Winter Weeds as Inoculum Sources of Tomato Spotted Wilt Virus and as Reservoirs for Its Vector, *Frankliniella fusca* (Thysanoptera: Thripidae) in Farmscapes of Georgia

RAJAGOPALBABU SRINIVASAN,1,2 DAVID RILEY,1 STAN DIFFIE,1 ANITA SHRESTHA,1 AND ALBERT CULBREATH3

Environ. Entomol. 43(2): 410–420 (2014); DOI: http://dx.doi.org/10.1603/EN13288

ABSTRACT Thrips-transmitted Tomato spotted wilt virus (TSWV) has a broad host range including crops and weeds. In Georgia, TSWV is known to consistently affect peanut, tomato, pepper, and tobacco production. These crops are grown from March through November. In the crop-free period, weeds are presumed to serve as a green bridge for thrips and TSWV. Previous studies have identified several winter weeds as TSWV and thrips hosts. However, their ability to influence TSWV transmission in crops is still not completely understood. To further understand these interactions, population dynamics of two prevalent vectors, viz., *Frankliniella fusca* (Hinds) and *Frankliniella occidentalis* (Pergande), on selected winter weeds were monitored from October through April in four counties from 2004 to 2008. Peak populations were typically recorded in March. *F. fusca* and *F. occidentalis* adults were found on winter weeds and their percentages ranged from 0 to 68% in comparison with other adults. Immatures outnumbered all adults. Microcosm experiments indicated that the selected winter weeds differentially supported *F. fusca* reproduction and development. The time required to complete one generation (adult to adult) ranged from 11 to 16 d. Adult recovery ranged from 0.97 to 2.2 per female released. In addition, transmission assays revealed that thrips efficiently transmitted TSWV from peanut to weeds, the incidence of infection ranged from 10 to 55%. Back transmission assays with thrips from TSWV-infected weeds resulted in up to 75% TSWV infection in peanut. These whole-plant transmission and back transmission assays provide the basis for TSWV persistence in farmscapes year round.

KEY WORDS tospovirus, alternate host, vector reservoir, inoculum source

Tomato spotted wilt virus (Family Bunyaviridae; Genus Tospovirus) is transmitted by thrips in a persistent and propagative manner (German et al. 1992; Ullman et al. 1993, 2002; Whitfield et al. 2005; Pappu et al. 2009). Approximately 10 species of thrips are known to transmit Tomato spotted wilt virus (TSWV; Mound 1996, Riley et al. 2011). Of those, the tobacco thrips, *Frankliniella fusca* (Hinds), and the western flower thrips, *Frankliniella occidentalis* (Pergande), are considered as important vectors of TSWV in Georgia (Todd et al. 1995, 1996; Riley et al. 2011). TSWV is predominantly a spring season threat in Georgia, and *F. fusca* and *F. occidentalis* populations in crops are often associated with early season TSWV infections and late season infections, respectively (Riley et al. 2012). TSWV has been increasing in importance since its introduction to the southeastern United States in the 1980s (Black et al. 1986). Crop losses in Georgia alone, due in large part to TSWV, have exceeded >US$326 million from 1996 to 2006 (Riley et al. 2011).

TSWV has a broad host range extending >900 plant species in multiple families (Tsompana and Moyer 2008, Pappu et al. 2009). It severely affects crops such as peanut (*Arachis hypogaea* L.), pepper (*Capsicum annum* L.), tobacco (*Nicotiana tabacum* L.), and tomato (*Lycopersicon esculentum* Miller) (Gitaitis et al. 1998, McPherson et al. 1999, Garcia et al. 2000, Riley and Pappu 2000, Culbreath et al. 2003, Culbreath and Srinivasan 2011). Besides the crops mentioned above, it is also known to infect numerous weed species in the southeastern United States (Groves et al. 2001, 2002; McPherson et al. 2003, Mullis and Martinez 2009).

