Efficiency of north-western Italian thrips populations in transmitting tospoviruses

Tavella L.[1], Tedeschi R.[1], Mason G.[1], Roggero P.[2]
[1] Di.Va.P.R.A. Entomologia e Zoologia applicate all'Ambiente "Carlo Vidano", Università di Torino, Italy;
[2] Istituto di Fitovirologia Applicata, C.N.R. Torino, Italy.
E-mail: tavella@agraria.unito.it

Abstract: Laboratory trials were carried out on *Frankliniella occidentalis* and *Thrips tabaci* populations from north-western Italy to assess their ability in transmitting the tospoviruses *Tomato spotted wilt virus* (TSWV) and *Impatiens necrotic spot virus* (INSV). For both thrips species, two different populations were tested, from Liguria and Piedmont; two virus isolates for TSWV and one isolate for INSV were used. Transmission experiments were made setting newly hatched larvae (≤ 2 hours) to acquire on systemically infected leaves of *Datura stramonium*. For inoculation, the emerged adults were assayed individually on leaf disks of pepper, petunia or tomato. Among the test plants, the highest efficiency was observed with pepper. *F. occidentalis* proved to be the best vector of both TSWV and INSV; particularly the Ligurian population was able to transmit a local isolate of TSWV with an efficiency of 96%. Both populations of *T. tabaci* were thelytokous; they transmitted both TSWV isolates even if with a low efficiency, but failed to transmit INSV.

Key words: *Frankliniella occidentalis*, *Thrips tabaci*, *Tomato spotted wilt virus*, *Impatiens necrotic spot virus*, tospovirus vector.

Introduction

The genus *Tospovirus* (Bunyaviridae) (Elliott *et al.*, 2000) includes some of the most devastating plant viruses, able to cause diseases to many economically important crops throughout the world. These viruses are exclusively transmitted by thrips (Thysanoptera Thripidae) in a persistent propagative manner (Ullman *et al.*, 1997). Transmission modalities, investigated mainly for the type species of the genus, *Tomato spotted wilt virus* (TSWV), are peculiar. Only the larvae can acquire the virus and, after a latent period during which the virions replicate and circulate in the vector, the mature larvae and, above all, the adults can inoculate the virus (Linford, 1932; Ullman *et al.*, 1992; van de Wetering *et al.*, 1996; Wijkamp *et al.*, 1993).

Among the reported vectors of tospoviruses, *Frankliniella occidentalis* is now considered one of the most efficient (Ullman *et al.*, 1997), although the first species recognized to transmit TSWV was *Thrips tabaci* Lindeman (Pittman, 1927; Sakimura, 1962; 1963). In fact, the spread of TSWV epidemics, reported in several countries, can be related to the world-wide dispersal of *F. occidentalis* from North America, its area of origin. In recent years, this species has reached an almost global occurrence with the increased international trade of vegetables and ornamentals, and also thanks to its great polyphagy, adaptability to different environmental conditions, high rate of reproduction and rapidity in developing resistance to insecticides (Tommasini and Maini, 1995).

In Italy, *F. occidentalis* was recorded at first in floral crops, in 1987, and subsequently has become a major pest (Arzone *et al.*, 1989; Tommasini and Maini, 1995). In north-western Italy, TSWV and *Impatiens necrotic spot virus* (INSV) were detected in floral and vegetable crops starting from 1989 (Lisa *et al.*, 1990; Vaira *et al.*, 1993). Previous studies showed that in our regions *F. occidentalis* was the most abundant thrips throughout the cultural cycle, whereas *T. tabaci*, nearly always present, was

usually the first thrips to colonise the cultivated plants (Tavella *et al.*, 1997). Thus, the present research was carried out to verify the vector efficiency of populations of *F. occidentalis* and *T. tabaci* collected in north-western Italy in transmitting different tospovirus isolates.

