Potential of Tomato Spotted Wilt Tospovirus Plant Hosts in Hawaii as Virus Reservoirs for Transmission by *Frankliniella occidentalis* (Thysanoptera: Thripidae)

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ABSTRACT

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Five host plant species of tomato spotted wilt tospovirus (TSWV) were evaluated for their potential as virus acquisition and inoculation hosts for transmission by *Frankliniella occidentalis*. In addition, the feeding and oviposition preferences of *F. occidentalis* adults (nonviruliferous) for virus-infected and noninfected plants were compared. The presence of TSWV in *F. occidentalis* and host plants was detected by double antibody sandwich-enzyme-linked immunosorbent assay. Based on the efficiency of TSWV acquisition by *F. occidentalis*, the various host plants were ranked as follows (in descending order): jimson weed, *Datura stramonium*; romaine lettuce, *Lactuca sativa* var. *longifolia*; and burdock (gobo), *Arctium lappa*. On the other hand, TSWV transmission by thrips inoculation was more efficient in *D. stramonium*, *A. lappa*, and golden crown-beard, *Verbesina enceloides*; than in *L. sativa* or cheeseweed, *Malva parviflora*. TSWV transmission from *L. sativa* to *L. sativa* showed the ability of *F. occidentalis* to initiate secondary spread within the crop. Likewise, virus transmission from *D. stramonium* to *L. sativa* and *A. lappa* or from *L. sativa* and *A. lappa* to noncrop hosts showed that *F. occidentalis* could spread TSWV from a weed to cultivated crops or vice versa. The presence of virus-infected plants has the potential to dramatically influence viruliferous thrips populations, because colonizing adults preferred landing and feeding on TSWV-infected plants. In addition, larval yields were significantly larger on diseased plants. The implications of these findings in the epidemiology of TSWV are discussed.

Additional keywords: host preferences, virus-thrips-host plant interaction, western flower thrips.

Tospoviruses, the only known plant viruses in the family *Bunyaviridae*, cause worldwide epidemics in vegetable crops and ornamentals in North America, Asia, Europe, and Australia (14,15). To date, there are only two distinct tospoviruses that have been serologically identified: the tomato spotted wilt tospovirus (TSWV) and the impatiens necrotic spot virus (INSV) (13). However, other distinct tospoviruses have been reported in watermelon (18) and peanuts (24). In Hawaii, where only TSWV is present, crop losses from severe disease epidemics have reached 60 to 70% in lettuce, tomato, and pepper (8,12).

Tospoviruses have an unusual and specific relationship with their thrips vectors (order: Thysanoptera), the only insect group that can transmit TSWV. The larva is the only stage that can acquire the virus and virus spread is more common by winged adults (26). Thus, suitability of TSWV-infected hosts for thrips development is very critical in disease epidemiology. Plant hosts that are immune to TSWV infection, although they could support thrips development, are less epidemiologically important than plants that are both susceptible to the virus and suitable for thrips development (15,32).

Several species of thrips are known to transmit TSWV. In Hawaii, the western flower thrips, *Frankliniella occidentalis* (Pergande), is the most abundant (33). Its importance as primary vector of tospoviruses has grown worldwide because of its polyphagous feeding habits and widespread geographical distribution (1,7,27,31).

The properties of TSWV and its vector relationships have been extensively reviewed (15,26,32). Sakimura (28) first demonstrated how efficiency of thrips transmission of TSWV is influenced by plant acquisition and inoculation hosts. He showed that onion thrips, *Thrips tabaci* Lindeman, and tobacco thrips, *Frankliniella fusca* (Hinds), transmitted TSWV with greatest efficiency when Flora's paintbrush, *Emilia sonchifolia* L., was used both for acquisition and inoculation. In comparison, virus transmission was reduced by one half when virus acquisition was from *E. sonchifolia* and inoculation was to aster or vice versa. On the other hand, thrips rate of transmission of TSWV from aster to aster was very low or unsuccessful by either *T. tabaci* or *F. fusca*. Recently, the importance of host preference and sustained ingestion of virus-infected plant tissues to thrips acquisition was demonstrated. Ullman et al. (32) found that efficiency of virus acquisition by *F. occidentalis* larvae varies with plant species. These authors showed that the rate of virus acquisition was higher when larvae were fed on *D. stramonium* as compared to *A. lappa*. Likewise, the virus titer detected in the thrips larvae, as well as the proportion of viruliferous thrips adults that subsequently developed, were consistently higher in *D. stramonium* than in the other host plants.