The role of weed flora as hosts of the vector or the virus could be critical to virus incidence in crop hosts (Duffus 1971). TSWV is transmitted by thrips only if acquisition occurs at the first-instar larval stage (van de Wetering et al. 1996). Therefore, besides being infected with the virus, it is necessary for any plant host to support thrips vector populations for at least a generation to function as a TSWV inoculum source. TSWV occurs annually in Georgia. Nonetheless, the crops are only grown from March through November and TSWV is not seed-transmitted. This implies that the virus and the vector should survive on alternate hosts or winter weeds during the crop-free period. A
number of winter annual weed species have been identified as hosts of TSWV in the southeastern United States (Groves et al. 2001, 2002; Mullis and Martinez 2009). Groves et al. (2002), through sampling adults and immatures from winter weeds and rearing immatures to adulthood on green bean (Phaseolus vulgaris L.) pods, demonstrated that winter weeds could serve as hosts of F. fusca. Other field studies indicated that the winter weeds differed in their abilities to support thrips populations (Groves et al. 2001, Morsello and Kennedy 2009). However, biological fitness parameters of F. fusca on various winter weeds over an entire generation (adult to adult) were not exclusively studied in the laboratory. The potential of these weed hosts to serve as TSWV inoculum sources and affect TSWV transmission in crop hosts is not completely understood either. To our knowledge, only one study had conducted controlled transmission assays involving weeds as inoculum sources, tobacco as recipients, and onion thrips (Thrips tabaci Lindeman) as the vector (Chatzivassiliou et al. 2007). Even in that study leaf discs were used instead of whole plants to conduct transmission assays. Previous surveys have revealed high levels of TSWV infection (up to 32%) in several weeds such as narrow leaf cudweed (Ganochaeta falcata (Lamarck) Cabrera), chickweed (Stellaria media L.), VA pepperweed (Lepidium virginicum L.), and Carolina geranium (Geranium carolinianum L.) (Groves et al. 2001, 2002; Mullis and Martinez 2009). Similarly, one of our preliminary winter weed sampling surveys, followed by serological testing of foliar samples, also indicated that a high percentage (up to 20%) of certain species of winter weeds were infected with TSWV. However, when an attempt was made to phenotype the TSWV isolates from the weed samples through mechanical inoculation of an indicator host (tobacco), numerous inoculations did not produce TSWV infection. Careful examination with other detection techniques revealed that several of those samples were not infected with TSWV and were indeed false positives. TSWV infections in weed hosts are typically asymptomatic, and double antibody sandwich–enzyme-linked immunosorbent assay (DAS–ELISA) is routinely used for TSWV detection in weeds. It is not uncommon to observe high background absorbance in microtiter plates while attempting to test samples from multiple plant species or families (Timmerman et al. 1985, Smith et al. 2006). Even though there are suggestions to ameliorate the background issue (Towbin and Gordon 1984, Smith et al. 2006), it is recommended that ELISA results be confirmed by other concrete detection methods or through transmission assays (Timmerman et al. 1985).

Susceptible crops grown in spring, especially in their early stages, are very vulnerable to TSWV and seldom recover from infection (Culbreath et al. 2003, Joost and Riley 2004, Riley et al. 2012). Their yields are typically unmarketable. Thus, it is essential to understand how the green bridge of winter and spring weeds functions as a thrips reservoir and as a TSWV inoculum source. Very few studies in the laboratory have examined the biological fitness parameters of F. occidentalis and onion thrips (T. tabaci) on weeds (Bautista and Mau 1994, Chatzivassiliou et al. 2007), even fewer with F. fusca. However, a number of field studies have documented differential abilities of winter annual weeds to support F. fusca populations (Groves et al. 2001, Morsello and Kennedy 2009). To better understand the ability of winter weeds to serve as reservoirs of TSWV vectors in Georgia’s farmscapes, we monitored the population dynamics of thrips across four counties from 2004 to 2008. Further, we conducted microcosm studies to compare thrips fitness parameters on selected winter weeds in the laboratory. In addition, we conducted transmission assays using TSWV-infected peanut as an inoculum source and selected winter weeds as recipients. In these assays, to circumvent the background effects associated with DAS–ELISA, we used reverse transcriptase polymerase chain reaction (RT-PCR) and employed other techniques such as mechanical inoculation of an indicator host to confirm TSWV-infection in winter weeds. To examine the potential of TSWV spread from weeds to crops, we conducted back transmission assays with TSWV-infected weeds as inoculum sources and peanut plants as recipients.

Materials and Methods

Thrips Sampling in Winter Weeds. Five species of weeds prevalent in Georgia’s farmscapes and known to serve as TSWV hosts were selected for sampling in six flagged locations (≈6 m apart) adjacent to production fields in four counties (Brooks, Colquitt, Decatur, and Tift) at two fields per county. The weed species sampled from 2004 to 2008 were G. carolinianum, S. media, G. falcata, sowthistle (Sonchus asper (L.) Hill), and L. virginicum. In the first season (2004–2005), the samples were collected in monthly intervals from October to April. In the second season (2005–2006), the samples were collected in monthly intervals from December to April. In the third (2006–2007) and the fourth (2007–2008) seasons the samples were collected in January and March alone, as they were considered as key indicator months for winter and spring thrips. A maximum of six samples (one from each flagged location) of fresh foliage of each weed species, enough to fill to capacity a 3.755 liters (1-gallon)–clear ziplock plastic bag, were collected from each farm site in monthly intervals and returned to the laboratory. If a weed species was not present or not sufficiently abundant within a 6 m diameter from a sample location flag to fill a sample bag, then whatever amount was present was collected. In the laboratory, the fresh samples were weighed. The weed samples were subsequently placed in Berlese’s funnels for 7 d and thrips were collected in 70% ethanol. The collected adult thrips were identified to species and immature thrips were counted as a group.

