Distinct Levels of Specificity in Thrips Transmission of Tospoviruses

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ABSTRACT

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Various thrips species were tested for their ability to transmit different tospovirus species using a petunia leaf disk assay system. Transmission efficiencies were determined for four species of thrips and four tospovirus species: tomato spotted wilt virus (TSWV), impatiens necrotic spot virus, tomato chlorotic spot virus (TCSV), and groundnut ringspot virus (GRSV). *Frankliniella occidentalis* appeared to be the most efficient vector for the four tospovirus species tested. A dark form of *F. schultzei* transmitted three (TSWV, TCSV, and GRSV) of the four tospoviruses, whereas a light form of this species transmitted TSWV and TCSV rather poorly. *F. intonsa*, which has been documented as vector of TSWV, although transmission data were not presented, transmitted TSWV efficiently and TCSV at a very low frequency. Strikingly, only one of four populations of *Thrips tabaci* from different geographic regions was able to transmit one of the tospoviruses tested (TSWV) and this at a low efficiency. Enzyme-linked immunosorbent assay (ELISA) showed that virus could be readily detected in transmitting adult thrips. Viral antigen also could be detected in some individuals that did not transmit virus to petunia leaf disks, but the amount of virus detected was consistently lower than those of transmitters. Positive ELISA values were found only for thrips-tospovirus combinations in which virus transmission could occur, whereas negative ELISA scores were observed for all individuals from thrips-virus combinations in which no virus transmission took place, indicating that acquisition of the virus did not result in replication and accumulation of these viruses in thrips.

The genus *Tospovirus* within the family *Bunyaviridae* encompasses a group of viruses that cause devastating diseases of many economically important crops worldwide (12,26). These viruses are exclusively transmitted by thrips (Thysanoptera:Thripidae). Thus far, seven thrips species have been reported as vectors of the tospoviruses. They are *Frankliniella occidentalis* Pergande (11), *F. fusca* (33), *F. schultzei* Trybom (35), *Thrips tabaci* Lindeman (27), *T. setosus* (15), *T. palmi* (43), and *Scirtothrips dorsalis* (3). Tospoviruses, after ingestion by larvae, are transmitted by second larval instars and adults after circulation and replication in the vector (37,40,41).

Based on serological properties and nucleotide sequence data, at least five tospovirus species have been distinguished. The established species include tomato spotted wilt virus (TSWV), impatiens necrotic spot virus (INSV) (5,17), tomato chlorotic spot virus (TCSV), groundnut ringspot virus (GRSV) (6), and watermelon silver mottle virus (42). The S RNA of the latter shares an almost identical nucleotide sequence with the S RNA of an isolate referred to as Tospo-to (1). Tospo-to is serologically related to groundnut bud necrosis virus, a virus that severely affects groundnut cultures in India and Southeast Asia (28). The taxonomic status of other reported isolates, such as peanut yellow spot virus (29) and a virus from *Verbesina alternifolia* (13) have not been established yet.

Diseases of varying magnitude have been recognized in different geographic regions and are believed to be caused by different tospoviruses and spread by different thrips species. *T. tabaci* is the main vector of TSWV in tobacco in eastern Europe but does not play a principal role in the epidemiology of TSWV in other areas (12). The spread of INSV appears to be highly correlated with the worldwide expansion and occurrence of *F. occidentalis*. These and other observed correlations suggest the existence of specific and favored relationships between certain thrips and virus species.

To understand these relationships more thoroughly and to describe the epidemiology and spread of the tospoviruses quantitatively, factors such as vector competence and transmission efficiency have to be known in more detail. Vector competence, which lies at the base of the question of whether a species can transmit, has been the subject of some limited studies (10,25,32). This paper reports the results of a study on the competence of several populations of thrips species to transmit four tospoviruses and the efficiencies by which these viruses are transmitted.

MATERIALS AND METHODS

Thrips. Virus-free *F. occidentalis, F. schultzei,* and *F. intonsa* Trybom were reared on bean pods (*Phaseolus vulgaris* L. 'Prelude'). The *F. occidentalis* colony was begun with adults collected from a greenhouse infestation in the Netherlands during 1990. A dark form of *F. schultzei* was obtained from a nursery that imported Cactaceae from Brazil (39). A light form of *F. schultzei* was collected from beans imported from Northern Africa. The *F. intonsa* culture originated in Japan (21). Four *T. tabaci* populations were included in the study; two of which were collected in the Netherlands from natural field populations and consisted of females only. A third, thelotokous, culture originated in Japan. These three cultures were reared on leek. A fourth, arrhenotokous, *T. tabaci* population also was obtained from Japan and produced males and females (21). This culture was reared on bean pods. All thrips were reared at 27 ± 0.5°C with a 16-h photoperiod (16 h of light/ 8 h of dark).

