Intraspecific variation in transmission of TSWV by *Frankliniella occidentalis* results from distinct virus accumulation

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Abstract: The relationship between intraspecific variation in transmission of *Tomato spotted wilt virus* (TSWV) by adult *Frankliniella occidentalis* and virus accumulation in their bodies was studied using a petunia leaf disk assay and triple-antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA) against the nucleocapsid (N) protein of TSWV. Two types of intraspecific variation were observed: the efficiency of TSWV transmission varied for nine populations in Japan from 6.1 to 29.2%, and in any population males transmitted the virus with a higher rate than females. The transmitters had ELISA values higher than non-transmitters. However, there was a difference in the thresholds of ELISA values to transmit TSWV between sexes. Almost all the males transmitted the virus with ELISA values higher than 0.5, whereas in this experiment females needed values higher than 1.0. The frequency of individuals with ELISA values over the thresholds agreed with the frequency of transmitters in both sexes. For each population, the two frequencies were almost equal. These results suggest that inter-population and inter-sexual variation in TSWV transmission by adult *F. occidentalis* result from distinct frequencies of individuals that have enough accumulation of TSWV to transmit.

Introduction

The western flower thrips, *Frankliniella occidentalis* (Pergande), is one of the efficient vectors of *Tomato spotted wilt virus* (TSWV) (Wijkamp et al. 1995) that causes serious losses in a wide range of crops and flowers all over the world (Gouldbach and Peters 1994; Peters 1998). But the efficiency is not always stable. Recent studies showed that the efficiencies of TSWV transmission were different among populations (van de Wetering et al. 1999a) and between sexes (Sakurai et al. 1998; van de Wetering et al. 1999b). Information on such intraspecific variations is very important because they influence virus spread based on the vector biology. Also, the detection of intraspecific variations and the investigation of the causes are effective in revealing the interaction between TSWV and its vectors. Nevertheless, the causes of intraspecific variations are almost unknown although sexual difference in transmission is supposed to be due to distinct feeding behavior between male and female (van de Wetering et al. 1998). In the present study, we examined the relationship between intraspecific variations in transmission of TSWV (i.e. inter-population and inter-sexual variations) and virus accumulation in thrips using *F. occidentalis* originating from several populations in Japan.

Materials and Methods

Thrips collection and rearing

Insects used in this study were collected on various crops and flowers in nine populations in Japan from 1993 to 1999 (Table 1) and identified as *F. occidentalis* by an identification guide (Mound and Kibby 1998). Adult thrips originating from the different populations were confined and maintained on tea pollen in a cage, as described by Murai and Ishii (1982), and larvae and pupae were reared on germinated seeds of broad bean, *Vicia faba*, at 25 ± 1 °C with a 16-h-light photoperiod.

Virus and plant materials

The Japanese isolate TSWV-O, originating from green pepper, *Capsicum annuum*, in Ibaraki Pref.

Population	Region	Original host	Collection year	n	Transmission efficiency (%)[1]
Kochi	Southwest	Eggplant	1998	114	20.2
Shimane	West	Gerbera	1995	214	6.1
Hiroshima	West	Chrysanthemum	1997	110	8.2
Shizuoka	Central	Chrysanthemum	1993	117	16.2
Fukushima	Northeast	Eggplant	1998	120	19.2
Miyagi	Northeast	Cucumber	1998	226	29.2
Iwate	Northeast	Eggplant	1999	310	25.2
Akita	Northeast	Chrysanthemum	1999	86	20.9
Aomori	Northeast	Chrysanthemum	1996	267	25.8

[1]The efficiency was significantly different between populations (G2=67.4, P<0.0001).

Table 1. Efficiency of TSWV transmission onto petunia leaf disks by *F. occidentalis* adults originating from nine populations in Japan.

in the central district of the main island of Japan (Tsuda et al., 1993), was used in all experiments. The isolate was inoculated mechanically or by infected thrips onto *Datura stramonium* plants. The infected plants were maintained as TSWV acquisition hosts for thrips in an incubator at 22 ± 1 °C with a 16-h-light photoperiod.

