart10), and 3 and 4 (isolate John11), respectively.

Transmission to reproductive tissue. Transmission of TSWV to both Sw-5+ and Sw-5– genotypes occurred when infectious thrips were caged on blossoms with or without visible, young fruit developing within the blossom (Fig. 1). Symptomatic fruit infections developed following inoculation by F. occidentalis confined to blossom clusters, as did systemic infections of noninoculated foliage and fruit of the same plant caged to exclude thrips. With the exception of two Sw-5+ and two Sw-5– plants, all systemic infections were observed on plants exhibiting infections at the site of inoculation (blossom clusters). In these cases, the blossoms exposed to viruliferous thrips failed to set fruit. Since extensive thrips feeding can cause flowers or fruit to abort (Childers 1997), it is possible that virus spread systematically from infected tissue that subsequently aborted. The number of infected plants, indicated by a positive DAS-ELISA test from any plant tissue, differed significantly between Sw-5+ and Sw-5– hosts, with fewer infections among Sw-5+ plants (7 of 33 plants) than among Sw-5– plants (17 of 25 plants) (odds ratio for transmission to Sw-5+ versus Sw-5– plants = 0.116 [95% CI 0.033 – 0.405]; Wald X² = 11.42; df = 1; P = 0.0007). The incidence of infected fruit did not differ significantly between treatments in which thrips were released on to blossoms with or without small developing fruit at the time of release (Wald X² = 2.272; df = 1; P = 0.1318) (Supplementary Table S1).

The frequency of infections that became systemic, confirmed by a positive DAS-ELISA test of leaf tissue or noninoculated fruit caged on the same plant to exclude thrips, was similar in both Sw-5+ and Sw-5– genotypes (71% and 76%, respectively; Fig. 1). Of these systemic infections, nine resulted in infected fruit developing from blossoms that were caged prior to opening to exclude thrips and prevent any feeding on the blossoms. Three of these infections occurred on Sw-5+ and six on Sw-5– plants.

Typical symptoms of ringspots, necrosis, and abnormal growth were observed on the fruit (Supplementary Fig. S1). Symptom severity was in some cases extreme, causing fruit to wither and die. Foliar symptoms on blossom-inoculated Sw-5+ plants consisted of small necrotic lesions on leaves, generally one or two nodes above the inoculated blossom cluster; whereas symptoms on blossom-inoculated Sw-5– were characteristic of TSWV infections on tomato and consisted of chlorotic ringspots often surrounding small necrotic lesions.

Field experiments. In both years, TSWV infections were common, although less so in 2012. In 2011, all 10 Sw-5+ plants had at least some fruit that exhibited infection but none of the plants developed systemic infections. The mean percentage (±SE) of infected fruit per Sw-5+ plant in 2011 was 38.9 (4.41). In 2012, seven of 25 Sw-5+ plants (28%) were infected, six of which had infected fruit and three of which developed systemic infections. The mean percentage (±SE) of infected fruit per Sw-5+ plant in 2012 was 12.1 (4.05), respectively. One of the Sw-5+ plants became infected early in the season and developed symptoms typical of those observed in Sw-
5– plants. The other two infections were detected late in the season and infections were limited to the infected fruit and foliage located directly above on the same branch (Supplementary Fig. S2). Because most of the Sw−5– plants were infected early and developed severe systemic infections, they produced few fruit. In 2011, all 12 Sw−5– plants were systemically infected and produced no fruit. In 2012, 16 of 25 Sw−5– plants (64%) were systemically infected. Of these, only six of eight fruiting plants had symptomatic fruit. All field infections were confirmed by DAS-ELISA.

*F. occidentalis* was the predominant vector present during both years of the field experiment, comprising 89 ± 6.5% of the thrips population within the blossoms in 2011 and 49 ± 12.2% in 2012. The only other species present in the blossoms was *F. tritici*, a non-vector of TSWV. In 2011, *F. occidentalis* numbers remained high throughout the experiment, averaging 4.25 ± 0.25 adults per blossom, while in 2012, *F. occidentalis* were less abundant, averaging 1.2 ± 0.33 adults per blossom (Fig. 2).

