Variation in tospovirus transmission between populations of *Frankliniella occidentalis* (Thysanoptera: Thripidae)

F. van de Wetering[1], M. van der Hoek[1], R. Goldbach[1], C. Mollema[2] and D. Peters[1]*

[1]Laboratory of Virology, Wageningen Agricultural University, Binnenhaven 11, 6709 PD Wageningen, The Netherlands: [2]Department of Research Strategy (DLO), PO Box 59, 6700 AB Wageningen, The Netherlands

Abstract

Fourteen populations of the western flower thrips *Frankliniella occidentalis* Pergande, originating from different hosts and countries in Asia, Europe, North America and New Zealand, were analysed for their competency and efficiency to transmit tomato spotted wilt virus (TSWV). All populations acquired and subsequently transmitted the virus, and were thus competent to transmit. They show marked differences in their efficiency, expressed as the percentage of transmitting adults. Efficiencies varied from 18% for a *F. occidentalis* population from the USA (US2) to 75% for a population from Israel (IS2). The differences between populations were not affected by the amount of virus ingested or by the host plant used. However, the tospovirus species studied and age at which the larvae acquired the virus affected the efficiency to transmit. First instar larvae of the NL3 population from The Netherlands were able to acquire tomato spotted wilt virus, whereas second instar larvae failed to do so. However, both instars of this population acquired impatiens necrotic spot virus (INSV), another tospovirus. This and tomato spotted wilt virus were both acquired by both larval stages of the populations IS2 and US2, although their ability to acquire virus decreased with their age. Hence, it is likely that, in general, both instar larvae of most *F. occidentalis* populations are competent to acquire both tospoviruses. These results show that large differences exist in the efficiency by which tomato spotted wilt is transmitted by the various *F. occidentalis* populations and that the ability to acquire tospovirus decreases with the age of the larvae

Introduction

Tospoviruses cause serious diseases in crops cultivated in the open field and in greenhouses throughout tropical, subtropical and temperate climate zones (German *et al*., 1992; Peters *et al*., 1996). Of all tospoviruses, tomato spotted wilt virus (TSWV) (Bunyaviridae, Tospovirus) is the most predominant one. Due to its large host range, rapid expansion and poor control of its vectors, this virus ranks among the top ten most economically important plant viruses (Goldbach & Peters, 1994).

Tospoviruses are transmitted by thrips (Thysanoptera: Thripidae), minute insects approximately 1 mm long. In the 1930s and 1940s, the major vector of tomato spotted wilt virus was considered to be the onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) (Pittman, 1927). Diseases caused by this virus faded almost completely away in western Europe in the 1950s, probably due to effective and intensive chemical control of this species, or to changes in agricultural and horticultural practices. A renewed incidence of this virus in the 1980s has been attributed to unintentional

*Author for correspondence.
Fax: +317 484820
E-mail: dick.peters@medew.viro.wau.nl

Table 1. Geographical origin and host plants of the *Frankliniella occidentalis* populations tested for their transmission efficiency.

Population	Country	Crop	Collector
DK	Denmark	Bean	H.F. Brødsgaard
FR	France	Bean	F. Leclant
IS1	Israel	Strawberry	M. Klein
IS2	Israel	Mango	M. Klein
IT	Italy	Bean	M.G. Tommasini
JA	Japan	Chrysanthemum	T. Murai
NL1	The Netherlands	Cucumber	C. Mollema
NL2	The Netherlands	Chrysanthemum	E.R van Dijken
NL3	The Netherlands	Bean	I. Wijkamp
NL4	The Netherlands	Tomato	P.M.J. Ramakers
NL5	The Netherlands	Rose	J.J. Fransen
NZ	New Zealand	Egg plant	N.A. Martin
US1	United States	Gloxinia	J.R. Baker
US2	United States	Chrysanthemum	E.A. Shearin

import, rapid dispersal and increased pesticide resistance of a new effective vector, the western flower thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) (Brødsgaard, 1994; Robb *et al*., 1995; Wijkamp *et al*., 1995; Zhao *et al*., 1995). This thrips species was originally known as a local pest in the western part of the USA (Moulton, 1931). To date, its occurrence has been reported throughout North and Central America, Africa, Australia, Europe, the Middle East, south-east Asia and New Zealand (Brødsgaard, 1989; Anon., 1993; Dal Bó *et al*., 1995). The expansion of *F. occidentalis* was associated with the occurrence of another, but distinct tospovirus, impatiens necrotic spot virus (INSV), in the United States (Law & Moyer, 1990) and Europe (de Ávila *et al*., 1992). To date, at least eight distinct tospovirus species have been reported (Kormelink *et al*., 1998), each is transmitted by one or more thrips species out of the eight known as vectors of tospoviruses (Wijkamp *et al*., 1995; Webb *et al*., 1998). *Frankliniella occidentalis*, one of these vectors, is one of the most efficient transmitters of tomato spotted wilt and impatiens necrotic wilt virus (Wijkamp *et al*., 1995).

