Accumulation and transmission of TSWV at larval and adult stages in six thrips species: distinct patterns between *Frankliniella* and *Thrips*

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Abstract: To examine whether the specificity of TSWV transmission by thrips is due to differences in the virus accumulation during the developmental stage, the accumulation of TSWV-N protein in thrips at larval and adult stages was compared among six thrips species, *F. occidentalis, F. intonsa, T. tabaci, T. setosus, T. palmi*, and *T. hawaiiensis.* A triple antibodies sandwich enzyme-linked immunosorbent assay (TAS-ELISA) indicated that the titers of virus N protein were almost the same or increased in two species of the genus *Frankliniella* from the second larval to adult stage, but decreased significantly in all species of the genus *Thrips.* The proportion of ELISA-positive thrips decreased from the larval to adult stage in the four species of *Thrips*, but was scarcely less in the two species of *Frankliniella*. The transmission efficiencies of TSWV for the petunia leaf disk by the two species of *Frankliniella* (ca. 30%) were higher than any of the species of *Thrips* (0 to 8.6%) at adult stage of three or four days after emergence. These results suggest that the virus accumulation from the larval to adult stage has a distinct pattern between these genera, and the distinct virus accumulation at adult result in the difference in transmission efficiency.

Introduction

Thrips species may show vector specificity in the transmission of *Tomato spotted wilt virus* (TSWV) (Wijkamp et al. 1995). Specificity might be closely related to the change or multiplication of virus in the vector from larvae to adults, because thrips acquire virus in their larval stages, but individuals subsequently transmit TSWV at second larval and adult stages with circulation and replication of viruses (Sakimura 1962; Ullman et al. 1992; Wijkamp and Peters 1993; van de Wetering et al. 1996). The virus multiplication in thrips is likely to be related to virus accumulation (Wijkamp et al. 1993). Although several researchers showed the change of accumulation for the TSWV-N protein during developmental stages of thrips vectors (Tsuda et al. 1996; van de Wetering et al. 1996; Ohnishi 1998), little information about differences of virus accumulation among thrips species is available. In the present study, the accumulation and transmission of TSWV by second larval and adult thrips were investigated among six thrips species, *F. occidentalis*, *F. intonsa*, *T. tabaci*, *T. setosus*, *T. palmi*, and *T. hawaiiensis*.

Materials and Methods

Thrips: Virus-free thrips were reared on germinated broad beans (*Vicia faba*). Each culture was begun with adults collected from a crop at a location in the west distinct of Japan (Table 1). All laboratory-reared cultures were maintained at 25 ± 0.5ºC with a 16-h light photoperiod.

Virus isolate and plant material: The TSWV isolate used was from sweet pepper in Ibaraki Pref. in Japan (Tsuda et al. 1993). This isolate was maintained by thrips inoculation on *Datura stramonium* plants. The inoculated plants were maintained in an incubator at 22 ± 1ºC with a 16-h light photoperiod. For the transmission assay by thrips, *Petunia* x *hybrida* cv. Polo Blawu was used 40-45 days after sowing, in a growth chamber at 22 ± 1ºC with a 10-h light photoperiod.

Species	Host plant	Location	Year
F. occidentalis	Solanum melongena	Nankoku C. Kohchi Pref.	1998
F. intonsa	Capsicum annuum	Nankoku C. Kohchi Pref.	1998
T. tabaci	Allium cepa	Izumo C. Shimane Pref.	1996
T. setosus	Glycine max	Izumo C. Shimane Pref.	1997
T. palmi	Solanum melongena	San-yo cho T. Okayama Pref.	1994
T. hawaiiensis	Rosa spp.	Kurashiki C. Okayama Pref.	1996

Table 1. The origin of thrips species used in the present study

Virus acquisition by thrips: Newly hatched larvae, 0-4 h old, were placed on systemically TSWV-infected *D. stramonium* detached leaves in the cages (Tashiro 1967) with an acquisition access period (AAP) of 4 h, and then reared on healthy germinated broad beans until adult emergence. Newly hatched larvae (0-4 h old) of thrips caged on virus-free *D. stramonium* leaves were used as controls. All experiments were carried out at 25 ± 0.5ºC with a 16-h light photoperiod.

Virus detection in whole thrips body by ELISA: The 4-day-old larvae or adults 5 to 6 days after emergence were individually examined to detect N protein in a whole body with antibodies against TSWV-N protein by a triple antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA) (Clark and Adams 1977; Roggero et al. 1996). Six healthy thrips as negative control were applied in a plate. Samples that gave ELISA reading values of greater than the mean of healthy control readings plus 3 times the standard deviation were considered as positive. The ELISA values were corrected by subtracting the mean of buffer control absorbance values from sample values.

