Research

Western Flower Thrips Can Transmit *Tomato spotted wilt virus* From Virus-infected Tomato Fruits

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Abstract

In this study, we demonstrate that western flower thrips (WFT, *Frankliniella occidentalis*) can acquire and transmit *Tomato spotted wilt virus* (TSWV) from symptomatic tomato fruits. TSWV and other thrips-transmitted tospoviruses have long been known to spread via plant propagation material such as transplants. Global dissemination of tospoviruses has also been linked, in part, to transport and trade of thrips-infested and virus-infected horticultural products. However, the role of tomato fruits transported across state and national borders has not previously been examined as a means of virus spread or as a source for thrips acquisition of virus. Tomato fruits displaying typical tospovirus

symptoms were purchased from several Washington State grocery stores. Many of these symptomatic fruits tested positive for TSWV and some for *Pepino mosaic virus* (PepMV). First instar larvae of WFT successfully acquired TSWV from these infected tomato fruits and transmitted the virus as adults to *Emilia sonchifolia* plants. Symptomatic *E. sonchifolia* plants were confirmed positive for TSWV by lateral flow immunoassays and sequence analysis of a portion of the nucleocapsid gene. These results suggest the dissemination of TSWV (and likely other tospoviruses) and PepMV to new geographic areas by human-assisted transport of infected tomato fruits.

Tospoviruses (family *Bunyaviridae*, genus *Tospovirus*) are one of the most damaging groups of plant viruses affecting a wide range of agricultural, floricultural, and horticultural crops grown in greenhouses or fields (Gilbertson et al. 2015). Unlike other vector-borne plant viruses, tospoviruses display several unique features that include an ambisense, tripartite RNA genome and circulative, propagative transmission by thrips (Thysanoptera: Thripidae) in a developmental stage-specific manner (Rotenberg et al. 2015). Besides their negative effects on crop yield (Sevik and Arli-Sokmen 2012), tospovirus infections can cause pronounced impacts on produce quality, leading to nonmarketable fruits, leaf vegetables, and ornamentals (Funderburk 2009). The striking symptoms produced by several tospoviruses on fruits include chlorotic and necrotic rings, irregular blotches, and deformation (Fig. 1). These symptomatic fruits could further reduce shelf-life in supermarkets and be cause for rejection by consumers due to poor aesthetic appeal and potentially reduced nutritional quality, resulting in financial losses to producers (Spence et al. 2006).

Tomato spotted wilt virus (TSWV) is the most ubiquitous of the tospoviruses, causing major losses in multiple crops worldwide, and is recognized as one of the top 10 most economically destructive plant viruses (Scholthof et al. 2011). The worldwide distribution of TSWV and that of its principal vector-thrips species, the western flower thrips (WFT, *Frankliniella occidentalis*), have been attributed, in part, to their exceedingly wide host ranges, which include many cultivated crops, ornamentals, and uncultivated plant species (Parrella et al. 2003; Reitz 2009) and to increased transport of vector thrips-

infested and virus-infected horticultural and floricultural products through global trade and commerce (Kirk and Terry 2003; Gilbertson et al. 2015). Consequently, preventing the invasion of both the virus and the vector into new locations has become a challenge.

Globalization of agriculture has, in part, contributed to an increased risk of cross-border and cross-continental spread of pests and diseases (Hulme 2009). Regional and international trade of agricultural produce is often suspected to be one of the primary means for pathogen and pest movement. As noted in Lecoq et al. (2003), there is limited experimental evidence to support the assumption that viruses spread via vegetables and fruits, such as tomato. Some examples of viruses thought to have moved via tomato fruit include *Tomato yellow leaf curl virus* (TYLCV) into northern Europe (Just et al. 2014) and *Pepino mosaic virus* (PepMV) into and within Europe (Staniulis et al. 2012; Steffen et al. 2015). Likewise, *Plum pox virus* (PPV) was detected in peach fruits imported from Chile into Brazil (Rezende et al. 2016) and in intercepted fruit at an international airport in Australia (Davis et al. 2002).