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Virus isolates. The TSWV isolate BR-01 and TCSV isolate BR-03 from tomato in Brazil, the GRSV isolate SA-05 from groundnut in South Africa, and the INSV isolate NL-07 from an *Impatiens* sp. in the Netherlands, described previously by De Ávila et al. (6,7), were used in the current study.

Virus detection by enzyme-linked immunosorbent assay (ELISA). The antigen titer in leaf extracts from plants used as virus sources for acquisition was determined by ELISA. This assay also was used to confirm the infection of petunia leaf disks showing local lesions after inoculation. The extracts were prepared by grinding leaf tissue at a ratio of 15 mg per ml of PBS-T (0.14 M NaCl, 1 mM KH_2PO_4, 8 mM Na_2HPO_4, 2.5 mM KCl, and 0.05% Tween 20). Leaf disks from healthy plants were used as controls.

A panel of two polyclonal and two monoclonal antisera was used to differentiate the four virus species. Polyclonal antisera, raised against the nucleocapsid (N) protein (anti-N-serum) of INSV isolate NL-07 and TCSV isolate BR-03, were used in a double-antibody sandwich-ELISA format as described previously (8,14). Monoclonal antibodies N1 and N2, prepared against the N protein of TSWV isolate BR-01 (formerly CNPH1) (14), were used in a triple-antibody sandwich-ELISA as described by De Ávila et al. (8). The discriminative reactions of the four tospovirus species are summarized in Table 1.

Individual thrips were analyzed by cocktail-ELISA, with amplification of the enzyme reaction, for their N protein content as described previously (41), with one modification: individual thrips were ground in 80 µl of sample buffer (2% polyvinylpyrrolidone [M_r is approximately 44,000] and 0.2% ovalbumin in PBS-T) and mixed with 20 µl of 2.5 µg of anti-N-conjugate per ml in sample buffer. The suspension was incubated overnight at 4°C. Anti-N-sera of TSWV isolate BR-01, INSV isolate NL-07, TCSV isolate BR-03, and GRSV isolate SA-05 were used to detect the N protein of the respective viruses in thrips. Absorbance values were read on a Titertek Multiskan colorimeter (Flow Laboratories, McLean, VA) at 492 nm. Absorbance values were corrected for blank values read for wells that contained only sample buffer in the sample incubation step.

Selection of plant species used in virus acquisition studies. To quantify and compare transmission efficiencies of all tospoviruses tested, it is desirable to use the same host plant for acquisition feeding by thrips. Source plants should both display high virus titers and be suitable for thrips feeding. Host plants that react with systemic symptoms when infected with a virus and that display high virus titers for the four tospoviruses tested are *Nicotiana benthamiana* Domin., *N. clevelandii* A. Gray, *Emilia sonchifolia* (L.) DC. ex Wight, and *Impatiens* sp. (7). The suitability of these plant species to support thrips development was tested by monitoring the performance of thrips. Larvae of *F. occidentalis*, *F. schultzei* (dark and light form), *F. intonsa*, and *T. tabaci* (four populations), 0- to 12-h old, were confined to healthy leaves of these host plants. Their survival and developmental stage were checked every 24 h. *Datura stramonium* L. was included as a control since this species is an adequate host for thrips feeding and development.

TABLE 1. Reaction of tospovirus species with a panel of antisera consisting of two monoclonal antibodies directed to the nucleocapsid protein of tomato spotted wilt virus (TSWV) (N1 and N2) and two polyclonal antisera directed to impatiens necrotic spot virus (INSV) and tomato chlorotic spot virus (TCSV) nucleocapsids

	Tospovirus[a]			
Antisera	TSWV	INSV	GRSV	TCSV
TSWV-N1	+	–	+	–
TSWV-N2	+	–	–	–
INSV	–	+	–	–
TCSV	±	–	±	+

[a] –: no reaction (0 to 0.05), ±: weak to moderate reaction (0.05 to 0.5) and +: strong reaction (0.5 to 3.00) in enzyme-linked immunosorbent assay.

