Transmission of Tomato Spotted Wilt Virus from Pepper and Three Weed Hosts by *Frankliniella fusca*

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ABSTRACT

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We tested the ability of the tobacco thrips (*Frankliniella fusca*) to transmit tomato spotted wilt virus (TSWV) from bell pepper (*Capsicum annuum*) and three wild hosts of the virus in Louisiana—spiny-leaved sowthistle (*Sonchus asper*), wild lettuce (*Lactuca floridana*), and buttercup (*Ranunculus sardous*). In experiments with five thrips per test plant, *F. fusca* was able to transmit TSWV from all four host species to *S. asper* and bell pepper test plants. *F. fusca* was able to reproduce in the laboratory on plants of bell pepper and each of the three weed hosts. These results indicate that *F. fusca* can acquire TSWV from the three weed species and bell pepper in the field and transmit it to pepper and other hosts.

Tomato spotted wilt virus (TSWV) causes serious disease problems in solanaceous crops in Louisiana. TSWV was first identified in Louisiana in 1972 (1), and its increased incidence now limits pepper, tomato, and tobacco production in the state.

Spiny-leaved sowthistle (*Sonchus asper* (L.) J. Hill), wild lettuce (*Lactuca floridana* (L.) Gaertner), and buttercup (*Ranunculus sardous* Crantz) are among the most commonly infected wild hosts of TSWV found in the vicinity of solanaceous crop fields in Louisiana (2). Three species of thrips that have been reported as vectors elsewhere (9) occur in Louisiana: *Frankliniella fusca* (Hinds), the tobacco thrips; *F. occidentalis* (Pergande), the western flower thrips; and *Thrips tabaci* Lindeman, the onion thrips. *F. fusca* is the only one of the three whose population abundance is significantly correlated with TSWV incidence in solanaceous crop fields in Louisiana (L. L. Black, R. N. Story, W. P. Bond, and J. M. Gatti, Jr., *unpublished*). We conducted laboratory experiments to determine the ability of *F. fusca* to acquire and transmit TSWV from bell pepper and the three weed hosts.

MATERIALS AND METHODS

Host plants. *S. asper, L. floridana,* and *R. sardous* were grown from field-collected seed. Seeds of *S. asper* and *L. floridana* were germinated in moist petri plates on a laboratory bench under continuous fluorescent lights. The seed coats of *S. asper* seeds were nicked with a razor blade before the seeds were placed in the petri plates; scarification of seed was necessary for good germination. *R. sardous* seeds germinated successfully in moist petri plates after two cycles of alternating temperatures of 5 C and 24 C (room temperature) with approximately 1 wk at each temperature. Young seedlings were transplanted into methyl bromide-fumigated soil in 10-cm-diam clay pots and maintained in a growth chamber with a photoperiod of 8 or 10 hr and a constant temperature of 25 $\pm$ 2 C. Bell pepper (*Capsicum annuum* L. 'Yolo Wonder') was seeded directly into pots with soil and maintained in the same growth chamber.

Thrips colonies. *F. fusca* individuals were obtained from gardenia (*Gardenia jasminoides* J. Ellis), rose (*Rosa* sp.), and white clover (*Trifolium repens* L.) in the Baton Rouge area. Colonies were established and maintained in Plexiglas sleeve cages on white clover, *S. asper, L. floridana, R. sardous,* or bell pepper plants. Openings cut into the top and sides of the sleeve cages were covered with 230-mesh PeCap monofilament polyester screen (Tetko Inc., Elmsford, NY). The cages were kept at approximately 24 C in the laboratory under continuous lighting from a bank of fluorescent lights.

Virus isolates. Two TSWV isolates used in thrips transmission tests were obtained from *S. asper* plants growing at different locations in Louisiana in the vicinity of TSWV-infected pepper or tomato plants. One isolate (S-BR) was obtained near a garden in Baton Rouge in 1990, and the other (S-NR) was obtained on a farm near New Roads in 1991. Both isolates were used in transmission tests with *S. asper* as virus acquisition host; only isolate S-NR was used in tests with *L. floridana* and bell pepper as acquisition hosts. In tests with *R. sardous* as acquisition host, a tomato isolate (T-G) obtained from a farm in Gilbert, LA, in 1992 was used in addition to S-NR. Another tomato isolate (T-NR) obtained from New Roads in 1989 was used for antiserum production.