Maintenance of Nonviruliferous F. fusca. A colony of F. fusca was established in 2009 on noninfected peanut leaflets of the cultivar Georgia Green with thrips collected from peanut blooms from the Bel-
flower Farm, UGA Tifton Campus. Since then, thrips were maintained in Munger cages (Munger 1942) on noninfected Georgia Green leaflets. Munger cages were maintained in a growth chamber (Thermo scientific, Dubuque, IA) at 25–30°C with a photoperiod of 14:10 (L:D) h. The leaflets in each cage were dusted with ≈0.05 g of pine (Pinus taeda L.) pollen (Angelella and Riley 2010). All laboratory and greenhouse experiments were performed only with F. fusca.

**F. fusca Reproduction and Development on Winter Weeds.** F. fusca reproduction and development were evaluated on four winter weed species, viz., G. carolinianum, G. falcata, S. asper, and S. media. Seedlings of weeds were collected from the borders of crop fields during the months of January and February. The seedlings were held in the greenhouse in 47.5 cm³ thrips-proof cages (Megaview Science Co., Taichung, Taiwan) for approximately 2 wk. If thrips larvae, adults, or feeding injuries were noticed before commencement of the experiment, then the plants were discarded. Ten Munger cages were set up with foliage with inoculated tobacco (Nicotiana tabacum var. Miller, TWP, Pittsburgh, PA) by a paintbrush. Thrips were subsequently released on weeds (10 potentially viruliferous thrips per plant) that were dusted with ≈0.05 g of pine pollen per plant. The plants at the time of inoculation were ≈5–10 cm in height with up to 10 fully expanded true leaves. All the weed species that were inoculated remained in the vegetative stage. Each plant was enclosed in a Mylar film (Grafix, Cleveland, PA) cage with a copper mesh top (mesh pore size-170 μm; TWP, Berkeley, CA). Plants were maintained in the greenhouse for 3 wk, after which TSWV infection status was assessed by RT-PCR (Jain et al. 1998, Shrestha et al. 2013).

Total RNA was extracted from foliar weed samples using RNeasy kit (Qiagen, Valencia, CA) according to the manufacturer’s recommendation. RT-PCR was performed by OneStep RT-PCR kit (Qiagen, Valencia, CA). The reaction volume was 50 μl, which included 0.6 μM of each forward and reverse primer (Jain et al. 1998). 2 μl of one-step RT-PCR enzyme mix, 10 μl of RT-PCR buffer, 10 μl of Q solution, and 400 μM of each deoxyribonucleotide triphosphates. Reverse transcription was performed in an automated thermal cycler (Eppendorf, Hamburg, Germany) programmed at 50°C for 30 min. Initial PCR activation was conducted at 95°C for 15 min followed by 35 cycles at 94°C for 1 min, 56°C for 45 s, and 72°C for 1 min, and a final extension step at 72°C for 10 min. The amplicons were assessed by electrophoresis on a 1% agarose gel. Statistical analysis was performed to compare the incidence of TSWV infection among different weed species. A completely randomized design was used. Treatments (weeds) were considered as fixed effects and replications were considered as random effects. Data were pooled from both repeats of the experiment using experiment as the blocking variable. TSWV infection was treated as a binomial response (positive or negative), and differences among treatments were estimated by logistic regression analysis using PROC GENMOD with logit link function in SAS. The statistical significance of differences between treatment pairs was estimated using pairwise contrasts.

**Mechanical Inoculation of Tobacco Plants.** Foliar samples from field-collected, TSWV-infected weeds (infection confirmed by RT-PCR) in the first repeat of the transmission assay were used as inoculum sources for mechanical inoculation (Mandal et al. 2002). Foliation from each weed species was ground in 1:10 ratio (wt:vol) in 0.1 M phosphate buffer pH 7.0, containing 0.2% sodium sulfite, 0.01 M mercaptobenzothiazole, Celite 545 (Acros Organics, Geel, Belgium), and 0.01 g/ml of carborundum (320 grit, Fisher, Fair Lawn, NJ). The extract was then rubbed onto leaves of 3-wk-old tobacco seedlings of the cultivar NC-71 that had been previously dusted with 1 ml carborundum per plant. Tobacco seedlings are very susceptible to TSWV and are often used as indicator hosts. Inoculated tobacco seedlings were placed in thrips-proof cages at 25–30°C, 80–90% relative humidity, and a photoperiod of 14:10 (L:D) h in the greenhouse. The presence or absence of TSWV symptoms was visually observed at 1 and 2 wks postinoculation.