Selection of plant species to be used in transmission experiments. To compare the suitability of different host plants in virus transmission tests, systemically infected leaves of *D. stramonium*, *Impatiens* sp., and *N. benthamiana* were used for the acquisition of TSWV, and *Impatiens* sp. and *N. benthamiana* were used for acquisition of INSV. *N. benthamiana* was included as a control for both viruses, because it displays high virus titers after inoculation by TSWV and INSV, and the transmission efficiencies are high when *F. occidentalis* acquires virus from this host. *D. stramonium* and *N. benthamiana* plants were mechanically inoculated 2 to 3 weeks and *Impatiens* sp. 4 to 6 weeks prior to the experiments. The plants were kept in a greenhouse at approximately 22°C with a 16-h photoperiod (16 h of light/8 h of dark). Systemically infected leaves that showed high virus titers in a dilution series in ELISA were used for acquisition feeding.

F. occidentalis larvae, 0- to 12-h old, were given an acquisition access period (AAP) of 24 h on TSWV- or INSV-infected *D. stramonium*, *Impatiens* sp., and *N. benthamiana* leaves in cages (36). Larvae confined to healthy leaves served as controls. After the AAP, larvae were transferred to healthy *D. stramonium* leaves to complete their development. Adults, 1 day after emergence, were transferred to fresh leaf disks (13 mm diameter) of *Petunia* × *hybrida* Hort. Vilm.-Andr. 'Blue Magic' in a 1.5-ml Eppendorf tube to test their infectivity in three successive inoculation access periods (IAPs) of 48 h. All experiments with thrips were performed at 25 ± 0.5°C with a 16-h photoperiod (16 h of light/8 h of dark). After each IAP, the leaf disks were incubated at 27°C in 24-well plates (Costar Europe, Ltd., Badhoevedorp, the Netherlands) while floating on water to develop local lesions as described previously (40).

Comparison of acquisition by larvae of different thrips. A prerequisite for successful virus transmission is the availability of virus that can be acquired by thrips larvae. In addition, because feeding preference or behavior of different thrips species may vary, it was verified whether larvae of all the species tested were able to ingest a virus from infected plant tissue. Virus uptake by larvae of *F. occidentalis, F. schultzei* (light and dark forms), *F. intonsa*, and *T. tabaci* (four populations) was recorded by establishing the virus titers in larvae after they had access to virus-infected leaves. Larvae (0- to 12-h old) were given an AAP of 12 h on TSWV-infected *D. stramonium* and *Impatiens* sp. leaves in cages and were collected directly after the AAP, and stored at –70°C for later testing in ELISA. An average of 20 larvae was tested for each thrips species. Larvae confined to healthy *D. stramonium* leaves served as controls.

Testing of transmission frequencies. Virus isolates were maintained in stock plants of *D. stramonium* (TSWV, TCSV, and GRSV) and *Impatiens* sp. (INSV) by thrips transmission using *F. occidentalis* as a vector. Prior to transmission tests, each isolate was mechanically inoculated from the stock plants onto either *D. stramonium* (TSWV, TCSV, and GRSV) or *Impatiens* sp. (INSV) as described previously. Systemically infected leaves with comparable high virus titers in a dilution series in ELISA were used for acquisition feeding. First instars (0- to 12-h old) were given an AAP of 72 h. First instars caged on virus-free leaves were used as controls. After the AAP, larvae were transferred to healthy *D. stramonium* leaves to complete their development. Adults, 1 day after emergence, were tested individually on petunia leaf disks as described previously. Each virus-vector combination was tested three times, with a minimum of 30 adults per repetition. The numbers of infected leaf disks were converted to transmission rates. Adult thrips, after the IAPs on petunia, were collected and stored at –70°C to be tested in ELISA to quantitatively compare virus transmission and virus content of adults. ELISA values for individual thrips were categorized in two classes based on their ELISA readings. Thrips that gave readings higher than average

the readings from healthy control thrips + 3 times standard deviation were considered positive; those with lower readings were negative.

RESULTS

Selection of plant species for virus acquisition. Distinct tospoviruses differ in symptom expression and host range, and each thrips species shows different feeding preferences. Therefore, preliminary experiments were conducted to find an adequate host plant on which the thrips could acquire the viruses to be tested. Healthy plants of *N. benthamiana*, *N. clevelandii*, *E. sonchifolia*, *Impatiens* sp., and *D. stramonium* were tested for suitability to support thrips development. All larvae of *F. occidentalis* and the dark form of *F. schultzei* survived on *N. benthamiana* until the adult stage. The mortality in the juvenile stages of the other thrips species reached levels of 90 to 100% on this plant species. Similar results were obtained when thrips were kept on *N. clevelandii* and *E. sonchifolia*. *Impatiens* sp. and *D. stramonium* were the only hosts on which no significant mortality of larvae from all the thrips species was observed. These species were chosen as hosts for the acquisition of tospoviruses by thrips.