Materials and methods

Thrips stock colonies. Transmission trials were carried out with four populations, two of *F. occidentalis* and two of *T. tabaci*. Provenance localities and crops are given in Table 1. Adults collected in the field were allowed to oviposit on cucumber leaves in cages (Tashiro, 1967) and, after hatching of the first larvae, were identified using the key of Mound *et al.* (1976). Thrips colonies, starting from these larvae, were reared in gauze-covered glass jars, with corrugated cardboard on the bottom to provide a suitable pupation site. Every two days, fresh green bean pods were introduced into the jars as a food source and oviposition substrate, together with pollen as additional food. Each population was reared for at least 3 months before using it in transmission trials. Moreover, the two populations of *T. tabaci* were periodically observed to check their sex-ratio. The mass rearings were maintained in climatic chambers at 25±1°C, 60±5% r.h. and 16:8 L:D.

Transmission trials. The experiments were performed following Wijkamp *et al.* (1995). Larvae no older than two hours were allowed to acquire the virus on an infected leaf in Tashiro cages for 48 hours; then they were transferred to other cages on healthy cucumber leaves until adult emergence. The transmission ability of the adults was tested in two successive inoculation access periods (IAPs), each of 48 hours; 24 hours after emergence, adults were put individually into plastic tube (1.5 ml capacity) with a virus-free leaf disk (12 mm diameter). At the end of each IAP, each disk was floated on water in 24-well plates for 96 hours; then they were analyzed by TAS (Triple Antibody Sandwich) ELISA to check the presence of virus. TAS ELISAs were carried out using polyclonal and monoclonal antibodies against TSWV and INSV nucleocapsid proteins as reported in Roggero *et al.* (1998). Leaf disks were homogenized with 0.5 ml of PBS-Tween and 2% polyvinylpyrolidone (PVP, Mr ~30,000). Samples having absorbance values at least three times that of healthy controls were considered positive.

Virus isolates, source and test plants. For transmission, the following tospovirus isolates were used: TSWV P105 from pepper in Liguria, Italy; TSWV BR-01 from tomato in Brazil; INSV P125 from pepper in Emilia Romagna, Italy. Plants of *Datura stramonium* were mechanically inoculated separately with the virus isolates. To avoid the generation of defective interfering elements, original virus cultures were kept under liquid nitrogen and transmitted by sap inoculation only a few times. For acquisition, *D. stramonium* leaves with systemic symptoms, generally occurring about 15 days after the mechanical inoculation, were used. To compare different test plants in the IAPs, a transmission experiment was carried out assaying the inoculation capacity of the population WFT-L on leaf disks of three plants: sweet pepper cv "Quadrato di Asti", petunia cv "Blue Magic", and tomato cv "Marmande". In the trials to verify the transmission efficiency of the four NW Italian populations, adults were tested on leaf disks of sweet pepper.

Species	Population	Locality	Crop	Date
F. occidentalis	WFT-L	Albenga (SV), Liguria	pepper	1997
	WFT-P	Venaria (TO), Piedmont	chrysanthemum	1998
T. tabaci	OT-L	Albenga (SV), Liguria	cineraria, cyclamen	1998
	OT-P	Carmagnola (TO), Piedmont	pepper	1999

Table 1. Source of *Frankliniella occidentalis* and *Thrips tabaci* populations tested in the transmission trials.

Statistical analysis. In test plant preference and transmission experiments the data were performed by χ^2 analysis. The efficiency of *F. occidentalis* males and females was compared using a one-way ANOVA analysis after transforming transmission percentages in arcsin square root.

Results

Test plant preference experiments. In the experiments to verify the favourite test plant, *F. occidentalis* fed preferably on pepper and tomato rather than on petunia. The peculiar leaf depigmentation, symptoms due to the nutritional activity of thrips, were more abundant on the two vegetables. By the χ^2 analysis, the transmission rate was significantly higher in pepper than in tomato, and in tomato than in petunia (Fig. 1).