Tobacco plants were maintained in Munger cages (Munger 1942) on noninfected Georgia Green leaflets. Munger cages were maintained in a growth chamber (Thermo scientific, Dubuque, IA) at 25–30°C with a photoperiod of 14:10 (L:D) h. The leaflets in each cage were dusted with ≈0.05 g of pine (Pinus taeda L.) pollen (Angelella and Riley 2010). All laboratory and greenhouse experiments were performed only with F. fusca.

**F. fusca Reproduction and Development on Winter Weeds.** F. fusca reproduction and development were evaluated on four winter weed species, viz., G. carolinianum, G. falcata, S. asper, and S. media. Seedlings of weeds were collected from the borders of crop fields during the months of January and February. The seedlings were held in the greenhouse in 47.5 cm³ thrips-proof cages (Megaview Science Co., Taichung, Taiwan) for approximately 2 wk. If thrips larvae, adults, or feeding injuries were noticed before commencement of the experiment, then the plants were discarded. Ten Munger cages were set up with foliage with inoculated tobacco (Nicotiana tabacum var. Miller, TWP, Pittsburgh, PA) by a paintbrush. Thrips were subsequently released on weeds (10 potentially viruliferous thrips per plant) that were dusted with ≈0.05 g of pine pollen per plant. The plants at the time of inoculation were ≈5–10 cm in height with up to 10 fully expanded true leaves. All the weed species that were inoculated remained in the vegetative stage. Each plant was enclosed in a Mylar film (Grafix, Cleveland, PA) cage with a copper mesh top (mesh pore size-170 μm; TWP, Berkeley, CA). Plants were maintained in the greenhouse for 3 wk, after which TSWV infection status was assessed by RT-PCR (Jain et al. 1998, Shrestha et al. 2013).

Total RNA was extracted from foliar weed samples using RNeasy kit (Qiagen, Valencia, CA) according to the manufacturer’s recommendation. RT-PCR was performed by OneStep RT-PCR kit (Qiagen, Valencia, CA). The reaction volume was 50 μl, which included 0.6 μM of each forward and reverse primer (Jain et al. 1998). 2 μl of one-step RT-PCR enzyme mix, 10 μl of RT-PCR buffer, 10 μl of Q solution, and 400 μM of each deoxyribonucleotide triphosphates. Reverse transcription was performed in an automated thermal cycler (Eppendorf, Hamburg, Germany) programmed at 50°C for 30 min. Initial PCR activation was conducted at 95°C for 15 min followed by 35 cycles at 94°C for 1 min, 56°C for 45 s, and 72°C for 1 min, and a final extension step at 72°C for 10 min. The amplicons were assessed by electrophoresis on a 1% agarose gel. Statistical analysis was performed to compare the incidence of TSWV infection among different weed species. A completely randomized design was used. Treatments (weeds) were considered as fixed effects and replications were considered as random effects. Data were pooled from both repeats of the experiment using experiment as the blocking variable. TSWV infection was treated as a binomial response (positive or negative), and differences among treatments were estimated by logistic regression analysis using PROC GENMOD with logit link function in SAS. The statistical significance of differences between treatment pairs was estimated using pairwise contrasts.

**Mechanical Inoculation of Tobacco Plants.** Foliar samples from field-collected, TSWV-infected weeds (infection confirmed by RT-PCR) in the first repeat of the transmission assay were used as inoculum sources for mechanical inoculation (Mandal et al. 2002). Foliation from each weed species was ground in 1:10 ratio (wt:vol) in 0.1 M phosphate buffer pH 7.0, containing 0.2% sodium sulfite, 0.01 M mercaptobenzothiazole, Celite 545 (Acros Organics, Geel, Belgium), and 0.01 g/ml of carborundum (320 grit, Fisher, Fair Lawn, NJ). The extract was then rubbed onto leaves of 3-wk-old tobacco seedlings of the cultivar NC-71 that had been previously dusted with 1 ml carborundum per plant. Tobacco seedlings are very susceptible to TSWV and are often used as indicator hosts. Inoculated tobacco seedlings were placed in thrips-proof cages at 25–30°C, 80–90% relative humidity, and a photoperiod of 14:10 (L:D) h in the greenhouse. The presence or absence of TSWV symptoms was visually observed at 1 and 2 wks postinoculation.
Back Transmission of TSWV from Weeds to Peanut. Four TSWV-infected winter weed species described in the previous experiment served as inoculum sources and 1-wk-old peanut seedlings served as recipients. Ten potentially viruliferous second-instar *F. fusca* larvae that developed on TSWV-infected weeds were transferred to a 1.5-ml microcentrifuge tube (Fisher, Pittsburgh, PA) by a paintbrush. Thrips were subsequently released on noninfected 1-wk-old peanut seedlings (10 potentially viruliferous thrips per plant) that have been dusted with 0.05 g pine pollen per plant. Each plant was enclosed in a Mylar film cage as described above. Plants were maintained in the greenhouse for 3 wk, after which TSWV infection status was assessed by RT-PCR. Ten peanut plants were inoculated per each inoculum source (weed species) and the experiment was repeated once (N = 20 peanut plants for each weed species). Total RNA extraction, RT-PCR, and statistical analyses were conducted as described previously.