Impatiens sp. is susceptible to all four virus species, but TCSV and GRSV do not spread uniformly throughout the plant. *D. stramonium* is infected systemically by TSWV, TCSV, and GRSV but not by INSV. Based on these findings, *Impatiens* sp. was chosen as the acquisition host for INSV; in this species, INSV is evenly distributed throughout the plant. *D. stramonium* was selected for the acquisition of TSWV, TCSV, and GRSV because these three viruses display high virus titers, as can be concluded from dilution end-points in ELISA and the development of nonnecrotic symptoms in this plant species.

Comparison of different host plants in transmission experiments. The performance of *D. stramonium*, *Impatiens* sp., and *N. benthamiana* as hosts for virus acquisition by *F. occidentalis* was studied in a preliminary transmission experiment. TSWV was acquired from *D. stramonium*, *Impatiens* sp., and *N. benthamiana*; INSV was acquired from the latter two species. To compare the amount of viral antigen present in leaves for acquisition feeding, dilution series of plant tissue in PBS-T were analyzed by ELISA (Fig. 1). At lower dilutions, ELISA values for TSWV in *N. benthamiana* and *D. stramonium* were higher than in *Impatiens* sp., but virus titers were comparable, as can be concluded from dilution end-points that were very similar at around 3 log. INSV titers in *N. benthamiana* and *Impatiens* sp. were comparable; dilution end-points were approximately 3.5 log.

F. occidentalis larvae were confined for 24 h on infected leaves, and subsequently, adults were tested for virus transmission to petunia leaf disks (Table 2). Transmission efficiencies ranged from 30.4 to 35.0% for TSWV and from 84.0 to 91.7% for INSV, demonstrating that *D. stramonium*, *Impatiens* sp., and *N. benthamiana* were equally suited for virus acquisition by *F. occidentalis*.

Acquisition of viral antigen by larvae of different thrips. The quantity of virus available for acquisition by thrips larvae was measured by allowing larvae an AAP of 12 h on leaves of *D. stramonium* and *Impatiens* sp. The amount of viral antigen present in larvae directly after the AAP was monitored by ELISA. The average ELISA values and standard deviations calculated for each thrips species that had fed on *Impatiens* sp. or *D. stramonium* are presented in Figure 2. The results show that 1-day-old larvae of all thrips species acquired viral antigen from both *Impatiens* sp. and *D. stramonium*. The amount of virus ingested per individual, however, varied dramatically, as indicated by the high standard deiations.

Testing of transmission frequencies. Larvae (0- to 12-h old) that were confined for an AAP of 72 h on infected leaves in cages were tested in the adult stage on petunia leaf disks for the efficiency with which the virus was transmitted (Table 3). The percentage of viruliferous thrips for each thrips-virus combination was calculated after three IAPs of 48 h. *F. occidentalis* was the only species that could transmit all four virus species. TSWV and INSV were transmitted very efficiently to petunia leaf disks, reaching efficiencies of 66.0 and 85.4%, respectively. TCSV and GRSV