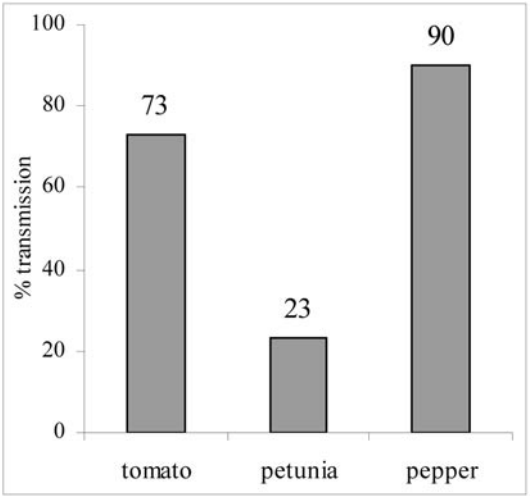

Figure 1. Rate of transmission on different test plants by the population of *Frankliniella occidentalis* coming from Liguria, NW Italy.

Thus, this last plant can be considered the least appreciated by the tested population WFT-L for nutrition and, consequently, also for transmission.

Efficiency in transmitting TSWV and INSV. Results of transmission trials are reported in Table 2. *F. occidentalis* proved to be the more efficient vector species, especially the population WFT-L able to transmit TSWV P105, isolate coming from the same area as the thrips, with an efficiency of 96%. The inoculation rate for *F. occidentalis* was generally higher than 50% except for the transmission of INSV P125 by the population WFT-L. The two populations of *T. tabaci* were thelytokous: no males were observed during both field collection and transmission trials; however, they could transmit the two TSWV isolates even if with a low efficiency, but they failed to transmit INSV. For *T. tabaci* the highest transmission rate of 17% was assessed in the experiments with the population OT-L and the isolate TSWV P105, again both coming from the same area.

F. occidentalis *Transmission pattern*. The transmission behaviour of the two *F. occidentalis* populations during the successive IAPs is shown in Fig. 2. Generally the transmitter thrips infected the leaf disk in both IAPs, although a discontinuity in transmitting tospovirus was recorded above all for the population WFT-L. In the laboratory trials the females transmitted the viruses with efficiency higher than, or at least equal to, males, except for population WFT-L and isolate TSWV BR-01 (Table 3). However, the one-way ANOVA analysis showed no significant differences in efficiency of males and females; thus, the transmission ability of the local populations of *F. occidentalis* did not seem to be influenced by sex.

Species	Population	TSWV P105		TSWV BR-01		INSV P125	
		no. thrips	% transm.	no. thrips	% transm.	no. thrips	% transm.
F. occidentalis	WFT-L	50	96 a	55	51 b	54	41 b
	WFT-P	60	62 b	50	62 b	48	60 b
T. tabaci	OT-L	54	17 a	50	2 b	50	0 b
	OT-P	50	4 b	52	4 b	66	0 b

Table 2. Transmission efficiency of *Frankliniella occidentalis* and *Thrips tabaci* populations during the two inoculation access period (IAPs). Values following by same letters, per each species, are not significantly different (χ^2 analysis; $P \leq 0.01$)