Reports of vector acquisition of viruses from infected fruits and transmission to noninfected plants are also rare. Lecoq et al. (2003) demonstrated that potyvirus-infected melon fruits could serve as a source for aphid transmission of *Papaya ringspot virus* and *Zucchini yellow mosaic virus* to young cucurbit plants. Similarly, aphid transmission of PPV from infected peach and apricot fruits has been demonstrated (Labonne and Quiot 2001; Gildow et al. 2004; Lowery et al. 2015). Whitefly transmission of TYLCV from infected tomato fruit to noninfected tomato plants was demonstrated by Delatte et al. (2003). Culled pepper fruits from TSWV-infected plants were implicated as a source of both virus and viruliferous thrips (Okazaki et al. 2007). Onions and onion transplants have been implicated in the spread of *Iris yellow spot virus* into new

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FIGURE 1

Representative tomato fruits with chlorotic spots and blotches characteristic of *Tomato spotted wilt virus* infection purchased from grocery stores.

regions of the United States. (Hsu et al. 2011; Nischwitz et al. 2007; Schwartz et al. 2014).

Import of fresh-market tomato fruits into the United States has grown dramatically in recent years and imported tomato fruits currently account for about one-third of tomato consumption (Zahniser et al. 2015). Consequently, alien pests and diseases carried via different types of tomato fruits, including truss tomatoes, could pose phytosanitary concerns and threats to agricultural security in the country (Gregory et al. 2006; Ferrier 2014; Perrings 2016). Since visual inspection of large volumes of imported tomatoes for pathogens, especially viruses, can be challenging and unreliable, infected fruits may escape scrutiny passing through the supply chain of international trade and inadvertently move across national or state borders (Wylie et al. 2014). Thus, international trade could be an additional avenue for long-distance dispersal of viruses via infected tomato fruits.

Tomato fruits displaying symptoms consistent with tospovirus infection (Fig. 1) were observed at several grocery stores in eastern Washington State in the United States. These observations led us to test the hypothesis that infectious tospoviruses may be present in these fruits, and these fruits may contribute to the dissemination of tospoviruses by serving as a source for thrips acquisition.

Virus Detection in Symptomatic Tomato Fruits

During June and July of 2014, 38 tomato fruits (Roma type) displaying chlorotic spots and blotches characteristic of TSWV were purchased from 10 grocery stores in six locations in the Yakima Valley of Washington State (Table 1). In addition, three non-Roma-type tomato fruits displaying virus-like symptoms were purchased from a grocery store in New Orleans, LA (Table 1). According to the produce labels, these tomatoes were produced in Mexico, Canada, or the United States, and subsequently transported to retail stores for sale. Symptomatic slices were cut from each tomato fruit, homogenized in 0.01M phosphate buffer, pH 7.0, and tested for TSWV, *Impatiens necrotic spot virus* (INSV), and

PepMV in lateral flow immunoassays (ImmunoStrip for TSWV STX 39300/0025, ImmunoStrip for INSV STX 20500/0025, ImmunoStrip for PepMV STX 13001/0025, Agdia, Inc., Elkhart, IN). Of the 41 fruits tested, 29 were positive for TSWV, 13 for PepMV, and none for INSV. Four tomato fruits were positive for both TSWV and PepMV. The remaining three fruits were negative for the three viruses. We did not test these three fruits for other viruses known to infect tomato plants (Hanssen et al. 2010).

It has been reported recently that TSWV lateral flow immunoassays can react with other tospoviruses, such as *Tomato chlorotic spot virus* (TCSV) and *Groundnut ring spot virus* (GRSV) (Adkins et al. 2015). Therefore, additional molecular diagnostic assays were used to confirm lateral flow immunoassay results. Total nucleic acids were extracted from three tomato fruits positive for TSWV in lateral flow immunoassays using a previously described protocol (Bagewadi et al. 2015). The denatured extract was used as a template in one-step RT-PCR to amplify an approximately 620-bp fragment of the nucleocapsid (N) gene encoded by the S-RNA segment using primers TSWV723 and TSWV722 (Adkins and Rosskopf 2002). RT-PCR products were resolved in 1.5% agarose gels, stained with GelRed (Biotium, Hayward, CA), and visualized under UV light. The amplicons were gel eluted using the QIAquick Gel Extraction kit (Qiagen Inc., Valencia, CA) and cloned in pCR2.1 (Invitrogen Corp., Carlsbad, CA). Two clones per amplicon were sequenced in both orientations (Retrogen, Inc., San Diego, CA). Sequences of cDNA clones from two fruits were identical (GenBank Accession No. KY349923) and showed 99% nucleotide sequence identity with corresponding N gene sequence of TSWV isolates (GenBank Accession Nos. AY744468 to AY744473) reported from California (Tsompana et al. 2005). These results confirmed immunoassay results for the presence of TSWV in symptomatic tomato fruits.