Isolates were identified as TSWV on the basis of symptoms in a mechanically inoculated set of host range plants, which included tobacco, tomato, *Datura stramonium,* and various pepper cultivars, in conjunction with positive reactions to TSWV-L antiserum in a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Agdia, Inc., Elkhart, IN). Isolates were maintained through mechanical inoculation and thrips transmission.

Transmission tests. Virus acquisition plants for thrips transmission tests were mechanically inoculated either from plants infected by viruliferous thrips or from plants that had been mechanically inoculated from plants infected by viruliferous thrips. Therefore, isolates had undergone only one or two cycles of mechanical transmission at the time of each individual thrips transmission test.

Systemically infected leaves with symptoms were detached from the acquisition host plants 10–20 days after mechanical inoculation and placed in modified Tashiro cages (11,12). First- and second-stage larvae of *F. fusca* were removed from colony cages and transferred with a fine-tipped brush to the Tashiro cages for a 4-day virus acquisition feeding period. Tashiro cages were maintained under continuous lighting from a bank of fluorescent lights in a laboratory at room temperature (about 24 C). The thrips were then transferred to Tashiro cages containing healthy leaves and maintained until they reached adult stage.

Adult thrips were aspirated into 00-size gelatin capsule halves attached to a

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fungal spore collector (ERI Machine Shop, Iowa State University, Ames). Open capsules containing five thrips were then placed at the base of individual young, healthy test plants for a test feeding period of 2 days in sealed 5.7- or 7.5-L (6- or 8-qt) white plastic containers. The groups of five thrips were all females, females and males, or all males. Little effort was made to group thrips according to gender, as both sexes of *F. fusca* are reported to have similar transmission capabilities (7). Males are small and yellow, as contrasted with the larger, dark brown females.

After the 2-day test feeding, thrips were removed from the test plants, and plants were sprayed with abamectin insecticide (Avid 0.15 EC) at the rate of 0.4 ml/L. The plants were sprayed twice more at 4- to 5-day intervals to prevent development of larvae from hatching eggs. Test plants were maintained in a growth chamber with a photoperiod of 8 or 10 hr at 25 ± 2 C for about 3 wk to allow disease symptom expression. Data were combined from two or three separate experiments for each combination of acquisition host and test host.

ELISA. Test plants were assayed for TSWV by direct double-antibody sandwich (DAS) ELISA (4). The T-NR isolate of the virus was purified from mechanically inoculated tobacco (*Nicotiana tabacum* L. 'Havana 425') by a purification method similar to that of Sreenivasulu et al (10), and antiserum to this isolate was produced in a rabbit. The rabbit was injected intramuscularly in the hind legs three times at 2-wk intervals with a 1:1 emulsion of purified TSWV and Freund's incomplete adjuvant, followed by a booster injection 4 wk later. A small-volume test bleeding was done 8 days after the third injection. After the antiserum was processed and tested for its suitability for use in DAS ELISA, four major bleedings were done at intervals of about 2 wk.

Table 1. Transmission of tomato spotted wilt virus by *Frankliniella fusca* from *Capsicum annuum* 'Yolo Wonder,' *Lactuca floridana, Ranunculus sardous*, and *Sonchus asper*[a]

	Test host	
Virus acquisition host	***S. asper***	***C. annuum***
C. annuum	6/13	4/17
L. floridana	9/13	7/18
R. sardous	11/13[b]	—[c]
S. asper	12/13[b]	13/17

[a]Five adult thrips per test plant, which had been caged as larvae on detached leaves from virus acquisition host plants infected 10–20 days earlier by mechanical inoculation. Data are number of test plants infected/total number of test plants and are the combined results of two or three experiments for each combination of acquisition host and test host. All data are based on the results of both double-antibody sandwich enzyme-linked immunosorbent assay and symptom development.

[b]Combined data from two virus isolates. For *R. sardous*, transmission was 5/6 with isolate S-NR plus 6/7 with isolate T-G. For *S. asper*, transmission was 6/6 with isolate S-NR plus 6/7 with isolate S-BR. Isolate S-NR was used in all other tests reported in this table.

[c]Transmission from *R. sardous* to *C. annuum* was not tested.