Thrips preference for TSWV-infected plants was first reported by Carter (6). He observed that colonizing onion thrips, *T. tabaci*,

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preferred *E. sonchifolia* infected with yellow spot of pineapple (=TSWV) to noninfected weeds. This observation was subsequently confirmed on romaine lettuce by Yudin et al. (33), who recorded larger numbers of *F. occidentalis* on TSWV-infected than on healthy lettuce plants. Besides changes in the physiology of virus-infected plants such as increased concentration of nitrogenous compounds (free amino acids) in debilitated tissues (20,30), it has been speculated that the yellow hue of chlorotic leaves that progressed 4 to 5 weeks after infection attracted more thrips than did healthy plants (33). *F. occidentalis* preference for diseased plants, however, is neither a rare phenomenon nor unique to thrips-TSWV infected plant system. For instance, aphid preferences for potatoes and sugar beets infected with potato leaf roll virus or yellows virus, respectively, have been documented elsewhere (2,5,22). Moreover, other investigations have focused on the suitability and possible effects of virus-infected plants on the longevity and reproductive potential of colonizing insect vectors (17,19,21,23,25).

We reported earlier the suitability of some TSWV host plants for the development of *F. occidentalis* (4), however, it is not known whether TSWV acquisition and inoculation by *F. occidentalis* from these plants are equally efficient. Moreover, the preference of adult thrips for virus-infected plants has not been previously demonstrated. Therefore, this study was undertaken in the laboratory and greenhouse to compare the efficiency of TSWV acquisition by *F. occidentalis* from several host plants, to demonstrate thrips transmission of TSWV by varying the plant acquisition and inoculation hosts, and to generate information on the feeding and oviposition preferences of *F. occidentalis* for TSWV-infected over noninfected plants.

MATERIALS AND METHODS

Production of thrips and test plants. Stocks of nonviruliferous *F. occidentalis* were taken from a laboratory colony collected originally from Kamilo Iki valley on the island of Oahu. Thrips were reared on pods of green beans, *Phaseolus vulgaris* L. ('Green Crop') and maintained in a controlled growth chamber at a mean temperature of 29 ± 0.1°C. Thrips were multiplied continuously by serial transfer of adults in well-aerated plastic containers.

TABLE 1. Efficiency of tomato spotted wilt tospovirus (TSWV) transmission by *Frankliniella occidentalis* (Pergande) adults in a no-choice test following thrips larval acquisition from three TSWV hosts

Virus acquisition hosts	Inoculation host plants[x, y]	Infected plants (%)[z]
Lactuca sativa var. *longifolia*	*L. sativa* (12)	8.3 bc
	M. parviflora (12)	0 c
	D. stramonium (25)	44.0 a
	A. lappa (16)	37.5 a
	V. enceloides (12)	33.3 a
	Control Plants (18)	0
Datura stramonium	*L. sativa* (12)	0 d
	M. parviflora (12)	16.3 cd
	D. stramonium (21)	76.2 a
	A. lappa (12)	41.7 b
	V. enceloides (12)	58.3 ab
	Control Plants (18)	0
Arctium lappa	*L. sativa* (12)	0 a
	M. parviflora (12)	8.3 a
	D. stramonium (24)	12.5 a
	A. lappa (12)	8.3 a
	V. enceloides (12)	0 a
	Control Plants (18)	0

[x] Values in parenthesis are total number of plants tested.
[y] Control plants within each acquisition host consisted of one plant for each species per replication × three replications.
[z] Within each virus acquisition host, means in a column followed by the same letter are not significantly different at 5% by least significant difference methods (LSD). Values are means of three replications.