**Results**

**Thrips Sampling in Winter Weeds.** Adult TSWV vectors, viz., *F. fusca* and *F. occidentalis*, were collected from the foliage of selected winter weeds by using Berlese’s funnels during the months of October through March. Other adults and immatures were also collected from the weeds during that period in all 4 yr. Other adults included *Frankliniella tritici* (Fitch), *Frankliniella bispinosa* (Morgan), and members of Phlaeothripidae (Thysanoptera: Tubulifera). Although the densities of thrips varied from year to year, their temporal distribution pattern was similar (Fig. 1). The number of thrips (adults and immatures) was typically greater in March than in any other sampling month in all 4 yr (Fig. 1). More thrips (adults and immatures) were found on *G. carolinianum* than on other weeds in all 4 yr (Fig. 2). In general, the number of immatures was typically much greater than the number of *F. fusca* or *F. occidentalis* adults (Fig. 2). The immatures were not identified to species in any of the weed species sampled. Among adults, irrespective of the host, on several occasions, the percentages of *F. fusca* or *F. occidentalis* adults were less than the other adults (Fig. 3). The percentages of *F. fusca* and *F. occidentalis* varied with weed species and sampling year (Fig. 3). The weights of plant materials collected in clear plastic bags indicated that the relative abundance of winter weeds varied with time (Fig. 4). At the...
time of peak thrips incidence (in March and April), the foliar weight of *G. carolinianum* was more than any other weed species collected in all 4 yr of sampling (Fig. 4).

*F. fusca* Reproduction and Development on Winter Weeds. The number of adults produced per 10 adults released varied with winter weed species (*F* = 4.54; df = 3, 76; *Pr > F* = 0.0090). The number of adults ranged from 6.06 ± 1.25 (mean ± SE) to 22.18 ± 4.02 (Fig. 5). The number of adults produced on *S. media* foliage was significantly greater than the number of adults produced on *G. carolinianum* and *G. falcata* foliage (Fig. 5). Few adults emerged in Munger cages with *G. falcata* foliage; however, it was not different from the number of adults that emerged from Munger cages with *G. carolinianum* and *S. asper* foliage (Fig. 5). Median developmental time also varied with weed species (*χ^2^* = 9.01; df = 3, 76; *Pr > χ^2^* = 0.0292). The developmental time on *G. carolinianum*, *G. falcata*, *S. asper*, and *S. media* foliage were 12, 14, 16, and 11 d, respectively. The time required to complete one generation (adult to adult) was the shortest on *S. media* and the longest on *S. asper*.

TSWV Transmission to Winter Weeds. Potentially viruliferous *F. fusca* that developed on TSWV-infected peanut foliage transmitted TSWV to winter weeds. TSWV infection in winter weeds was identified by 770 bp RT-PCR amplicons (Fig. 6a). The incidence of TSWV infection varied with weed species (*χ^2^* = 12.40; df = 3, 76; *Pr > χ^2^* = 0.0061). The percentages of TSWV infection in *G. carolinianum*, *S. media*, *G. falcata*, and *S. asper* were 10.00 ± 10.00 (mean ± SE), 55.00 ± 5.00, 40.00 ± 14.14, and 20.00 ± 10.00, respectively. The incidence of TSWV infection in *S. media* was greater than in *G. carolinianum* and *S. asper* (Table 1), but was not different from the incidence of TSWV infection in *G. falcata* (Table 1). The incidence of TSWV infection in *G. falcata* was greater than in *G. carolinianum* (Table 1). The incidences of TSWV infection in *G. carolinianum* and *S. asper* were not different from each other (Table 1).

TSWV infection in individual weeds was detected by RT-PCR and confirmed by mechanical inoculation of tobacco seedlings. Tobacco seedlings inoculated with foliage from all TSWV-infected weeds species, except for *S. media*, produced symptoms suggestive of TSWV infection. Detection of TSWV by RT-PCR and confirmation by mechanical inoculation were in agreement for *G. carolinianum*, *G. falcata*, and *S. asper*. The symptoms included necrotic spots, which later coalesced and spread systemically (Fig. 7). Infected plants were stunted or dead.