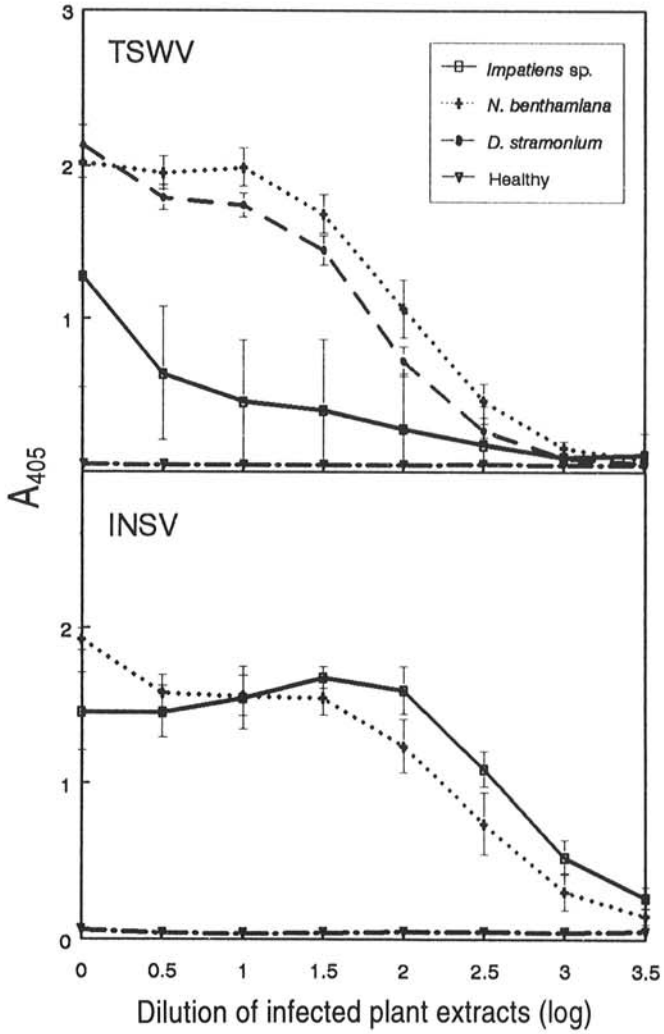

Fig. 1. Average enzyme-linked immunosorbent assay (ELISA) readings plus standard deviations of extracts from plant species used for acquisition feeding by *Frankliniella occidentalis*. Plants were infected with either tomato spotted wilt virus (TSWV) or impatiens necrotic spot virus (INSV). Polyclonal antisera against the nucleocapsid proteins of TSWV and INSV were used to detect the respective viruses. Leaf tissue of healthy *Impatiens* sp., *Datura stramonium*, and *Nicotiana benthamiana* (healthy) served as controls. Leaf tissue was ground in a ratio of 15 mg per ml of PBS-T (initial dilution; 0.14 M NaCl, 1 mM KH_2PO_4, 8 mM Na_2HPO_4, 2.5 mM KCl, and 0.05% Tween 20). The mean ELISA values and standard deviations are indicated with vertical bars.

TABLE 2. Comparison of transmission efficiency of tomato spotted wilt virus (TSWV) and impatiens necrotic spot virus (INSV) by *Frankliniella occidentalis* using different plant species as acquisition hosts[a]

Tospovirus	Acquisition host	n[b]	Transmission (%)
TSWV	*Impatiens* sp.	20	35.0
	Datura stramonium	24	33.3
	Nicotiana benthamiana	23	30.4
INSV	*Impatiens* sp.	25	84.0
	N. benthamiana	24	91.7

[a] Acquisition access period was 24 h on systemically infected leaves (Fig. 1). Transmission by adults was tested on leaf disks of petunia.

[b] Number of thrips tested per combination in one experiment. Thrips that were confined to noninfected leaves of *Impatiens* sp., *D. stramonium*, and *N. benthamiana* as larvae did not transmit virus.

were transmitted less efficiently by *F. occidentalis*, in ratios of 27.6 and 10.2%, respectively. The dark form of *F. schultzei* could transmit three (TSWV, TCSV, and GRSV) of the four tospoviruses. This vector transmitted TCSV at the highest rate (37.5%), whereas GRSV and TSWV transmission was lower (15.7 and 13.7%, respectively). The light form of *F. schultzei* appeared to be a rather inefficient transmitter of TSWV (2.3%) and TCSV (5.9%), whereas transmission experiments with GRSV and INSV were negative. *F. intonsa* efficiently transmitted TSWV (31.8%), whereas TCSV transmission was poor (0.7%). For *T. tabaci*, only the arrhenotokous form of *T. tabaci* could transmit TSWV (9.8%), whereas the three thelotokous populations of this species were unable to transmit the four tospoviruses.