Romaine lettuce, *Lactuca sativa* var. *longifolia* Lam.; cheeseweed, *Malva parviflora* L.; jimson weed, *Datura stramonium* L.; golden crown-beard, *Verbesina enceloides* (Cav.) Benth. & Hook. ex Gray; and burdock, *Arctium lappa* L., were used in the tests because they are known hosts of TSWV in Hawaii and are commonly associated with *F. occidentalis* (9,33). Also, weeds are commonly found along borders and within plantings of cultivated lettuce or burdock, a noxious weed elsewhere but grown commercially as a crop in Hawaii. Because of differences in germination time, seeds were sown on staggered dates to produce plants of uniform size and development.

Production of plant virus acquisition hosts. An isolate of TSWV was collected from tomato, *Lycopersicon esculentum* Miller, on the island of Maui and used in all tests. The virus was maintained serially by thrips transmission to *E. sonchifolia*. However, the virus acquisition plants intended for comparison were inoculated mechanically with sap obtained from *E. sonchifolia*. Prior to inoculation using the methods of Cho et al. (9,12), test plants at the four- to five-leaf stage were held for 24 h out of direct sunlight. The newly inoculated plants were immediately rinsed with tap water, then held under reduced light for another 24 h before they were placed on sunlit benches in the greenhouse.

Efficiency of TSWV acquisition by *F. occidentalis* from various host plants. Virus acquisition by *F. occidentalis* was compared among *L. sativa*, *D. stramonium*, and *A. lappa*. *M. parviflora* and *V. enceloides* were not used in these studies. Systemically infected plants were selected at random, uprooted from pots, trimmed or completely removed symptomless foliage, then grouped into a bouquet of five to six plants by wrapping strips of Parafilm M (American National Can Co., Greenwich, CT) around the stems. Each bouquet was placed inside a modified virus-acquisition cage, which consisted of two plastic containers placed on top of one another. The upper container, which served as the acquisition chamber for the thrips, was a cylindrical plastic cup with a lid fitted with a 180-mesh nylon screen for aeration. A piece of plastic tubing (0.5 by 8 cm) was inserted through a hole cut in the bottom center of the cage. Stems of the acquisition bouquet were pushed down through the tube until the roots were completely immersed in the water. With this method, acquisition bouquets remained turgid for 3 to 4 days.

Cohorts of newly hatched larvae were placed on TSWV-infected plants and allowed an acquisition access feeding period of 2 days. Larvae were transferred to another container containing fresh bean pods (*P. vulgaris*) and allowed to develop into adults.

To test for larval acquisition and transstadial passage of the virus to adults, cohorts of second instar larvae and 5- to 6-day-old adults (20 to 80 thrips per sampling × 4 to 13 replications) were taken at random from each virus acquisition host. Thrips were killed by immersion in 70% ethyl alcohol, then stored in an extraction buffer at 4°C until assayed for virus presence (10). Larval and adult thrips from each acquisition host were pooled to estimate the proportion of viruliferous thrips in the cohort. Thrips were assayed within 7 days after sampling. Equal numbers of virus-free thrips of both life stages were likewise sampled from a separate set of noninfected plants for use as negative controls. The remainder of thrips adults that emerged from each virus acquisition host was subsequently used for transmission tests.

TSWV transmission by *F. occidentalis*. Thrips transmission was conducted using no-choice and choice situations. In TSWV transmission to a single host (no-choice), six adult thrips 5- to 6-day postemergence were placed inside a ventilated plastic cup and allowed a 2-day inoculation access period on one test plant. For controls, the same number of nonviruliferous thrips were caged with one plant of each species. Treatment and control plants were kept in a rearing room with fluctuating temperatures of 30 to 32°C and a 12 h photoperiod. The test was repeated three times with subsamples of four plants of each species. Occasion-

ally, more than four plants of each species were exposed to thrips, as in the case of *D. stramonium* (Tables 1 and 2).