Back Transmission of TSWV from Weeds to Peanut. Potentially viruliferous thrips that developed on TSWV-infected winter weeds transmitted TSWV to peanut. TSWV infection in peanut was identified by 770 bp RT-PCR amplicons (Fig. 6b). Typical TSWV symptoms, such as necrotic spots, yellowing, and ter-
minal wilting, were also observed in infected plants. The incidence of infection varied based on the inoculum source ($\chi^2 = 14.18; \text{df} = 3, 70; \text{Pr} > \chi^2 = 0.0027$). The percentages of TSWV infection in peanut after inoculation with viruliferous thrips from G. carolinianum, S. media, G. falcata, and S. asper were 62.50 ± 12.50 (mean ± SE), 14.28 ± 0, 60.00 ± 10.00, and 75.00 ± 15.00, respectively. The infection percentage was the lowest when potentially viruliferous thrips from S. media were used for inoculation (Table 2). The incidences of TSWV infection in peanut plants did not vary from each other when potentially viruliferous thrips that developed on G. carolinianum, G. falcata, and S. asper were used for inoculation (Table 2).

**Discussion**

Adult TSWV vectors, viz., F. fusca and F. occidentalis, were found on all the sampled winter weeds over a 4-yr period. Groves et al. (2002) sampled numerous species of winter weeds and collected adult thrips and immatures from them using Berlese’s funnels in North Carolina. They also reared immatures to adulthood using green bean pods. Their results indicated that 77% of total thrips trapped comprised TSWV vectors, of which 96% was F. fusca. Our data over the sampling periods indicated that only up to 68% of the trapped adults were TSWV vectors. Of which, 0–67% were F. fusca and 0–47% were F. occidentalis. In our case, the immatures were not reared to adulthood. This precluded us from evaluating what percentage of trapped immatures were F. fusca or F. occidentalis. Several preliminary attempts to rear immatures from weed foliage to adults using green bean pods resulted in the death of a very high percentage of immatures before reaching adulthood.

To further examine the ability of these weeds to support thrips reproduction and development, Munger cage studies were conducted in the laboratory with weed foliage using F. fusca. F. fusca reproduction and development were monitored for an entire generation (adult to adult) in this study. Although both F. fusca and F. occidentalis can efficiently transmit TSWV (Whitfield et al. 2005, Pappu et al. 2009, Riley et al. 2011), F. fusca is documented to colonize crops, such as peanut and tobacco, in their early stages in Georgia (Chamberlin et al. 1992, McPherson et al. 1999). However, F. occidentalis populations on crop hosts seem to...
Fig. 4. Percentages of weights of foliage from five weed species. Foliage from six sampling points in two fields from each county was brought to the laboratory and weighed.

Fig. 5. Percentage survival to adult *F. fusca* on weed foliage in Munger cages. Adults were removed daily beginning 5 d after release of thrips. Observations were taken until there were no alive larvae.
be closely associated with flowers later in the season in Georgia (Riley and Pappu 2000, Joost and Riley 2004, Riley et al. 2010). Early season TSWV infections are known to cause more yield losses in crops than late season infections (Chaisuekul et al. 2003, Culbreath et al. 2003, Riley et al. 2012). Because of these reasons and the maintenance of a F. fusca colony in our laboratory, fitness studies were only conducted with F. fusca. Munger cage studies indicated that winter weeds differentially supported F. fusca reproduction and development. The number of adults developed per female adult released was greater on S. media than on G. carolinianum, G. falcata, and S. asper. These results should be cautiously interpreted, as the fitness experiment was conducted using detached foliage. Foliage detachment could lead to induction of defense responses otherwise absent in intact leaves and affect insect feeding (Reymond et al. 2000). Despite that, these results reiterate that winter weeds could support F. fusca populations for at least a whole generation. Our field sampling counts indicated that numerically more F. fusca, and thrips in general, were recovered from G. carolinianum than from other weed species. This might be due, in part, to the amount of sample available and collected from each location. The sample weights clearly indicated that the weight of G. carolinianum foliage was greater than the foliage from any other weed species sampled in all 4 yr. Thrips median developmental time (adult to adult) was the shortest on S. media and the longest on S. asper. This revealed that not only was the reproduction rate greater on S. media, but also the median developmental time was the shortest on S. media, suggesting that S. media might be a more suitable host for F. fusca than the others tested in this study.