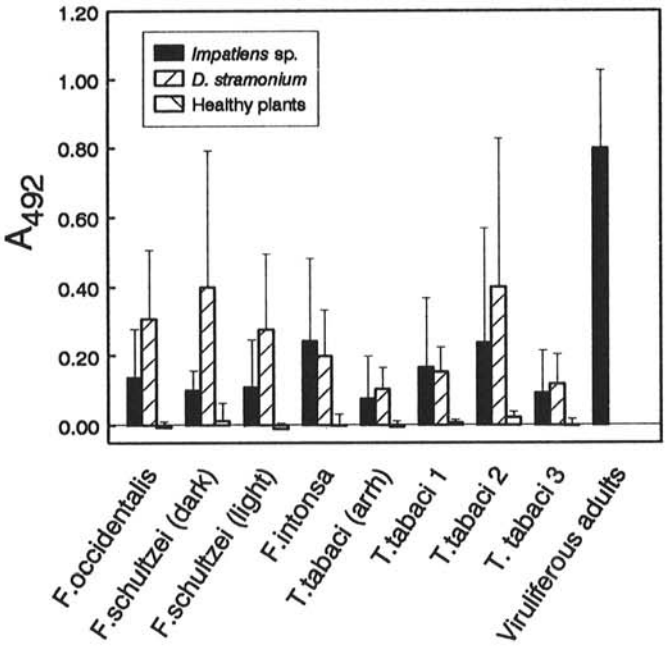

Fig. 2. Acquisition of tomato spotted wilt virus (TSWV) by larvae of different thrips species after an acquisition access period (AAP) on leaves of infected *Impatiens* sp. or *Datura stramonium*. Larvae of each species also were confined to healthy leaves of *D. stramonium* (healthy plants). *Thrips tabaci* (arrh) represents an arrhenotokous population, whereas *T. tabaci* 1, 2, and 3 represent three thelotokous populations: one thrips culture from Japan (1) and two from the Netherlands (2 and 3). Larvae were sampled 12 h after the beginning of the AAP. The mean enzyme-linked immunosorbent assay values and standard deviations (vertical bars) obtained for larvae that were tested singly are given.

Antigen titer in viruliferous and nonviruliferous thrips. The adults tested on petunia leaf disks for virus transmission were individually ground in sample buffer to assay the amount of antigen by ELISA. Results of virus transmission to leaf disks were correlated to readings in ELISA (Fig. 3). Thrips could be categorized in three classes on the basis of transmission and ELISA readings. Thrips that did not transmit virus to petunia leaf disks were divided into two classes based on their ELISA readings. The first class of thrips did not transmit virus, and ELISA values were comparable to those found for healthy thrips (petunia negative; ELISA negative). In the second class, viral antigen was detected in individuals that did not transmit virus to petunia after they were given access to virus-infected leaf tissue during the larval stages (petunia negative; ELISA positive), indicating that virus replication occurred, but virus titers were lower than those of transmitting thrips. The values below the bars in Figure 3 represent the percentage of nontransmitter thrips that scored positive in ELISA. The third class consisted of thrips that could transmit virus to petunia leaf disks, and viral antigen could be detected readily by ELISA (petunia positive; ELISA positive).

The experiments with four virus species and six thrips cultures resulted in 24 possible combinations of tospovirus-thrips species. In each of the 24 combinations in which virus transmission occurred, ELISA values for thrips were either positive or negative, whereas in combinations in which no virus transmission took place, ELISA readings for individual adults were always negative, which shows that acquisition of virus from infected plants by these species did not result in replication and accumulation of virus in thrips.

DISCUSSION

For a reliable comparison of the transmission efficiency of different tospovirus isolates, experiments have to be performed using a plant species that will react systemically upon infection with all virus isolates and support development and feeding of all thrips species tested. No single plant species was found that could meet all the requirements; therefore, we chose *D. stramonium* as a host from which the thrips could acquire TSWV, TCSV, and GRSV, and *Impatiens* sp. was used for the acquisition of INSV.

We found that *F. occidentalis* was the only species able to transmit the four tospoviruses included in this study. Efficient transmission of TSWV by *F. occidentalis* has been reported previously (2,25,31,40). *F. occidentalis* is the only thrips species that has been tested so far for INSV transmission (4,40). The current results demonstrate that the other thrips species tested are unable to transmit INSV and apparently are unable to acquire or replicate

TABLE 3. Efficiency of tospovirus (tomato spotted wilt virus [TSWV], tomato chlorotic spot virus [TCSV], groundnut ringspot virus [GRSV], and impatiens necrotic spot virus [INSV]) transmission by several thrips species[a]

Thrips species	Tospovirus species[b]			
	TSWV	TCSV	GRSV	INSV
Frankliniella occidentalis[c]	66.0 ± 0.8 (140)	27.6 ± 2.7 (109)	10.2 ± 5.8 (99)	85.4 ± 5.8 (83)
F. schultzei (dark)[d]	13.7 ± 2.3 (168)	37.5 ± 0.4 (157)	15.7 ± 2.0 (174)	0 (179)
F. schultzei (light)[e]	2.3 ± 1.3 (161)	5.9 ± 4.8 (123)	0 (95)	0 (>200)
F. intonsa	31.8 ± 8.1 (103)	0.7 ± 0.5 (130)	0 (115)	0 (>200)
Thrips tabaci (arrhenotokous)[f]	9.8 ± 3.3 (178)	0 (132)	0 (117)	0 (119)
T. tabaci (thelotokous)[g]	0 (>200)	0 (>200)	0 (>200)	0 (>200)

[a] Acquisition access period was 72 h on systemically infected *D. stramonium* (TSWV, TCSV, and GRSV) and *Impatiens* sp. (INSV) leaves. Adult thrips were tested individually on leaf disks of petunia for virus transmission.