In the choice test, five plants of each species at the four- to five-leaf stage were arranged in a completely randomized design inside a cubical cage (60 by 60 by 60 cm). Approximately 300 to 400 viruliferous thrips at age 5- to 6-day postemergence were released in the cage and allowed a 2-day inoculation access period on test plants. All tests were done in the laboratory at 23 to 24°C. Subsequently, plants were removed and sprayed with 0.15 EC Avid (Merck & Co., Inc., Rahway, NJ) at a rate of 0.5/500 ml water to kill the thrips. A second application was made 3 days later to disinfest plants of eclosed and emerging larvae. Inoculated plants were maintained for symptom observation in the greenhouse for 30 days with ambient day and night temperatures of 37 and 22°C, respectively. The tests were repeated three times. Control plants (one plant of each species) were exposed in a separate cage to the same number of nonviruliferous thrips.

Thrips and plants were assayed for TSWV by double antibody sandwich-enzyme-linked immunosorbent assay (DAS-ELISA) (9,10). Reactions were measured photometrically with an ELISA reader model 309 (Biotek Instruments, Winooski, VT) at 405 nm. Samples were considered TSWV-positive if their ELISA values were greater than twice that of the average buffer or healthy plant host control (whichever was higher). The apical leaves of the plant with visible or weakly expressed symptoms or manifested as chlorotic spots were assayed to detect systemic infection. Foliage of asymptomatic plants was likewise assayed to confirm if the plants were virus-negative.

Data analysis. Unless otherwise stated, data were analyzed by a SAS program for general linear model (PROC GLM) for unbalanced design (29). Percentages were transformed to arcsine square root proportion before analysis. Untransformed means were used in the presentation of results.

Thrips response to noninfected and TSWV-infected plants. Differences in thrips response to TSWV-infected and noninfected plants were compared using *L. sativa*, *D. stramonium*, and *A. lappa*. Four potted plants of each species (two healthy and two TSWV-infected) were arranged alternately in a semicircle inside a 45- by 45- by 61-cm cage with a clear glass top. Test plants were spaced apart to insure that leaves of adjacent plants did not touch one another. Cohorts of approximately 200 2-week-old nonviruliferous thrips were released in the cage and allowed to feed and oviposit on the plants for 3 days. Plants were retrieved from the cage, held in a controlled chamber with a mean temperature of 29 ± 0.6°C for larval emergence, and examined under a stereomicroscope to estimate leaf feeding damage and larval numbers. Leaf feeding injury was measured and expressed in square millimeters per plant using a quadrat method (3). Differences in feeding and oviposition preference were compared between TSWV-infected and noninfected plants among and within species using Student's *t* test for paired comparison. Each test was repeated twice for each plant species.

TABLE 2. Efficiency of tomato spotted wilt tospovirus (TSWV) transmission by *Frankliniella occidentalis* (Pergande) adults in a choice test following thrips larval acquisition from three TSWV hosts

Virus acquisition hosts	Inoculation host plants[x, y]	Infected plants (%)[z]
Lactuca sativa var. *longifolia*	*L. sativa* (15)	26.7 bc
	M. parviflora (15)	20.0 c
	D. stramonium (30)	80.0 a
	A. lappa (15)	26.7 bc
	V. enceloides (15)	46.7 b
	Control Plants (18)	0
Datura stramonium	*L. sativa* (15)	20.0 bc
	M. parviflora (15)	6.7 c
	D. stramonium (30)	66.7 a
	A. lappa (15)	53.3 a
	V. enceloides (15)	60.0 a
	Control Plants (18)	0
Arctium lappa	*L. sativa* (15)	0 a
	M. parviflora (15)	6.7 a
	D. stramonium (24)	22.2 a
	A. lappa (15)	20 a
	V. enceloides (15)	6.7 a
	Control Plants (18)	0

[x] Values in parenthesis are total number of plants tested.