TSWV transmission tests

First instar larvae up to 6 h old were confined with pieces of TSWV-infected leaves in a glass container for an acquisition access period of 24 h. After this period, the larvae were reared on healthy germinated broad bean seeds until adults. The transmission efficiency of TSWV was determined using petunia leaf disks (Wijkamp and Peters, 1993). Three days after emergence, adults were individually tested for their competences to transmit the virus onto leaf disks with an inoculation access period of 24 h. After inoculation, these leaf disks were floated on water for 2 days in 24-well plates for symptom development. The percentage of leaf disks that developed local lesions was used to calculate the transmission efficiency of TSWV. After the leaf disk assay, TAS-ELISA was conducted on each individual adult.

TAS-ELISA

TAS-ELISA with commercially available polyclonal and monoclonal antibodies was conducted to detect N protein of TSWV in individual thrips. After the substrate reaction was allowed to proceed for 30 min at room temperature, absorbance values were determined with an ELISA reader at 405 nm (A_{405}). Samples that gave ELISA values of greater than 3 times the mean of healthy control thrips were considered to be positive. The A_{405} values were corrected by subtracting the mean of the buffer control absorbance values from sample values.

Statistics

Analyses of frequencies of transmission experiments were conducted by a *G*-test. Differences in TSWV-N protein accumulation between sources, and the effect of the interaction between sources were tested by one- or two-way analysis of variance (ANOVA) (Sokal and Rohlf, 1995).

Results

Inter-population variation

Table 1 shows that a significant difference in the efficiency of TSWV transmission was detected among nine populations from Japan. The two western populations, that is Shimane and Hiroshima, transmitted the virus with efficiencies of lower than 10%, whereas the south-western population, that is Kochi, and four of the five north-eastern populations, that is Miyagi, Iwate, Akita and Aomori, transmitted with efficiencies higher than 20%.

Inter-sexual variation

Males transmitted TSWV with higher efficiencies than females (Table 2). In five out of nine populations, that is Shimane, Shizuoka, Miyagi, Iwate and Akita, significant differences in the efficiency of virus transmission were observed between males and females.

N protein accumulation and transmission

To study the relationship between accumulation of N protein of TSWV in thrips and virus transmission by adults, ELISA values for N protein in individual thrips were examined for three populations of Miyagi, Iwate and Aomori whose transmission efficiencies were high. The absorbance values (A_{405}) of adult thrips that acquired TSWV as first instar larvae varied from 0 to 2.02 in this experiment. Effects of the transmission competence, sex and its interaction were all significant for the variation in amount of virus N protein (transmission competence: F=491.3, P<0.0001, sex: F=22.6, P<0.0001, interaction: F=10.86, P<0.01). Transmitters showed higher values than non-transmitters in both sexes, and transmitting females had more virus N protein than transmitting males (Table 3).

The frequency distributions of ELISA values for N protein of TSWV in individual thrips are shown in Table 4. No thrips with values lower than 0.5 transmitted the virus in both sexes except for a female with a value of 0.27. On the other hand, all males and females with values higher than 1.0 were transmitters. In a range of values of 0.5 to 1.0, however, most males (91%) had a competence of TSWV transmission, whereas no females transmitted the virus, suggesting that thresholds of ELISA values to transmit TSWV are different between sexes: 0.5 for males and 1.0 for females.

The percentage of adult thrips with ELISA values over these thresholds was significantly higher for males than for females (G^2=6.55, P<0.05) and almost agreed with that of transmitters in both sexes (Table 5). Compared with four populations of Kochi, Shimane, Hiroshima and Shizuoka, which significantly showed distinct transmission efficiencies (G^2=26.4, P<0.0001), the percentage of thrips with the values over thresholds was also significantly different among the populations (G^2=20.9, P<0.001) and coincided with that of transmitters in each population (Table 6).