**Discussion**

Use of the Sw−5 resistance allele has provided significant protection in reducing losses to TSW (Riley and Joseph 2011; Riley and Pappu 2004); however, there are numerous reports of reduced efficacy due to occurrence of resistance-breaking (Aramburu and Martí 2003; Ciuffo et al. 2005; Latham and Jones 1998) and frequent reports of late-season expression of TSW symptoms in mature fruits of Sw−5+ tomato cultivars (Aramburu and Rodríguez 1999; Moriones et al. 1998). The failure of Sw−5 to prevent late-season fruit infections is poorly understood. This study extends previous research by Aramburu et al. (2000) demonstrating the ability of *F. occidentalis* to transmit TSWV to nearly mature excised fruit by demonstrating that fruit infections can result from feeding on blossoms by infectious *F. occidentalis* and that the Sw−5 resistance allele can be circumvented by NRB isolates leading to systemic infections when inoculated into blossoms by *F. occidentalis*. The Sw−5 resistance was effective at preventing infections resulting from inoculation of foliar tissue and reduced the probability of infection resulting from inoculation of blossoms and/or very small fruit relative to Sw−5– hosts in our experiments. Thrips transmission to blossoms apparently provides a gateway for NRB isolates of TSWV to infect Sw−5+ plants and move systematically.

Systemic infections not attributed to resistance-breaking isolates have been considered to result from incomplete penetration of Sw−5, resulting in approximately 2% of plants becoming infected (Aramburu et al. 2000; Stevens et al. 1992). Our experiment revealed a much higher incidence of such infections. Although we found significantly fewer total infections (i.e., fruit limited, systemic, or both) of Sw−5– than Sw−5+ plants following thrips inoculation of blossoms/fruit, we did not detect a significant difference in incidence of systemic infections between Sw−5+ and Sw−5– plants. However, the numbers of infected Sw−5– plants were low (seven total, five systemic infections) compared with Sw−5+ plants (17 total, 13 systemic infections) and it remains possible that additional replication to achieve higher incidences of total and systemic infections would reveal differences between the Sw−5+ and Sw−5– plants.

To ensure that our results were not unique to a specific TSWV isolate, we included different isolates that failed to infect Sw−5+ plants following mechanical or thrips inoculation of foliar tissue and found that all were able to infect Sw−5+ plants when inoculated by *F. occidentalis* into blossoms. In the field, we also observed infections of Sw−5+ plants that were limited to foliage directly above symptomatic fruit. Several isolates collected from a grower’s planting of the Sw−5+ cultivar Redline in Candor, NC, were tested for RB. Infections from Sw−5+ foliage were often unsuccessful. However, mechanical inoculations of foliage of Sw−5+ Mountain Spring seedlings using isolates obtained from infected fruit from two Sw−5+ plants resulted in seven and two infected plants per 15 inoculated seedlings, respectively; similar inoculations of Sw−5– seedlings with an isolate obtained from infected foliage of a third Sw−5+ plant resulted in nine infected plants per 24 inoculated seedlings. Mechanical inoculations of seedlings of 15, 15, and 24 Sw−5+ Mountain Glory plants using these isolates, respectively, failed to produce any infections. This suggests that the isolates infecting the resistant plants are unlikely to be resistance-breaking. One likely hypothesis explaining our results is that the Sw−5 gene is not expressed in blossoms and fruit.

In our field experiments, *F. occidentalis* was the only vector observed during the bloom period and was more abundant in 2011 when TSW prevalence was high than in 2012 when prevalence was low. This difference in vector abundance during the bloom period likely accounts for the differences in TSWV prevalence between years. Previous studies have shown that while the tobacco thrips, *F. fusca*, is generally the primary vector of TSWV during the spring (April through early June), *F. occidentalis* is the primary vector during the summer months (Beaudoin 2011). Our results suggest that controlling *F. occidentalis* populations throughout the season to prevent populations from developing in the blossoms and feeding on newly set fruit may prove important in reducing late-season spread and prevalence of infected fruits in Sw−5+ varieties. Although tomato plants that become infected later in the season produce significantly more fruit than plants infected early, the fruits often express symptoms of infection and are unmarketable (Moriones et al. 1998). A further concern when thrips populations develop within a planting is the potential for secondary spread. Since immature *F. occidentalis* can be found consistently in tomato blossoms throughout the season (Beaudoin 2011), and have been shown to acquire TSWV from infected tomato fruits and subsequently transmit it to susceptible plants (Szoetsk et al. 2017), it may be possible for them to acquire TSWV and spread it among fruit clusters within a field of Sw−5+ plants, even in the absence of systemic infection.

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**Literature Cited**