[y] Control plants within each acquisition host consisted of one plant for each species per replication × three replications.

[z] Within each virus acquisition host, means in a column followed by the same letter are not significantly different at 5% by least significant difference method (LSD). Values are means of three replications.

RESULTS

Efficiency of TSWV acquisition by *F. occidentalis* from various host plants. The proportion of larvae that tested positive for TSWV was significantly different among the three plant acquisition hosts ($F = 3.58$; df = 2, 19; $P = 0.03$) (Fig. 1). Percent DAS-ELISA positive larvae was highest (81%) on *D. stramonium*, intermediate on *L. sativa* (55%), and lowest on *A. lappa* (36%). These results were consistent with the virus titers measured by DAS-ELISA from larval cohorts that fed from these plant acquisition hosts. The mean absorbance ± standard error of the mean at 405 nm of larvae sampled from *L. sativa* (0.20 ± 0.09, range = 0.05 to 0.45) and *D. stramonium* (0.28 ± 0.05, range = 0.02 to 0.55) were two to three times higher than those obtained for larvae fed on TSWV-infected *A. lappa* (0.10 ± 0.02, range = 0.03 to 0.13).

Transstadial passage of TSWV occurred within cohorts of *F. occidentalis* adults that fed as larvae on the three acquisition hosts. However, the proportion of virus-positive adults was much lower than the proportion of thrips assayed previously as larvae (Fig. 1). No significant differences were detected among the three acquisition hosts ($P = 0.26$). Nonetheless, larval cohorts that were given acquisition access feeding on *L. sativa* and *D. stramonium* produced nearly three to four times more viruliferous adults than those in *A. lappa*. However, except for *L. sativa* in which the mean virus titer was higher in the adults than the larvae, ELISA values for larvae were either comparable to those for adults (in *A. lappa*) or higher (in *D. stramonium*) (Fig. 1).

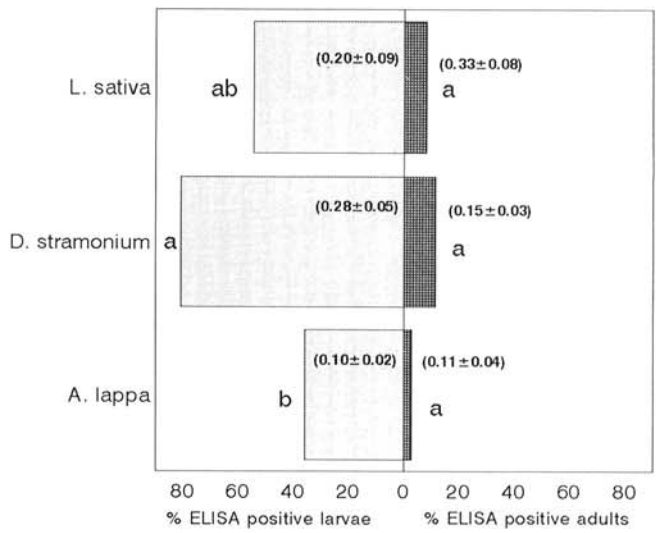

Fig. 1. Proportion of *Frankliniella occidentalis* (Pergande) larvae and adults that tested DAS-ELISA positive for tomato spotted wilt tospovirus following larval acquisition feeding from three TSWV hosts. Bars within each thrips stage topped with the same letter are not significantly different at 5% by Tukey's honestly significant difference method (HSD). Values in parenthesis indicate mean absorbance (A) ± standard error of the mean at 405 nm of thrips larvae or adults assayed as single thrips DAS-ELISA.