Transmission assays with F. fusca indicated that they transmitted TSWV from peanut to all weed species tested. The TSWV infection percentages ranged from 10 to 55%. The infection percentages in S. media and G. falcata were greater than the other two weed species tested. S. media, in addition to being a very suitable host for thrips, was also very susceptible to TSWV. Because the seedlings of weeds used for transmission assays were field-collected, to assess any initial TSWV infection, we tested at least 50% of the number of plants in each weed species by RT-PCR before inoculation. None of the plants tested were positive for TSWV infection before inoculation. Considering the infection rates obtained after inoculation and that the infection rates before inoculation was zero, it is highly likely that the observed TSWV infection in weeds was a direct result of thrips-mediated inoculations in the greenhouse. In general, serological detection techniques have been known to overestimate virus incidence in some crop and noncrop hosts (Timmerman et al. 1985, Smith et al. 2006). High levels of background absorbance induced by nontarget binding or because of other reasons have been associated with DAS–ELISA testing. To avoid overestimation of TSWV infection in inoculated weed hosts all the weeds were tested only by RT-PCR. Further, to confirm TSWV infection in inoculated weeds, mechanical

<table>
<thead>
<tr>
<th>Treatment pairs</th>
<th>df</th>
<th>χ²</th>
<th>P &gt; χ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. media versus G. falcata</td>
<td>1, 38</td>
<td>0.96</td>
<td>0.3273</td>
</tr>
<tr>
<td>S. media versus G. carolinianum</td>
<td>1, 38</td>
<td>10.34</td>
<td>0.0013</td>
</tr>
<tr>
<td>S. media versus S. asper</td>
<td>1, 38</td>
<td>5.65</td>
<td>0.0174</td>
</tr>
<tr>
<td>G. falcata versus G. carolinianum</td>
<td>1, 38</td>
<td>5.26</td>
<td>0.0218</td>
</tr>
<tr>
<td>G. falcata versus S. asper</td>
<td>1, 38</td>
<td>2.02</td>
<td>0.1548</td>
</tr>
<tr>
<td>G. carolinianum versus S. asper</td>
<td>1, 38</td>
<td>0.52</td>
<td>0.4653</td>
</tr>
</tbody>
</table>

Ten potentially viruliferous F. fusca adults that developed on TSWV-infected peanut plants were used for inoculating weeds. TSWV infection status of inoculated plants was tested by reverse transcriptase polymerase chain reaction 3 wk post inoculation. Differences in the incidence of TSWV infection between treatment pairs were assessed by pairwise contrasts following logistic regression in SAS using PROC GENMOD.
inoculation was adopted. Mechanical inoculation using TSWV-infected foliage from weeds produced infection in tobacco, except when TSWV-infected S. media foliage was used as an inoculum source. It is not clear as to why that was the case even though the incidence of TSWV infection in S. media was greater than all the other weed species. TSWV-infected S. media plants had extensive thrips feeding injuries. They were also substantially senescing when compared with other weed species that were inoculated at the same time. These factors might have contributed to a reduction in virus load in S. media and needs to be investigated in detail.

In the transmission assay, thrips introduced to inoculated weeds were not removed from those plants. Second-instar larvae that developed on TSWV-infected weeds were used for back transmission assay using peanut plants as recipients. Back transmission assay indicated that F. fusca were able to transmit TSWV from all the selected weed species to peanut. The incidence of TSWV infection varied from 14 to 75%. The incidence of infection was the lowest when S. media was used as the inoculum source. The incidence of TSWV infection in peanut was 3–5 times more when potentially viruliferous larvae from other weed species were used for inoculation. Despite the ability of S. media to serve as a good thrips reservoir and support high levels of TSWV infection, thrips were able to transmit TSWV from S. media to peanut only at a low efficiency when compared with other weed species. Extensive thrips feeding injuries observed on S. media foliage and increased senescence than the other weed species could have suppressed virus multiplication and accumulation, which in turn, could have negatively affected TSWV acquisition from S. media and inoculation of peanut plants by F. fusca. A previous survey on winter weed infection percentages revealed that TSWV infection in S. media was lower than several other winter weeds sampled (Groves et al. 2002). On the contrary, Groves et al. (2001) was able to recover more viruliferous F. fusca from caged S. media plants in the field than from Scleranthus annuus L. and S. asper. Planting of S. media as an inoculum source in the field also resulted in increased TSWV spread to Ranunculus sardous Crantz plants. More than 40% of the R. sardous samples were infected with TSWV (Groves et al. 2001), suggesting that S. media could be an efficient TSWV inoculum source.