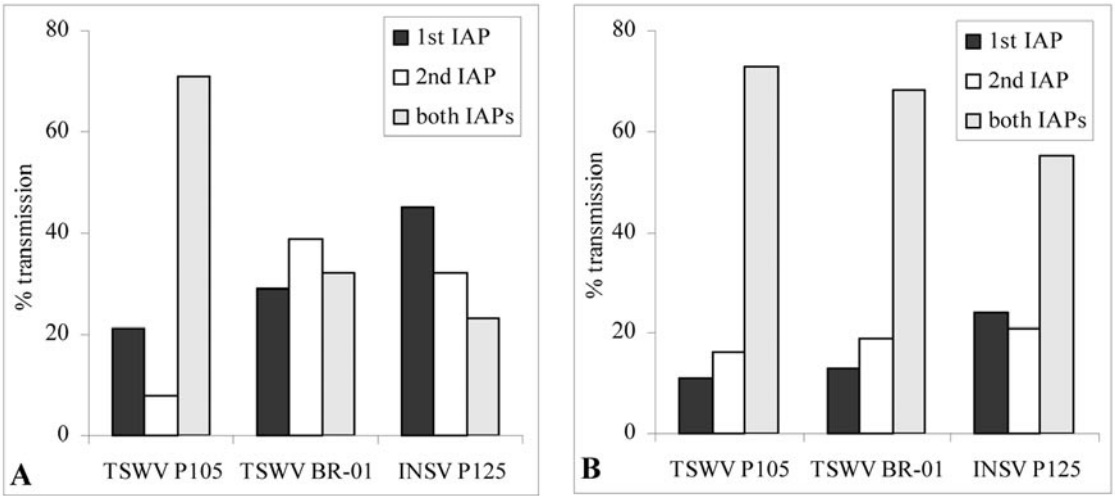

Figure 2. Transmission pattern of the two populations of *Frankliniella occidentalis* checked in laboratory trials: A, population WFT-L; B, population WFT-P.

Virus	Population WFT-L				Population WFT-P			
	Males		Females		Males		Females	
	no.	% transm.	no.	% transm.	no.	% transm.	no.	% transm.
TSWV P105	14	85.7	36	100.0	13	68.1	47	68.1
TSWV BR-01	21	52.4	34	50.0	24	57.7	26	57.7
INSV P125	18	33.3	36	44.4	18	55.6	30	63.3

Table 3. Comparison of transmission efficiency in males and females of the two *Frankliniella occidentalis* populations.

Discussion

All tested populations of both thrips species were able to transmit TSWV, whereas only the two populations of *F. occidentalis* could vector INSV. However, the efficiency in transmitting TSWV was very different: high from 51% to 96% in *F. occidentalis*, low from 2% to 17% in *T. tabaci*. Therefore, in north-western Italy with these two species present in crops, *F. occidentalis* can be considered the main vector of tospoviruses, as also reported in other studies (Wijkamp *et al.*, 1995; Chatzivassiliou *et al.*, 1999).

The transmission ability of *T. tabaci* is very controversial: previous research showed that the infectivity was related to the sex-ratio and only populations that were bisexual could transmit (Zawirska, 1976; Wijkamp *et al.*, 1995). In our experiments, both populations were unisexual, thelytokous, yet able to transmit TSWV with an efficiency comparable to that of bisexual ones (Chatzivassiliou *et al.*, 1999), whereas they failed to vector INSV, and previously also *Tomato chlorotic spot virus* and *Groundnut ringspot virus* (Tedeschi *et al.*, 2001), as other tested populations (Wijkamp *et al.*, 1995). Thus, the capacity of transmission would seem to depend on compatibility among thrips populations, virus isolates and plants used for both acquisition and inoculation. Moreover, the different competence in transmission could be related to tospovirus translocation mechanisms; a poor migration of the virus through the ligament to the salivary glands was observed in a population of *T. tabaci* able to acquire but not to transmit TSWV (Nagata *et al.*, 2002).

The importance of the plants used in transmission trials is confirmed in our experiments. Among the test plants, sweet pepper was the most susceptible to TSWV inoculation by *F. occidentalis*. This result is consistent with field observations: the most severe tospovirus infections in north-western Italy occurred in sweet pepper crops (Tavella *et al.*, 1997). This vegetable is probably strongly attractive for this nearctic thrips and, at the same time, highly susceptible to the virus.

Furthermore, in the present experiments, males and females of *F. occidentalis* showed a similar efficiency in transmitting both TSWV and INSV to pepper leaf disks. In contrast, males were more efficient than females in transmitting tospovirus in other studies carried out using petunia as test plants (van de Wetering *et al.*, 1998). The different efficiency between the sexes was correlated with their feeding behaviour: on petunia males usually made more feeding punctures but without inducing scarring, whereas females produced larger quantities of scars and so damaged irreversibly the cells that became unable to support viral infection.

In conclusion, the epidemics of tospoviruses in north-western Italy seem to be related to the introduction and consequently infestations in the area of the nearctic *F. occidentalis*, whereas the Mediterranean *T. tabaci* probably plays only a marginal role in transmitting TSWV, and none at all in transmitting INSV. Thus, in our regions strategies to control tospovirus infections in crops must focus mainly on the control of *F. occidentalis* populations.

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