TSWV transmission by *F. occidentalis*. The efficiency of TSWV transmission by *F. occidentalis* adults in a no-choice test is shown in Table 1. The incidence of infection varied significantly among plant inoculation hosts within each virus acquisition host ($F = 4.04$; df = 19, 44; $P < 0.0001$). The efficiency of virus transmission was greatest on *D. stramonium,* followed by *A. lappa* or *V. enceloides* when larval acquisition occurred from *L. sativa* or *D. stramonium*. Regardless of the virus acquisition host, *F. occidentalis* was less successful or did not transmit TSWV to *L. sativa* or *M. parviflora*. Furthermore, the transmission rate to all host plants tested was considerably reduced and no significant differences among plant species were found when larval virus acquisition took place from *A. lappa*.

Table 2 summarizes results of thrips transmission in the choice test. TSWV transmission from the same larval acquisition hosts, as assayed in the no-choice test, was successful to essentially all inoculation host plants. The incidence of infection among plants within each acquisition host, however, indicated variability in the transmission of TSWV by *F. occidentalis*. Interestingly, where *L. sativa* was the acquisition host, efficiency of virus transmission to *L. sativa* and *M. parviflora* improved from 8.3 and 0% (no-choice) to 27 and 20% (choice), respectively.

Thrips response to noninfected and TSWV-infected plants. Adults of *F. occidentalis* preferred to feed and oviposit on TSWV-infected plants (Table 3). Data pooled from TSWV-infected plants of the three species showed significantly more leaf feeding damage ($P = 0.03$) and larger larval yield ($P < 0.0001$) than noninfected plants. Within each plant species, symptomatic leaves of TSWV-infected plants with severe chlorotic mottling received more feeding scars and had more larvae than asymptomatic leaves. TSWV-infected plants had 17 to 47% more leaf feeding damage and 15 to 20% more larvae than noninfected plants.

DISCUSSION

Our study provided evidence that the variability of TSWV transmission by *F. occidentalis* was influenced by the plant virus hosts and the thrips feeding preferences. Furthermore, we demonstrated experimentally that adult nonviruliferous thrips had feeding and oviposition preferences for TSWV-infected plants.

We showed that *D. stramonium* was a better acquisition host than *A. lappa*. Likewise, we found *L. sativa* to be a better acquisition host than *A. lappa*, although not as good as *D. stramonium*. Our findings concurred with those of Ullman et al. (32) and Cho et al. (11) and supported their contention that the efficiency of *F. occidentalis* to acquire TSWV is governed by differences in virus host attributes. For example, plant factors such as susceptibility to TSWV and the ability to support high virus titer, as well as the distribution of virions within plant parts, particularly leaves, have been suggested to influence the efficiency of virus acquisition by larvae from plant hosts (15,32). In fact, evidence through serological tests by direct immunoblotting of the leaves revealed that the distribution of virus particles was even in *D. stramonium* leaves, but patchy in *A. lappa*, suggesting a higher probability of virus acquisition from *D. stramonium* than the latter plant species (32).

TABLE 3. Feeding and oviposition preferences of nonviruliferous *Frankliniella occidentalis* (Pergande) adults on TSWV-infected and noninfected plant hosts

Plant species assayed	Criteria used to measure thrips preferences: Leaf feeding scars (mm^2) per plant	Number of larvae per plant
Between treatments (Pooled)		
TSWV-infected	243.3	29.7
Noninfected	93.6	5.4
P-value	0.03	<0.0001
Within species		
TSWV-infected *L. sativa*	460.5	53.5
Noninfected *L. sativa*	201	14
P-value	0.04	<0.001
TSWV-infected *D. stramonium*	208	20.3
Noninfected *D. stramonium*	58.5	1.8
P-value	0.02	0.04
TSWV-infected *A. lappa*	61.5	9.3
Noninfected *A. lappa*	21.3	0.5
P-value	0.03	<0.0001

Although we encountered some difficulty in obtaining TSWV infection in *L. sativa* by sap inoculation (12), the virus titer estimated for *F. occidentalis* adults that fed as larvae on TSWV-infected *L. sativa* was two to three times higher than that for adults fed on *D. stramonium* or *A. lappa*. We reported previously that *L. sativa* was highly preferred and a very suitable host for development of *F. occidentalis* (4). Apparently, a relatively higher mean DAS-ELISA absorbance value obtained with adult thrips could be attributed to a larger amount of virus-infected tissues ingested by the larvae during their development on this host plant.