Examination for TSWV transmission efficiency: The petunia leaf disk assay was used for thrips given AAP (Wijkamp and Peters 1993). Three-day-old larvae or adults 4 to 5 days after emergence were individually given an inoculation access period (IAP) of 24 h on leaf disks (9 mm in diameter) of *Petunia* x *hybrida* cv. Polo Blawu. After the assay, the larvae and adults were stored at – 30C to be tested by ELISA. After the IAP, the leaf disks were floated on water for 48 h in 24-well plates for symptom development. The transmission efficiency of virus was calculated as the percentage of leaf disks that developed local lesions. All experiments were carried out at 25 ± 0.5ºC with a 16-h light photoperiod.

Statistical analysis: A one or two-way analysis of variance (ANOVA) was applied to detect differences in virus accumulation between sources, and the effect of interaction between sources. A Fisher's exact probability test or *G*-test was performed for comparison of frequencies of virus positive individuals in TAS-ELISA or leaf disk assay (Sokal and Rohlf 1995).

Results

Virus accumulation in six thrips species: The accumulation of TSWV-N protein is shown for all samples and for positive individuals only (Table 2). In all tested samples, the accumulation of virus was significant between six species ($F = 40.993$, df = 5, $p < 0.0001$), and between stages ($F = 132.456$, df = 1, $p < 0.0001$), and there was interaction between species and stage ($F = 45.670$, df = 5, $p < 0.0001$). In the positive only samples, the virus accumulation was not significant between five species except for *T. palmi* because of no positive adult in its species ($F = 1.583$, df = 4, $p = 0.1800$), significant between stages ($F = 18.013$, df = 1, $p < 0.0001$), and on the effect of interaction between species and stage ($F = 6.079$, df = 4, $p = 0.0001$).

The differences between stages in all samples were not significant in two species of *Frankliniella* (*F. occidentalis*: $F = 1.747$, df = 1, $p = 0.1893$; *F. intonsa*: $F = 2.578$, df = 1, $p = 0.1116$), although they were significant in four species of *Thrips* (*T. tabaci*: $F = 50.331$, df = 1, $p < 0.0001$; *T. setosus*: $F = 154.817$, df = 1, $p < 0.0001$; *T. palmi*: $F = 17.846$, df = 1, $p < 0.0001$; and *T. hawaiiensis*: $F = 444.519$, df = 1, $p < 0.0001$). This

Species	ELISA A_{405} values[a]			
	All tested samples		Positive individuals only[b]	
	larvae	adults	larvae	adults
F. occidentalis	0.166 ± 0.32 (40)	0.274 ± 0.447 (61)	0.392 ± 0.444 (15)	0.647 ± 0.504 (25)
F. intonsa	0.582 ± 0.561(34)	0.445 ± 0.302 (67)	0.654 ± 0.559 (30)	0.507 ± 0.275 (58)
T. tabaci [c]	0.513 ± 0.336 (18)	0.069 ± 0.115 (35)	0.573 ± 0.305 (16)	0.223 ± 0.141 (9)
T. setosus	0.626 ± 0.313 (7)	0.032 ± 0.086 (71)	0.626 ± 0.313 (7)	0.162 ± 0.156 (12)
T. palmi	0.065 ± 0.105 (47)	0.014 ± 0.014 (80)	0.206 ± 0.146 (11)	0.081 ± 0.033 (3)
T. hawaiiensis	0.844 ± 0.423 (48)	0.014 ± 0.016 (115)	0.917 ± 0.36 (44)	- (0)
control	0.021 ± 0.012 (72)	0.009 ± 0.009 (121)	-	-

[a]Mean ± SD. Numbers in parentheses show sample sizes.
[b]Thrips given ELISA reading values of more than the mean of the healthy control thrips plus 3 times standard deviation.
[c]*T. tabaci* population producing females only (thelytoky).

Table 2. Mean A_{405} values of triple antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA) in the second instar larval and adult stages of different thrips species, using antiserum against the N protein of *Tomato spotted wilt virus*

Species	Stage	Number of positive[a] /all tested samples	Pvalue[b]
F. occidentalis	larva	15/ 40	0.8359
	adult	30/ 34	
F. intonsa	larva	25/ 61	> 0.9999
	adult	58/ 67	
T. tabaci [c]	larva	16/ 18	< 0.0001
	adult	9/ 35	
T. setosus	larva	7/ 7	< 0.0001
	adult	12/ 71	
T. palmi	larva	11/ 47	< 0.0001
	adult	0/ 80	
T. hawaiiensis	larva	44/ 48	< 0.0001
	adult	3/115	

[a]Number of thrips given ELISA reading values of more than the mean of the healthy control thrips 3 times standard deviation.
[b]Fisher' s exact probability test between stages in each species. There was no significant difference between the stages for any test in the genus *Frankliniella*, whereas significant difference in the genus *Thrips.*
[c]*T. tabaci* population producing females only (thelytoky).