Population	Male		Female		G2-value[1]
	n	efficiency (%)	n	efficiency (%)	
Kochi	58	24.1	56	16.1	1.1
Shimane	101	11.9	113	0.9	12.9***
Hiroshima	51	9.8	59	6.8	0.3
Shizuoka	56	25.0	61	8.2	6.2*
Fukushima	37	29.7	83	14.5	3.6
Miyagi	104	37.5	122	22.1	6.4*
Iwate	144	37.5	166	14.5	22.0****
Akita	43	32.6	43	9.3	7.4**
Aomori	133	30.1	134	21.6	2.5

[1]*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

Table 2. Difference of TSWV transmission efficiency onto petunia leaf disks between males and females of *F. occidentalis* originating from nine populations in Japan.

Sex	Non-transmitter		Transmitter	
	n	A_{405}	n	A_{405}
Male	46	0.08 (0.15)	24	1.04 (0.29)
Female	58	0.15 (0.26)	13	1.46 (0.43)

Table 3. Average ELISA values (A405) for N protein of TSWV in individual F. occidentalis males and females in relation to virus transmission. Numbers in parentheses are standard deviation.

ELISA value (A_{405})	Number of males		Number of females	
	Non-transmitter	Transmitter	Non-transmitter	Transmitter
<0.1	37	0	42	0
0.1 to 0.5	8	0	7	1
0.5 to 1.0	1	10	9	0
1.0	0	14	0	12

Table 4. Frequency distributions of ELISA values (A_{405}) for N protein of TSWV in individual *F. occidentalis* males and females in relation to virus transmission. All healthy males (n=12) and females (n=12) used as control had less than 0.1 of ELISA value.

Sex	n	ELISA value over thresholds (%)	Transmitter (%)	G 2-value
Male	70	35.7	34.3	0.03
Female	71	16.9	18.3	0.05

Table 5. Percentages of adults with ELISA values over the thresholds to transmit TSWV (males: 0.5, females: 1.0) and the virus transmitters of *F. occidentalis* males and females.

Population	n	ELISA value over thresholds (%)	Transmitter (%)	G 2-value
Kochi	52	34.6	34.6	0.00
Shimane	54	3.7	1.9	0.31
Hiroshima	52	13.5	9.6	0.38
Shizuoka	51	25.5	23.5	0.05

Table 6. Percentages of adults with ELISA values over the thresholds to transmit TSWV (males: 0.5, females: 1.0) and the virus transmitters of *F. occidentalis* originating from different populations in Japan.

Discussion

TSWV is transmitted by seven thrips species of the genera *Frankliniella* and *Thrips* (Mound 1996; Webb et al. 1998) of which *F. occidentalis* is one of the most efficient vectors of this virus (Wijkamp et al. 1995). Recently, however, variations in transmission competence of TSWV within a species have been shown for *F. occidentalis* (Sakurai et al. 1998; van de Wetering et al. 1998, 1999a,b) and for *Thrips tabaci* (Chatzivassiliou et al. 1999). In the present study, we demonstrate two separate patterns of variation in TSWV transmission by *F. occidentalis* in Japan: inter-population and inter-sexual variation. This supports the view that such intraspecific variation is a general phenomenon, as shown among fourteen populations from eight countries by van de Wetering et al. (1999a), and between males and females by Sakurai et al. (1998) and van de Wetering et al. (1998, 1999b).

In Japan, *F. occidentalis* was first recorded in 1990 (Fukuda et al. 1991; Hayase and Fukuda 1991). From 1993 to 1996, areas infested by *F. occidentalis* immediately increased (Saeki 1998), and this thrips species has become one of the major insect pests of fruit trees (Masui 1998) as well as vegetables and ornamentals (Katayama 1998), and an efficient transmitter of TSWV (Kato and Katayama 1998). Therefore the relationship between TSWV and *F. occidentalis* is not so long in Japan, suggesting that inter-population variation in transmission of TSWV by *F. occidentalis* occurred for a short period in Japan. This is a significant problem for the control of TSWV and *F. occidentalis*, because transmission efficiency in a vector population is likely to affect the spread of TSWV in the field and greenhouses. Interestingly, few TSWV infections have been observed in the western district of main island of Japan (Hanada 1999), to which Shimane and Hiroshima populations with low transmission rates belong, whereas severe economic losses by TSWV have been reported in Kochi, Shizuoka and several areas of the northeastern district of

the main island of Japan (Kato and Katayama 1998) where populations with high transmission efficiencies were observed in this study.