There was variability in TSWV transmission by *F. occidentalis* with different combinations of virus acquisition and inoculation hosts. Variation in the transmission efficiency of TSWV by thrips from the three plant virus acquisition hosts was readily apparent when percent infection was pooled among the five inoculation hosts. Based on their potential as virus reservoirs for TSWV transmission, the acquisition hosts were ranked in the following descending order: *D. stramonium*, *L. sativa*, and *A. lappa*. Likewise, the highest incidence of infection was recorded for *D. stramonium*, followed by *V. enceloides* and *A. lappa*. Of 154, 81, and 85 total plants assayed for inoculation (observations pooled in the no-choice and choice tests), 43.2, 37.5, and 35.5% of test plants were infected by TSWV, respectively. The lowest TSWV incidence was associated with *L. sativa* (7.9%) and *M. parviflora* (10.8%).

Based on efficiency of virus transmission by thrips, *D. stramonium* was ranked as the best host, both for virus acquisition and inoculation. Hence, the ability of *F. occidentalis* to acquire the virus from *D. stramonium* and transmit it to *L. sativa* and *A. lappa* suggested that this weed species is a potential source of primary infection in these crops. Furthermore, our findings, that thrips adults reared as larvae on TSWV-infected *D. stramonium* readily transmitted TSWV to *V. enceloides* and *D. stramonium*, reinforced the epidemiological importance of *D. stramonium* as a virus reservoir source for noncrop hosts. On the other hand, despite relative suitability of *D. stramonium* for *F. occidentalis* development (4), this weed occurs only in clustered stands during the winter months; hence, we speculated that its overall importance in TSWV epidemiology could only be in early spring.

The ability of *F. occidentalis* to transmit TSWV from *L. sativa* to *L. sativa* supported the previous hypothesis that TSWV spreads in *L. sativa* plantings through secondary infection (8,34). Because *L. sativa* is highly suitable for *F. occidentalis* development (4) and reproduction (R. Bautista and R. Mau, *unpublished*), this would influence thrips population buildup and subsequent spread of TSWV in the crop. In fact, even after the *L. sativa* crop is harvested, as many as one million thrips per 0.4 ha were estimated to emerge from the soil during a 15-day period (8). This indicated that a plowed field may still be an important source of thrips adults for 2 to 3 weeks after harvest. Likewise, virus transmission by *F. occidentalis* from *L. sativa* to *A. lappa* and other weed species suggested that *L. sativa* was an important virus reservoir providing inoculum for the spread of TSWV to crops and noncrops alike.

F. occidentalis that previously acquired the virus as larvae from *A. lappa* transmitted TSWV poorly, or not at all, to the five inoculation hosts assayed. Moreover, even when this host plant was used both for acquisition and inoculation, *F. occidentalis* had only 8 to 20% success in transmitting TSWV. Thus, we considered *A.*

lappa a poor reservoir host for TSWV spread. On the other hand, even if *A. lappa* was a good virus source, its overall impact in the virus-vector-host chain is deemed not as important compared with *L. sativa*, because it is less suitable for development of *F. occidentalis* (4). Although trapping studies of *F. occidentalis* on a commercial farm of *A. lappa* showed substantial emigration of thrips adults (R. Mau, *unpublished*), data were not available to show how much of the migrating thrips population was in fact viruliferous.