[b] Transmission efficiency represents the mean percentage ± standard error of infected petunia leaf disks for three experiments. The number of insects tested per combination is indicated in parentheses; sum of three replications. Thrips that were confined to noninfected leaves of *Impatiens* sp. and *D. stramonium* as larvae did not transmit virus.

[c] The combination of TSWV isolate BR-01 and *F. occidentalis* was tested four times.

[d] A dark form of *F. schultzei*, consisting of males and females.

[e] A light form of *F. schultzei*, consisting of females only.

[f] *T. tabaci* colony producing males and females (arrhenotoky).

[g] *T. tabaci* colony producing females only (thelytoky); results of three populations consisting of females only.

virus, as can be concluded from the absence of detectable amounts of virus in adults using ELISA. The high efficiency of INSV transmission by *F. occidentalis* explains the rapid spread of INSV during the early 1990s in greenhouses in North America and Europe and in the open in more subtropical climates where this thrips species is presently the predominant tospovirus vector.

So far, the spread of TCSV and GRSV seems to be restricted to the (sub)tropics, because they have been found only in Brazil, South Africa (8), and Argentina (9). The natural thrips vectors for TCSV and GRSV are unknown. Our results show that *F. occidentalis* is a vector for these two tospoviruses, but transmission by the dark form of *F. schultzei* is the most efficient. TCSV, as well as some GRSV isolates, originates in Brazil, where these viruses frequently are found in tomato crops (22). *F. schultzei* is a common pest in the tropics (39) and may play an important role in the epidemiology of these viruses in Brazil. *F. schultzei* and *F. occidentalis* are both reported in South Africa and may contribute to the epidemiology of GRSV in that region.

The light form of *F. schultzei* appears to be an inefficient vector of TSWV and TCSV (Table 3). This conclusion confirms previous results of Sakimura (34) who could not demonstrate virus transmission by a pale form of *F. schultzei*. Some nontransmitting *F. schultzei* exhibited higher titers in ELISA than healthy thrips (Fig. 3), suggesting that after acquisition some replication of virus may occur, although not resulting in sufficient amounts to achieve virus transmission. Alternatively, the virus may not reach the proper tissues for successful transmission (e.g., the salivary gland).

Several earlier reports indicate that *T. tabaci* has been one of the most important TSWV vectors (19,30,31,32,33). Efficient transmission by this vector also has been reported for some specific cases such as transmission of a tospovirus from dahlia in Japan (10) and TSWV in Finland (18). However, other studies failed to show transmission of this virus by *T. tabaci* (12,20,24,25). One reason for the failure of *T. tabaci* populations to transmit virus may have been that the adults were tested before the latent period (LP) was completed. Sakimura (33) reported a LP of 18 days for *T. tabaci*. In the present experiments, however, individuals of nontransmitting *T. tabaci* populations, which were tested 18 to 20 days after the beginning of the AAP, exhibited ELISA values comparable to those found in healthy thrips, indicating that no virus accumulation or replication had taken place.

Incompatibility between virus isolate and thrips species, each originating in different locations, could be another reason for the observed nontransmissibility; this phenomenon was previously suggested by Paliwal (25). A third possibility is that races or ecotypes of vector species exist that cannot transmit the virus. In Poland, some races of *T. tabaci* were able to transmit TSWV, whereas others did not (44). Failure to transmit the virus in Poland has been correlated with the absence of males in local *T. tabaci* populations. Populations consisting of both males and females apparently did transmit TSWV, whereas populations consisting of females only did not (26,44). Furthermore, it has been claimed that the Mediterranean ecotypes of *T. tabaci* are unable to vector TSWV isolates (16,23). Our results seem to confirm the presence of different types of *T. tabaci* that exhibit different transmission characteristics. Of the four populations tested, only one was able to transmit virus. Strikingly, this population consisted of females and males, whereas the other, nontransmitting populations did not produce males. However, further testing is required to correlate virus transmission with the presence of males in *T. tabaci* populations.