Table 3. Frequencies of positive individuals in six thrips species for *Tomato spotted wilt virus* by triple antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA)

trend was also shown in positive samples only except for *T. palmi* (*F. occidentalis*: $F = 2.608$, df = 1, $p = 0.1146$; *F. intonsa*: $F = 2.712$, df = 1, $p = 0.1032$; *T. tabaci*: $F = 10.445$, df = 1, $p = 0.0037$; *T. setosus*: $F = 18.914$, df = 1, $p = 0.0004$; and *T. hawaiiensis*: $F = 15.851$, df = 1, $p = 0.0002$).

Proportions of ELISA positive thrips were compared between larval and adult stages in each species. Distinct results were shown between *Frankliniella* and *Thrips* (Table 3). Differences between stages were not significant in two species of the genus *Frankliniella*, although they were significant in all four species of the genus *Thrips*. Amongst the *Thrips* species, the most drastic change of positive ratios was shown in *T. hawaiiensis*, which is regarded as a non-vector species of TSWV.

Transmission efficiency of TSWV by thrips. All species did not transmit TSWV to petunia leaf disk at the three-day-old larvae. In the adult stage, four species, *F. occidentalis*, *F. intonsa*, *T. tabaci*, and *T. setosus*, transmitted virus, whereas *T. palmi* and *T. hawaiiensis* did not (Table 4). The most efficient transmitters were the two species of *Frankliniella*, and the most

Species	Number of positive[a, b] /all tested samples
F. occidentalis	19/ 61
F. intonsa	22/ 67
T. tabaci [c]	3/ 35
T. setosus	4/ 71
T. palmi	0/ 80
T. hawaiiensis	0/115

[a]Number of leaf disks infected.
[b]A *G*-test was conducted for the four species, *F. occidentalis*, *F. intonsa*, *T. tabaci* and *T. setosus* that transmitted the virus. There was a significant difference among them (G^2=25.723, df=3, p< 0.0001).
[c]*T. tabaci* population producing females only (thelytoky).

Table 4. Transmission efficiency of *Tomato spotted wilt virus* by six adult thrips species to petunia leaf disks

inefficient vectors two species of *Thrips*. The difference of frequencies of transmitter among the vector species was significant (Table 4).

Discussion

In this study, we examined the accumulation and transmission of TSWV by six species of thrips at the second larvae and adults. Serological evidence demonstrated that accumulation of TSWV-N protein was different not only among six species but between second larval and adult stage (Table 2). The differences between stages were not significant in two species of *Frankliniella*, although there were significant difference in four species of *Thrips* in all samples and positive samples. Similarly, frequencies of ELISA positive thrips showed distinct pattern between the genera. These results suggest that there are specificities in the replication of the virus in thrips during their developmental stage. As a result, the transmission efficiency of virus by adult thrips may be different between species. These specificities, moreover, may exist at genus level.

Two species *Frankliniella* have no difference between stages in the virus accumulation and frequencies of ELISA positive, although these levels of *F. intonsa* were higher than those of *F. occidentalis* (Table 2, 3). These results suggest that they might be able to replicate the virus after acquisition from larval stage to adult. In contrast, in four species of *Thrips* significant differences were observed between stages in virus accumulation and frequencies of ELISA positive (Table 2, 3). *T. hawaiiensis* , a non-vector of tospoviruses (Mound 1996; Mumford et al. 1996; Ullman 1996; Ullman et al. 1997), showed the most noticeable difference between stages. Its ELISA-value the second larvae to adults decreased about one-tenth and also its frequency of ELISA-positive decreased dramatically from 91.7% to 2.6%. Therefore, it is possible that most of the virus acquired by this species disappears in adults even if the virus replicates in larvae. We found different TSWV transmission efficiency between genera of vector species; not only were efficiencies not different between species within a genus (Table 4), but they were higher in *Frankliniella* than in *Thrips*. These data between genera corresponded to some extent with the accumulation of virus in the positive adult (Table 2). Hence, higher accumulation in adults might affect virus transmission. However, none of the species were transmitters as second instars. This may be due to insufficient multiplication of the virus in the salivary glands at the 3-day-old larvae. Our data indicated that *F. intonsa* could be an efficient vector of TSWV, as reported by Wijkamp *et al*. (1995). This species is likely to have high ability for TSWV-accumulation, but it is not always an efficient virus vector. Although ELISA-positive of adult *F. intonsa* had high frequency than that of *F. occidentalis*, the transmission efficiency of these species was almost the same at the adult stage, suggesting that *F. occidentalis* is able to transmit virus very effectively.