We could not demonstrate the efficiency of virus acquisition by *F. occidentalis* from *M. parviflora* and *V. enceloides*. However, when these host plants were used for virus inoculation, a higher incidence of TSWV infection was obtained with *V. enceloides* than with *M. parviflora*. Although both plant species have been reported to be systemic hosts (9), systemic infections were seldom induced in either host in the greenhouse. On *V. enceloides,* 98% of the plants assayed exhibited only local lesions (two to three concentric ring spots) on the leaves, that subsequently faded as plants matured. In the case of *M. parviflora*, no symptoms were readily apparent except for very faint chlorosis on young leaves. More often than not, plants that tested DAS-ELISA positive for the virus were asymptomatic (31). Nonetheless, the unsuitability of *V. enceloides* for *F. occidentalis* development (4) and reproduction (R. Bautista and R. Mau, *unpublished*) further reduces the contribution it may have in perpetuating the disease cycle. On the other hand, despite some difficulty encountered in inoculating *M. parviflora*, we still considered this host plant to be an important TSWV reservoir because of other attributes. Besides a high incidence of TSWV infection in the field (9), *F. occidentalis* had strong predilection for this species at all stages of plant phenology (vegetative and flowering) and the foliage was suitable for *F. occidentalis* development (4).

We demonstrated the efficiency of TSWV transmission by *F. occidentalis* to an array of test plants. Nonetheless, there were at least two inconsistencies that we observed in our results. First, there was a wide overlapping in the separation of treatment means, which could be attributed to a small sample size compared between treatments. For example, 0% TSWV-infected plants did not differ significantly from 8%, which represented one TSWV-infected plant out of 12 plants assayed. Although this mean overlap was not statistically significant, within the context of TSWV disease spread, one TSWV-infected plant becomes epidemiologically important particularly when thrips preference for TSWV-infected plants is taken into account. Secondly, in the no-choice test, 16.7% of recovered thrips adults that tested DAS-ELISA positive for TSWV did not transmit TSWV to the inoculation hosts. Furthermore, the adult thrips (13.3%) that were DAS-ELISA negative successfully transmitted the virus. These observations may be explained as follows. First, although DAS-ELISA is a useful serological technique in determining the presence of virus antigen, it does not distinguish between infective and inactive TSWV virions (16). Secondly, viruliferous thrips that we failed to recover could have escaped or died. Probably the latter event occurred, because of the reported TSWV pathogenicity to virus-infected *F. occidentalis* adults (25).

Although *M. parviflora* and *L. sativa* were relatively difficult to infect, we observed that, when thrips transmission was successful, the incidence of infection in these plant species increased markedly when assayed in a choice test compared with a no-choice test. Such improvement in the efficiency of TSWV transmission could have been a product of strong feeding and oviposition preferences of *F. occidentalis* for these host plants (4). Given access to a mixed cultivar of host plants (choice test), greater numbers of thrips probably landed and fed on the more preferred *L. sativa* and *M. parviflora*, resulting in an increase in the frequency of inoculation and success of TSWV transmission. A similar phenomenon (host plant preference) may be occurring in the field where high incidences of TSWV have been recorded on *L. sativa* and *M. parviflora* (9).

Evidence was provided that TSWV-infected plants were not only attractive to landing thrips, but were preferred for feeding and, more importantly, for oviposition by the females. The significance of this finding could impact the abundance of the thrips vectors in the field. The preference of dispersing thrips for TSWV-infected plants could effect explosive increases of vector populations when environmental conditions are optimal for thrips reproduction. Thus, where host plants susceptible to TSWV and suitable for *F. occidentalis* development are available, the biased behavior of nonviruliferous thrips for TSWV-infected plants could ensure perpetuation of the TSWV life cycle during periods of low disease incidence.

We realize the difficulty of managing the disease induced by TSWV in Hawaii because of a wide overlapping host range of the virus and thrips vectors. Moreover, the semitropical condition throughout the Hawaii farmlands favor multiplication and buildup of *F. occidentalis* populations all year round. Nonetheless, the information provided in this study could serve as a basis for more concrete strategies and recommendations aimed at mitigating the impact of TSWV.

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