Thrips Acquisition and Transmission of TSWV From Infected Tomato Fruits

WFT must acquire the virus as larvae to transmit as adults (van de Wetering et al. 1996); therefore, for tomato fruits to serve as a source of inoculum, infectious TSWV must be present in the fruits and available to WFT larvae for acquisition. To test this hypothesis, three additional tomato fruits with typical TSWV symptoms were purchased from local grocery stores and TSWV infection was confirmed with lateral flow immunoassays as above. These fruits were then used to determine whether TSWV could be acquired by thrips larvae and transmitted to indicator plants under laboratory conditions using the protocol described by Naidu et al. (2005).

First-instar thrips larvae (100 to 300 synchronous-aged, 4-h old) from a colony of WFT maintained on green bean pods in the laboratory were released onto each of three tomato fruits in separate thrips-proof containers and allowed to feed for approximately 24 h (Fig. 2). After this acquisition access, the larvae were transferred to and maintained on green bean pods until development into adults. These potentially viruliferous adult thrips were released onto young *Emilia sonchifolia* plants (3 to 5 true leaf stage) for approximately 48 h of inoculation access feeding in separate thrips-proof containers. The cohort of adult thrips fed as larvae on the first tomato fruit was divided into two groups of ~50, and one group was released onto each of two *E. sonchifolia* plants. Cohorts of adult thrips (~50) fed as larvae on the second or third tomato fruits were released onto each of two additional *E. sonchifolia* plants. Subsequently, the plants were sprayed with a pyrethrin-based home garden insecticide to kill adult thrips. Plants were maintained in an isolated area in the laboratory for about two weeks. Leaves with thrips feeding damage were removed as

TABLE 1
Tomato spotted wilt virus and Pepino mosaic virus detection in store-bought symptomatic tomato fruits

Purchase location ^a	Symptoms	Origin	Virus ^b
Sunnyside, WA	Green rings	Baja California, Mexico	TSWV
Sunnyside, WA	Light green	Baja California, Mexico	TSWV
Sunnyside, WA	Green spots and rings	Baja California, Mexico	-
Sunnyside, WA	Green and yellow rings	Baja California, Mexico	-
Prosser, WA, Grocery 1	Chlorotic rings	Mexico	TSWV
Prosser, WA, Grocery 1	Chlorotic ring, clear spots	Mexico	PepMV
Prosser, WA, Grocery 1	Green rings, chlorotic spots	Mexico	PepMV
Prosser, WA, Grocery 1	Chlorotic rings	Mexico	PepMV
Yakima, WA, Grocery 1	Chlorotic ring, brown spots	Mexico	PepMV
Yakima, WA, Grocery 1	Chlorotic spots	Mexico	PepMV
Yakima, WA, Grocery 1	Chlorotic spots, green spots	Mexico	TSWV & PepMV
Yakima, WA, Grocery 1	Chlorotic spots	Mexico	TSWV & PepMV
Toppenish, WA, Grocery 1	Green spots, chlorotic spots	Mexico	TSWV & PepMV
Toppenish, WA, Grocery 1	Chlorotic spots	Mexico	TSWV & PepMV
Toppenish, WA, Grocery 1	Chlorotic ring	Mexico	TSWV
Toppenish, WA, Grocery 1	Severe chlorotic spots	Mexico	TSWV
Sunnyside, WA	Chlorotic spots with green spots	Mexico	TSWV
Sunnyside, WA	Chlorotic blotches, red spots	Mexico	TSWV
Sunnyside, WA	Chlorotic spots	Mexico	TSWV
Sunnyside, WA	Chlorotic spot	Mexico	TSWV
Sunnyside, WA	Chlorotic spots, green spots	Baja California, Mexico	TSWV
Sunnyside, WA	Chlorotic spots	Baja California, Mexico	TSWV
New Orleans, LA ^c	Chlorotic spots, green spots	Canada	PepMV
New Orleans, LA ^c	Chlorotic blotches	Canada	PepMV
New Orleans, LA ^c	Chlorotic spots, green spots	Canada	TSWV
Union Gap, WA	Chlorotic blotches	Canada	PepMV
Toppenish, WA, Grocery 2	Chlorotic rings, green spots	California, USA	TSWV
Toppenish, WA, Grocery 2	Chlorotic rings	California, USA	PepMV
Toppenish, WA, Grocery 2	Green, yellow rings	California, USA	TSWV
Toppenish, WA, Grocery 2	Chlorotic rings	California, USA	TSWV
Toppenish, WA, Grocery 2	Chlorotic spots, rough skin	California, USA	TSWV
Yakima, WA, Grocery 2	Chlorotic ring	California, USA	TSWV
Yakima, WA, Grocery 2	Chlorotic rings	California, USA	TSWV
Yakima, WA, Grocery 2	Chlorotic rings	California, USA	-
Richland, WA	Chlorotic rings, spots	Unknown	TSWV
Richland, WA	Chlorotic rings	Unknown	TSWV
Prosser, WA, Grocery 2	Chlorotic rings	California, USA	TSWV
Yakima, WA, Grocery 3	Chlorotic coat, chlorotic spots	Mexico	TSWV
Yakima, WA, Grocery 3	Chlorotic rings	Mexico	TSWV
Yakima, WA, Grocery 3	Chlorotic rings	Mexico	TSWV
Yakima, WA, Grocery 3	Chlorotic spots	USA	TSWV