Unfortunately, tospovirus spread can not be efficiently controlled by the pest management strategies (Todd *et al*., 1996; Daughtrey *et al*., 1997). The development of alternative and durable strategies to control tospoviruses and their vectors also depend on a better understanding of tospovirus epidemics, and hence of tospovirus-vector interactions. Although many transmission parameters have been studied, including virus propagation in *F. occidentalis* (Ullman *et al*., 1993; Wijkamp *et al*., 1993) after acquisition by young larvae (van de Wetering *et al*., 1996), information on possible differences in virus transmission efficiencies by thrips populations from different origins is lacking. Variations exist between the various *F. occidentalis* populations in their ribosomal RNA (Gillings *et al*., 1995), reproduction and damaged caused on cucumber plants, body size and host adaptation (de Kogel *et al*., 1997). The ability to acquire and transmit tospoviruses may also differ for the various *F. occidentalis* populations.

This report presents the results of a study on the adults of 14 *F. occidentalis* populations, collected from different hosts and regions of the world, to transmit tomato spotted wilt virus. Efficiency was analysed using two different tospoviruses after being acquired by specimens of both instars from three of the populations studied. The effects of virus source and test plant on the transmission were also compared in this study.

Materials and methods

Origins and maintenance of thrips colonies

Samples of the different *F. occidentalis* populations were originally collected from various crops and locations throughout the world (table 1). These samples were designated as 'populations' in this report as they did not derive from a single female or from a single male and female. Most of these populations were maintained at the Centre for Plant Breeding and Reproduction Research (CPRO-DLO) Wageningen for at least two and half years. The Dutch populations were kept for two to seven years in culture in our laboratory. They were all identified by Mr Vierbergen (Plant Protection Service, Wageningen) as *F. occidentalis*. Virus-free cultures were continuously reared on bean pods (*Phaseolus vulgaris* L. cv. Prelude) in glass jars at 27 ± 0.5°C and a16:8 h light:dark period (de Kogel *et al*., 1997; Peters *et al*.,1997). To avoid contamination with thrips from other origins, the pods were washed in water, incubated at 27 ± 0.5°C for four days, and washed again to remove any hatched larvae before the pods were used in the cultures. Contamination between the populations was prevented by methods described in detail by Peters *et al*. (1997).

Tospovirus populations and test plants

In this study, the Brazilian tomato spotted wilt virus isolate BR-01 (de Ávila *et al*., 1990) and the impatiens necrotic spot virus isolate NL-07 (de Ávila *et al*., 1992) were used. Both viruses were maintained by thrips inoculation on *Datura stramonium* L. (Solanaceae) and *Nicotiana benthamiana* Domin. (Solanaceae) plants, respectively. To infect the larvae with virus, they were either fed on *D. stramonium*, *Impatiens* sp. (Balsaminaceae) or *N. benthamiana* plants infected with TSWV, or on *Impatiens* sp. or *N. benthamiana* plants infected with INSV. These plants were mechanically inoculated on the first two true leaves with extracts from thrips-inoculated plants and kept in a greenhouse at approximately 22°C (16:8 h of light:dark).

Tospovirus detection by enzyme-linked immunosorbent assay (ELISA)

The viral nucleocapsid protein (N) content of the infected *D. stramonium* or *Impatiens* sp. leaves used as virus source, was determined with the double antibody sandwich (DAS)