From our experiments, it was evident that thrips were able to transmit TSWV to weed hosts and from weeds to peanut efficiently. Again, the caveat here is that the experiments were conducted in the greenhouse and in the laboratory where environmental conditions were relatively optimal for plant growth, thrips reproduction, and TSWV symptom expression. Experiments conducted by Morsello and Kennedy (2009) indicated that weather factors, such as rainfall timing and amount and temperature, could play a major role in the extent of TSWV spread in a weed species and the subsequently influence of TSWV infection in crop hosts. For instance, fewest number of TSWV-infected S. media plants were found in patches that received the highest rainfall. Further, rainfall also negatively affected the immature F. fusca populations (Morsello

Table 2. Differences in the incidence of TSWV infection in peanut plants with TSWV-infected winter weeds as inoculum sources

<table>
<thead>
<tr>
<th>Treatment pairs</th>
<th>df</th>
<th>$\chi^2$</th>
<th>$P &gt; \chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. media versus G. falcata</td>
<td>1, 32</td>
<td>7.67</td>
<td>0.0056</td>
</tr>
<tr>
<td>S. media versus G. carolinianum</td>
<td>1, 32</td>
<td>7.67</td>
<td>0.0056</td>
</tr>
<tr>
<td>S. media versus S. asper</td>
<td>1, 32</td>
<td>13.17</td>
<td>0.0003</td>
</tr>
<tr>
<td>G. falcata versus G. carolinianum</td>
<td>1, 38</td>
<td>0.04</td>
<td>0.8410</td>
</tr>
<tr>
<td>G. falcata versus S. asper</td>
<td>1, 38</td>
<td>1.03</td>
<td>0.3903</td>
</tr>
<tr>
<td>G. carolinianum versus S. asper</td>
<td>1, 38</td>
<td>1.03</td>
<td>0.3903</td>
</tr>
</tbody>
</table>

Ten potentially viruliferous F. fusca second-instar larvae that developed on TSWV-infected weeds were used to inoculate non-infected peanut plants. TSWV infection status of inoculated plants was tested by reverse transcriptase polymerase chain reaction 3 wk post inoculation. Differences in the incidence of TSWV infection between treatment pairs were assessed by pairwise contrasts following logistic regression in SAS using PROC GENMOD.

Fig. 7. Confirmation of TSWV infection in winter weeds by mechanical inoculation of tobacco seedlings. (A) noninoculated tobacco plant and (B) tobacco plant inoculated with TSWV-infected winter weed foliage. (Online figure in color.)
and Kennedy 2009). Not much information is available on how weather factors could influence thrips development and TSWV spread in other weed species. However, rainfall in general, is known to be a key factor in regulating the population dynamics of numerous thrips species including F. fusca (Kirk et al. 1997; Morsello et al. 2008, 2010). A recently developed epidemiological model for TSWV incidence in tobacco suggests a strong link between previous years thrips abundance, winter annual weeds, and weather factors such as winter temperature and precipitation (Chappell et al. 2013).

The results from this study clearly indicate that TSWV infection rates could be high in winter weeds and that F. fusca could efficiently transmit TSWV from peanut to weed hosts and vice versa. Besides F. fusca other vectors, such as F. occidentalis, could also influence TSWV epidemics in the southeastern United States by using weeds as hosts and as inoculum sources (Kahn et al. 2005). Given that TSWV is transmitted by more than one vector species and that several winter weeds can serve as a green bridge for TSWV vectors and the virus, this information could be used to reduce TSWV incidence in agricultural crops. Information on abundance of winter weeds in conjunction with weather parameters could be used in epidemiological models to forecast TSWV incidence in crops (Chappell et al. 2013). Management of winter annual weeds to mitigate TSWV incidence would be another pragmatic option to consider. For instance, a multidisciplinary effort including weed management was helpful in the management of TSWV in Hawaii (Cho et al. 1989, Bautista et al. 1996). However, studies addressing timing of removal, removal distance from crops, and economic feasibility should be undertaken to assess the usefulness of this tactic in the southeastern United States.

The findings in this manuscript substantiate the role of winter weeds as thrips reservoirs and TSWV inoculum sources. The experiments conducted in this manuscript clearly demonstrate that winter weeds in Georgia farmscapes could serve as hosts of TSWV vectors; however, their ability to support thrips populations could vary with species. The transmission and back transmission assays provide direct experimental evidence for movement of TSWV from crops to weeds and vice versa. The findings also emphasize the importance of suitable pathogen detection techniques and confirmatory tests while assessing the role of non-conventional hosts in virus epidemics.

Acknowledgments

We wish to express our sincere thanks to the County extension agents who assisted with sample collection. We also extend our gratitude to Simmy Mckeown and Sheran Thompson for their assistance with thrips rearing and in conducting transmission assays. This research was partially supported by research grants from U.S. Department of Agriculture Southern Region Integrated Pest Management Center and the National Peanut Board.

References Cited