^a LA = Louisiana, WA = Washington.

^b TSWV = *Tomato spotted wilt virus* and PepMV = *Pepino mosaic virus* detected in the tomato by lateral flow immunoassay. – = TSWV, INSV, PepMV not detected.

^c Non-Roma type tomato fruits.

new leaves emerged. The inoculated plants free of thrips (from visual observations) were subsequently maintained in a temperature-regulated greenhouse frequently sprayed and monitored for insects. Symptomatic leaves from each of the four plants subjected to thrips inoculations were subsequently tested for the presence of TSWV with lateral flow immunoassays. For further confirmation that the *E. sonchifolia* plants were infected with TSWV, an approximately 620-bp fragment of the N gene was amplified by RT-PCR from two F3 plants (Fig. 3), and cloned and sequenced as described above. A pairwise comparison of these sequences was identical (GenBank

Accession No. KY349924) and showed 99% nucleotide sequence identity with sequences obtained from corresponding tomato fruits. These results confirmed that TSWV was present in *E. sonchifolia* plants inoculated by thrips that had acquired the virus as larvae from infected tomato fruits.

Conclusions

In this study, about 71% of tomato fruits showing tospovirus-like symptoms purchased from grocery stores were found to be infected

FIGURE 2

First instar thrips larvae shown feeding on a tomato fruit, (A) magnified 20x and (B) 60x.

with TSWV. About 32% of these tomato fruits were infected with PepMV. These results indicated that virus-infected tomato fruits are being shipped thousands of miles from primary production areas. The likelihood of virus-infected, asymptomatic fruits being present in grocery stores was not determined. We also demonstrated for the first time that WFT are capable of acquiring TSWV from infected tomato fruits and transmitting the virus to indicator plants under laboratory conditions. The results from this study offer proof-of-concept that tomato fruits from TSWV-infected plants may function to introduce the virus to new geographic areas. Additionally, mixed infections of TSWV and PepMV observed in this study need to be considered when implementing strategies for prevention and management of these viruses.

Tomato cultivars carrying the *Sw-5* gene have been widely deployed for resistance against TSWV in commercial tomato production around the world (Riley et al. 2011; Turina et al. 2016 and references therein). The *Sw-5b* gene has been shown to confer broad resistance to other tospovirus species, such as TCSV, GRSV, INSV, and Chrysanthemum stem necrosis virus (Boiteux and de B. Giordana 1993; Turina et al. 2016 and references therein). It has been reported that *Sw-5* gene-mediated resistance is effective against TSWV (Spassova et al. 2001) and virus infections in tomato cultivars carrying the *Sw-5* gene were limited to fruits (Aramburu and

FIGURE 3

Detection of *Tomato spotted wilt virus* (TSWV) in *Emilia sonchifolia* plants inoculated by viruliferous thrips that acquired the virus as larvae from infected tomato fruits. (A) Foliar symptoms in *E. sonchifolia*. (B) A 620-bp DNA fragment of the TSWV nucleocapsid protein gene amplified from symptomatic leaves. M = molecular weight marker; - = negative control; + = positive control; 1 and 2 = duplicate samples from one plant; 3 and 4 = duplicate samples from a second plant.