The results in this paper indicate that *F. intonsa* can potentially act as a vector of tospoviruses. This species is restricted to the Northern Hemisphere, from eastern Asia to Europe and in one region of North America. In one report, this species was documented as a vector, but transmission data were not yet available (38). The results presented here definitely indicate that *F. intonsa* can transmit tospoviruses and, thus, may contribute to the natural spread of TSWV. In Europe, *F. intonsa* is a flower thrips, mainly feeding on pollen, and hence may play only a limited role in the epidemiology of TSWV. In Japan, however, *F. intonsa* is a pest on vegetables and ornamentals and may be important as a vector of tospovirus species present in Japan, in addition to *T. setosus*, *T. tabaci*, and *T. palmi* whose status as vectors has been confirmed previously (10,15).

From the results presented here, it is evident that specificity in transmission, which has been found for other plant viruses and

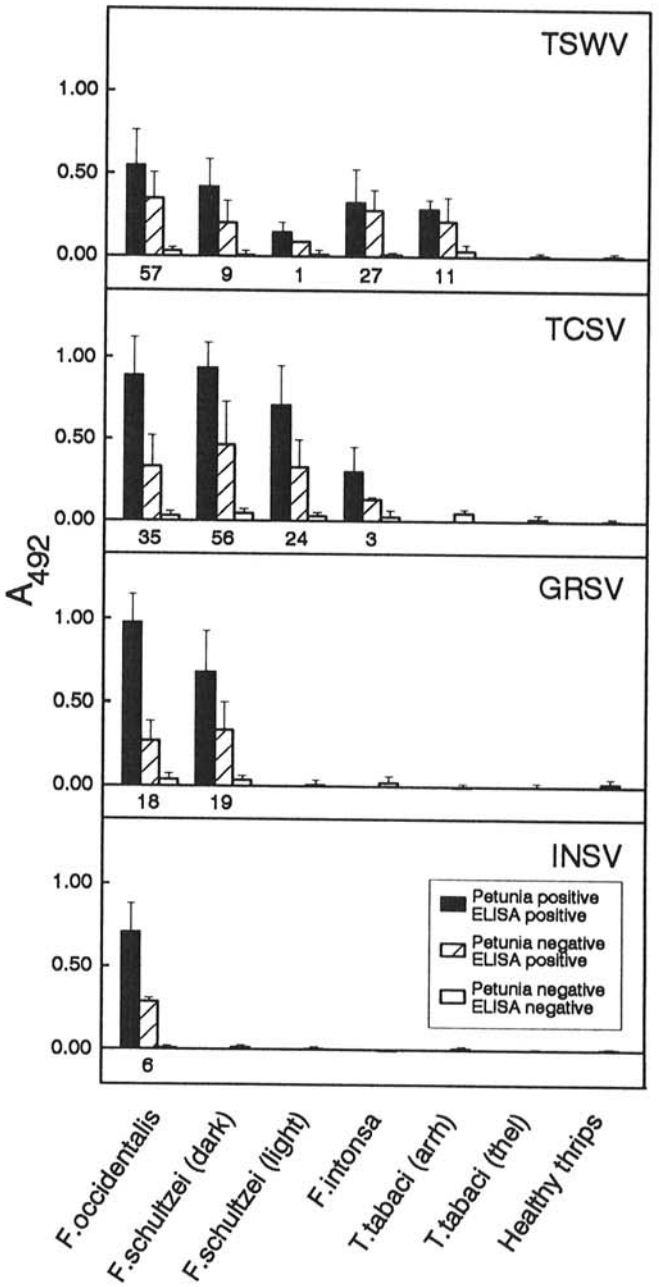

Fig. 3. Average enzyme-linked immunosorbent assay (ELISA) values plus standard deviation for different tospovirus-thrips combinations. Larvae were given an acquisition access period (AAP) of 72 h on virus-infected leaves: tomato spotted wilt virus (TSWV), tomato chlorotic spot virus (TCSV), groundnut ringspot virus (GRSV), and impatiens necrotic spot virus (INSV). Transmission by adults was tested on petunia leaf disks. Individual adults were assayed for antigen content by ELISA. Thrips were categorized in three classes based on their ability to transmit virus to leaf disks of petunia and their readings in ELISA. The minimum threshold values for positive thrips in ELISA consisted of healthy mean + 3 times standard deviation; all readings above this threshold were considered positive in ELISA; readings below this value were considered negative. Values below bars represent the percentage of nontransmitter thrips that scored positive in ELISA. Vertical bars indicate standard deviation.

their vectors, also exists between tospoviruses and their thrips vectors. In different geographic areas, the three components of virus epidemiology—plant species, virus isolate, and virus vector species—therefore, should be well characterized to establish the relative importance of each vector species in a particular area. New variants of tospoviruses usually are characterized rapidly, but information concerning the vector's role in the epidemiology of the virus is usually less clear or even lacking.

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