The interaction between TSWV and thrips is unique and complex (Peters et al. 1996; Nault 1997; Ullman et al. 1997). Acquisition, multiplication and circulation of TSWV in its vector, leading to successful virus transmission, are closely related to developmental stages of thrips (Ullman et al. 1992, 1993; Wijkamp et al. 1993; Ohnishi et al. 1996; Tsuda et al. 1996; van de Wetering et al. 1996; Nagata et al. 1999). Therefore several barriers for TSWV should occur during development and determine vector competence. If abilities to pass these barriers are different among thrips individuals, they should result in intraspecific variation in virus transmission. In addition, variation in transmission efficiency within a vector species may be due to differences in ecological factors such as dispersal, feeding and host preferences of thrips. Several factors that affect the transmission efficiency of TSWV have been reported in *F. occidentalis*. Although it is known that only larvae can acquire TSWV (Sakimura 1962; Ullman et al. 1992), virus acquisition ability varies from the first to the second instar larvae between populations from different countries (van de Wetering et al. 1996, 1999a). Transmission by second instar larvae depends on temperature: larvae reared at lower temperatures transmit at a higher efficiency than those reared at higher temperatures. This indicates that development might be relatively faster at higher temperatures than the progress of infectivity (Wijkamp and Peters 1993). The salivary glands of transmitting adults are infected heavier than those of non-transmitters probably by successful multiplication and circulation of virus (Nagata et al. 1999). It is also implied that distinct feeding behaviors between sexes might result in lower transmission efficiency for females (van de Wetering et al. 1998).

In the present study, almost all the larvae should have acquired TSWV, because they were young enough (up to 6 h) at the start of the acquisition access period (van de Wetering et al. 1996), and temperature was constant during experiments. Thus, these factors did not affect the variation observed in our study. ELISA values for N protein of TSWV were higher for transmitters than for non-transmitters in both sexes, whereas the threshold of ELISA values to transmit the virus was higher for female transmitters than for male ones, suggesting that females have to retain more virus to transmit than males. Higher thresholds for females might be due to their larger bodies including the midgut and salivary glands that are viral replication sites (Ullman et al. 1993; Wijkamp et al. 1993). Hence, virus content per unit in saliva might be similar for transmitting males and females, even if females have larger amounts of virus than males. The proportion of individuals with ELISA values over these thresholds, which were different between males and females, agreed with that of transmitters in both sexes respectively. These results show that inter-sexual variation in transmission efficiency of TSWV would be caused by distinct frequencies of males and females with enough virus to transmit, rather than distinct feeding behaviors between sexes as shown by van de Wetering et al. (1998). Similarly, in each population, the proportion of virus transmitters coincided with that of thrips with ELISA values over thresholds. Therefore, we may conclude that both inter-population and inter-sexual variation results from a common factor: variation in the frequency of individuals that have enough accumulation of virus to transmit. Whether a thrips has sufficient virus content or not may be affected by virus multiplication during development (Ullman et al. 1993, Wijkamp et al. 1993). Moreover, as Nagata et al. (1999) showed, successful transmission requires salivary glands to be infected heavily. So, infection of salivary glands is likely to be associated with not only virus circulation to the organ but also virus multiplication in thrips, although the relationship remains to be elucidated.

To carry out the efficient management of TSWV epidemics, we must understand better the biology of its vectors and the interactions between virus and thrips that are likely to affect the virus spread in a population. Inter-population and inter-sexual variation in *F. occidentalis* shown in this study indicate that it is essential for evaluating the spread of virus to investigate the sex ratio and the proportion of transmitting adults in the field or greenhouses in which this thrips species

is observed. In addition, we demonstrated that the intraspecific variation is probably related to the frequency of thrips with enough virus to transmit, which can play an important role to determine the barriers for TSWV multiplication in thrips.

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