Thrips tabaci is believed to have been a primary vector of TSWV worldwide for forty years (Sakimura 1962), but the vector status of

this species has become questionable recently at some localities (Mau et al. 1990; German et al. 1992). In the present study, a thelytokous population of *T. tabaci* transmitted TSWV, but at a low rate. Tedeschi et al. (2001) also confirmed transmission of TSWV by a thelytokous *T. tabaci*. However, some thelytokous populations were not able to transmit the virus (Wijkamp et al. 1995) although an arrhenotokous population transmitted (Wijkamp et al. 1995; Chatzivassiliou et al. 1999). This problem has been discussed in several reports (German et al. 1992; Ullman 1996; Ullman et al. 1997), but remains to be studied. While *T. palmi* was regarded as a vector of *Tospovirus* (Fujisawa et al. 1988; Yeh et al. 1992; Lakshmi et al. 1995; Kato et al. 2000), some previous reports considered this species a non-vector of TSWV (Mau et al. 1990; German et al. 1992). We were unable to transmit TSWV with this species. A lower virus titer was observed in *T. palmi* larvae and adults compared with vector species. Newly hatched larvae of *T. hawaiiensis* were not only able to acquire TSWV, but accumulate plenty of virus at the second larval stage. The accumulation in *T. hawaiiensis* larvae was higher than in others, the frequency of ELISA positive larvae reached approximately 90%. These results suggest that larvae of this thrips species are more suitable for TSWV than others. However, virus accumulation and transmission were not observed at the adult stage. These interspecific variations in vector abilities suggest that the accumulation of TSWV from larval to adult stages might be a key factor in the variation in transmission efficiency by vectors, and that the tendency of accumulation might be different between the genera *Frankliniella* and *Thrips*. It is noteworthy that the non-vectors of TSWV, *T. hawaiiensis* and *T. palmi*, had similar character of virus accumulation to vector species in the genus *Thrips*. Since we had only data of two species in the genus *Frankliniella*, virus accumulation for non-vector in this genus should be investigated in the same way.

Several factors may affect the difference in accumulation and transmission of TSWV between *Frankliniella* and *Thrips*. The genera originate from different geographical areas; more than 90% of *Frankliniella* species are from the Neotropics, although *Thrips* is absent from this area (Mound and Kibby 1998). Hence, the genera have possibly encountered the virus separately, resulting in differences in vector competence toward TSWV. Mound (1996) mentioned that vector competence has presumably arisen independently several times, and our results are not necessarily a denial of this possibility. Acquisition, multiplication and movement of virus within thrips involves the digestive system and some barriers for the virus (German et al. 1992; Ullman et al. 1992; Tsuda et al. 1996; Moritz 1997; Ohnishi 1998; Nagata et al. 1999). In particular, it is important for virus to enter the salivary glands for successful transmission (Wijkamp et al. 1993; Tsuda et al. 1996; Ohnishi 1998; Nagata 1999; Nagata et al. 1999). This may be related to the internal morphologiy of thrips, with morphologies varying widely among Thysanoptera (Ullman et al. 1989), including the genera *Frankliniella* and *Thrips*. On the one hand, an experiment using primary cell cultures from thrips embryos suggested some differences in virus multiplication between *F. occidentalis* and *T. tabaci* (Nagata et al. 1997). Nagata (1999) further compared the tissue tropism and virus movement between *F. occidentalis* as a vector and a non-vector thelytokous *T. tabaci*, and found differences in the process of salivary gland infection between two species.

The characters of TSWV-thrips relationship might be variable, because each vector species is highly polyphagous (Mound 1996; 1997) and TSWV has a very wide range of hosts (German et al 1992). A part of TSWV gene is very variable, for instance, variation of its N gene by genome reassortment (Qiu and Moyer 1999). However, in this study, the vector species within a genus have similar characteristics of virus accumulation in the combination of the virus isolate and thrips. The vector competence of thrips, therefore, may differ at genus level. Our results might provide a clue to understand TSWV-thrips interaction. Future researches should be conducted for various isolates of TSWV, and for the mechanisms of virus multiplication at tissue level in these thrips genera.

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