RESULTS AND DISCUSSION

Mechanically inoculated and naturally infected *S. asper* developed an initial mottle, followed by leaf distortion, mosaic, and stunting symptoms. Chlorotic or necrotic ring spots were observed occasionally in field-collected plants. Symptoms in field-infected and mechanically inoculated *L. floridana* plants included chlorotic or necrotic ring spots, mosaic, and leaf distortion. Mechanically inoculated *R. sardous* plants developed chlorotic spots, mosaic, and moderate leaf distortion.

F. fusca was able to transmit TSWV from *S. asper, L. floridana,* and bell pepper to test plants of both *S. asper* and bell pepper (Table 1). It was also able to transmit TSWV from *R. sardous* to *S. asper* (transmission from *R. sardous* to bell pepper was not tested). All test plants in each experiment were observed for symptoms and evaluated by ELISA. Only one ELISA-positive *Sonchus* test plant was symptomless; ELISA reaction and symptoms were perfectly correlated for the remainder of the *Sonchus* and bell pepper test plants. None of 18 control *S. asper* test plants exposed to groups of five adult *F. fusca* collected directly from the colony cages became infected.

All-female, all-male, and mixed groups of thrips were able to transmit TSWV (*data not shown*). Males were less abundant than females, making up only 20.9–32.5% of the populations used for each combination of acquisition host and test host. Most of the females and males used in transmission tests were brachypterous (short-winged), but a minority were macropterous (long-winged). We did not distinguish between short- and long-winged thrips in transmission experiments.

Because only larval thrips can acquire TSWV that is subsequently transmitted (6,9), an epidemiologically important virus source plant must also be able to sustain thrips reproduction. *F. fusca* populations were introduced into Plexiglas sleeve cages containing exclusively plants of *S. asper, L. floridana,* or *R. sardous*. Larvae were subsequently collected over a period of 1–2 mo from plants of all three weed hosts and were used in transmission studies and for other purposes. Because the two larval stages of *F. fusca* last a total of only about 1 wk under our laboratory conditions, these results demonstrate that *F. fusca* can reproduce on the three weed hosts and that *S. asper, L. floridana,* and *R. sardous* are capable of serving as virus acquisition sources in a field situation.

In Louisiana, pepper and tomato are transplanted into the field in March and April, and the cropping season is over by mid-July. Although long-distance movement of TSWV may occur, we believe that incidence in some Louisiana solanaceous crop fields is so high and develops in such a short period that only movement from local source plants can account for it. *S. asper, L. floridana,* and *R. sardous* may play such roles. *L. floridana* is a biennial that flowers in August and September and first appears as a seedling in mid-September. *S. asper,* a winter annual, also begins to emerge as a seedling during September, overwinters as a rosette, and usually flowers in March and April. *R. sardous,* also a winter annual, first emerges as a seedling in early December and generally flowers from late January to May (peaking in March or April).

F. fusca could also reproduce on bell pepper plants in Plexiglas sleeve cages in the laboratory. We have seen little or no indication of within-field spread of TSWV in pepper crops in Louisiana. However, the ability of *F. fusca* to colonize bell pepper suggests the possibility that bell pepper plants could serve as virus acquisition sources for infection of other hosts at the end of the cropping season.

S. asper and *L. floridana* both belong to the Compositae family. Other members of the Compositae have been reported as likely reservoirs of TSWV elsewhere. In Hawaii, *Emilia sonchifolia* was shown to be an important host of both TSWV and *T. tabaci,* the main vector of the virus to pineapple (6). In the vegetable-growing regions of Hawaii, five of 20 weed species believed to be important reservoirs of TSWV are composites (*Arctium lappa, Bidens pilosa, S. oleraceus, Verbesina enceloides,* and *Xanthium saccharatum*) (3). In India, one of the two species suspected of being important TSWV reservoirs, *Ageratum conyzoides,* is a member of the Compositae (8). In Japan, *S. oleraceus* and the closely related *Youngia japonica* are believed to act as virus sources for infection of tomato in Nara Prefecture (5).

In surveys of thrips populations and TSWV incidence in solanaceous fields in Louisiana, *F. fusca* was the only thrips species whose population abundance was significantly correlated with TSWV incidence (L. L. Black, R. N. Story, W. P. Bond, and J. M. Gatti, Jr., *unpublished*). However, other thrips species could be involved in disseminating TSWV among wild hosts and into crop plants. Therefore, other thrips species present in Louisiana are currently being evaluated for their ability to transmit TSWV.

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