Rodriguez 1999; Aramburu et al. 2000). Additionally, there is no apparent difference in field tolerance to thrips vectors, such as WFT and tobacco thrips (*F. fusca*), between tomato cultivars with or without the *Sw-5* resistance gene (Riley et al. 2011) because the mode of resistance is directly associated with TSWV (Spassova et al. 2001). It has also been observed that mature fruits (and sometimes foliage) of tomato plants expressing *Sw-5*-mediated resistance can exhibit symptoms as a result of viruliferous thrips feeding on different parts of flowers and young fruits (Houle and Kennedy, *In press*), further indicating differences in the expression of *Sw-5* gene-mediated resistance in vegetative and reproductive parts of the plant (Houle and Kennedy, *In press*). In recent years, the emergence of TSWV strains capable of overcoming resistance conferred by the *Sw-5* gene cluster has become a concern for durability of resistance against tospoviruses (Batuman et al. *In press*). In this context, results of our study imply that tomato fruits may be able to function as a conduit for dissemination of resistance-breaking strains of tospoviruses. Further studies are therefore required to examine whether fruits from TSWV-infected tomato plants carrying the *Sw-5* gene can provide a fitness advantage for resistance-breaking virus isolates and serve as a source for dissemination by vector thrips under field conditions.

It should be noted that tospovirus infection of tomato fruits is not limited to TSWV, since tospoviruses such as Peanut bud necrosis virus, Capsicum chlorosis virus, GRSV, and TCSV can also infect tomato fruits (Kunkalika et al. 2011; Webster et al. 2015). Similarly, peppers (*Capsicum annuum* L. cv. Dempsey) infected with INSV (Naidu et al. 2005) and watermelons (*Citrullus lanatus* [Thumb.] Matsun and Nakai) infected with *Watermelon bud necrosis virus* (Kunkalika et al. 2011) can produce fruits with a range of symptoms. The results of our study highlight the potential risk of

these tospoviruses also being moved to new geographic areas via infected fruit.

Under field conditions, WFT is known to cause goldfleck spots and halo rings due to direct feeding on green or red tomato fruits (Ghidiu et al. 2006). It has also been demonstrated that tomato fruits can exhibit symptoms when green fruits of cultivars carrying the *Sw-5* gene are inoculated with viruliferous thrips carrying TSWV (Aramburu et al. 2000). This would imply that *Sw-5* gene resistance can be circumvented when blossoms and green fruits are infected with TSWV leading to the production of symptomatic fruits during the latter part of the season. Since WFT can oviposit during feeding (Salguero Navas et al. 1991), any larvae emerging from these infected tomato fruits prior to harvest can acquire the virus and contribute to secondary spread as overwintering pupae and adults. Indeed, Okazaki et al. (2007) from Japan have reported that green pepper fruits discarded in the greenhouses and open fields were positive for TSWV and the adults of WFT that acquired virus from fruits during larval stages could transmit the virus to pepper seedlings. They also observed a decrease in TSWV incidence in greenhouses after removing virus-infected green pepper fruits from the previous season. Likewise, destruction of symptomatic tomato fruits at the point of origin in open-field and greenhouse-protected production systems may be similarly prudent to minimize the risk of infection of subsequent crops or introduction of tospoviruses into new geographic areas. Although infected tomatoes in imported consignments may be unlikely to serve as a source for virus acquisition, fruit-bearing trusses may offer a better habitat for thrips-mediated transmission as the stems remain attached to infected tomatoes, thereby contributing to long-range spread of tospoviruses. Our study also underscores the spread of PepMV through international trade of tomato fruits (reviewed in Hanssen and Thomma 2010). Three major genotypes of PepMV have been reported spreading through tomato seed supply systems (Ling et al. 2008). Due to the highly contagious nature of PepMV, the potential risk of these genotypes, either as single or coinfections, spreading through the import and distribution of tomato fruits needs further study.

In the case of vegetatively propagated crops and ornamentals, imported planting stock has most often been considered as a potential source of introduction of viruses and strains to new geographic areas (Kim et al. 2014; Gergerich et al. 2015). Our study, together with previous reports (Hanssen and Thomma 2010; Staniulis et al. 2012; Just et al. 2014; Steffen et al. 2015; Rezende et al. 2016), provides evidence that movement of infected fruits and vegetables to new locations is another mode of virus dispersal. Future studies should focus on risk analysis of viruses introduced via the transportation and supply chain systems from virus-infected areas and on improving international standards of phytosanitary measures to prevent movement of virus-infected tomato fruits destined for fresh market sale, repacking, or processing.

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