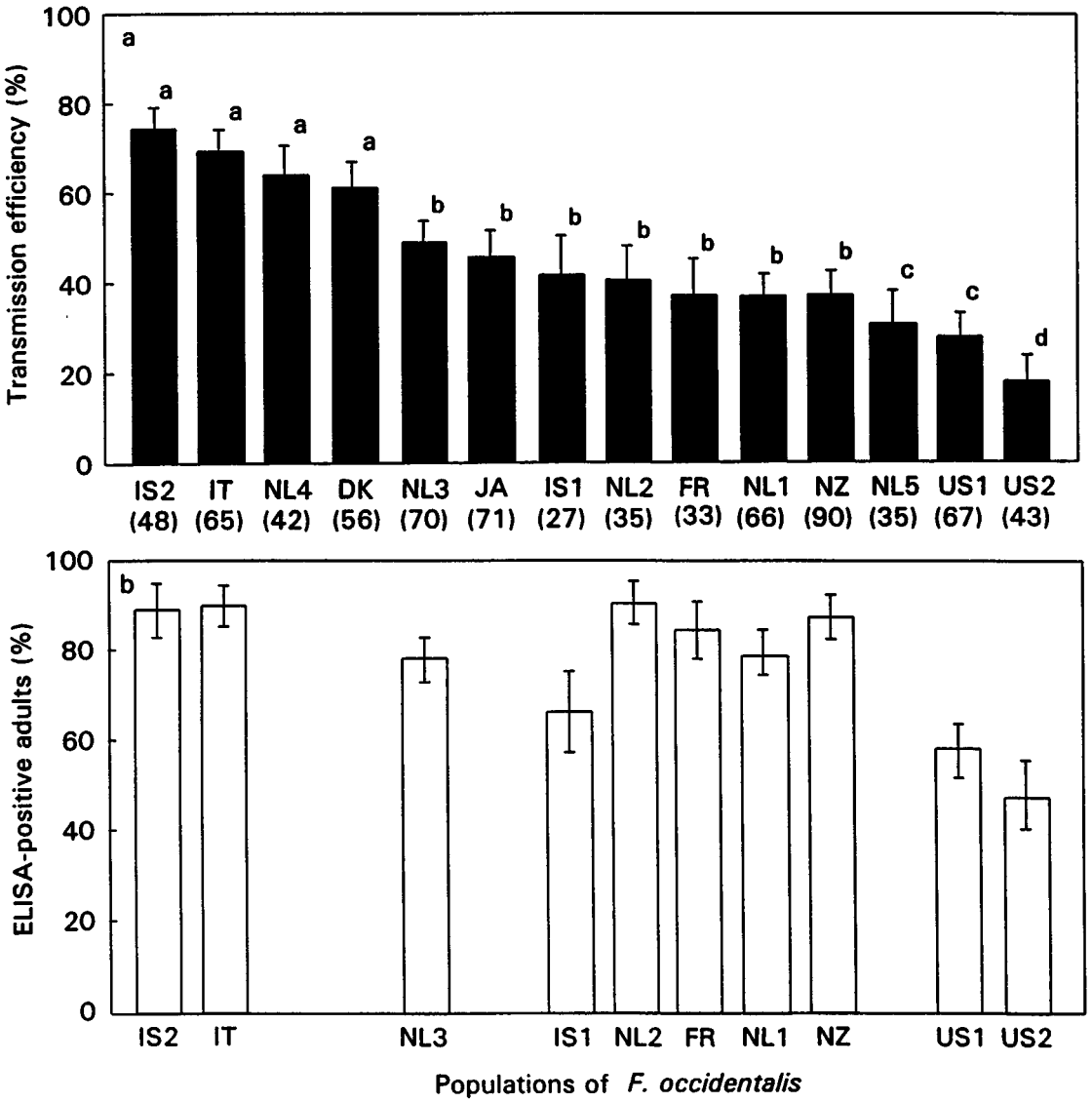

Fig.1. (a) Tomato spotted wilt virus (TSWV) transmission efficiency (± s.e.), as determined by petunia leaf disk assay, and (b) enzyme-linked immunosorbent assay (ELISA)-positive adult thrips after a 24 h acquisition access period on TSWV-infected plant material of *Datura stramonium* by different populations of *Frankliniella occidentalis*. Values in parentheses represent the number of thrips tested. Bars with a common letter are not significantly different (paired t-test, $P < 0.05$)

enzyme-linked immunosorbent assay (ELISA) (Clark & Adams, 1977; Resende *et al.*, 1991). Extracts were prepared by grinding of leaf disks, 5 mm in diameter, at a ratio 1:30 (w/v) in PBS-T (140 mM NaCl, 1 mM KH_2PO_4, 8 mM Na_2HPO_4, 2.5 mM KCl and 0.05% Tween-20). Extracts from non-infected plants were used as controls. Individual thrips were analysed for their TSWV-N protein content by a cocktail ELISA followed by enzyme amplification as described previously (van de Wetering *et al.*, 1996). Polyclonal antiserum, raised against the N-protein of BR-01 and NL-07, was used in both assays (van de Wetering *et al.*, 1996). ELISA readings were corrected for blanks read from wells containing only sample buffer in the incubation step. Readings higher than the average non-infected controls, either from plants or thrips, plus three times their s.d., were considered to be positive, those with lower readings as negative. These readings plus their s.d. ranged from 0.045 to 0.060 for healthy plant material and from 0.019 to 0.021 for healthy thrips.

Handling thrips in transmission experiments

To obtain cohorts of equally aged larvae, fresh bean pods were placed in *F. occidentalis* colonies. After 24 h, these pods were incubated at 27°C after removal of the thrips. The larvae, which emerged on these pods four days later, were

collected every 4 h and subsequently placed on the virus source or maintained in Tashiro cages (Tashiro, 1967; Peters *et al*., 1997) until they were given an acquisition feeding period. These cages consisted of three plexiglass plates of 10 cm long, 8 cm wide and respectively 6, 10 and 6 mm thick. A hole, surrounded by a rubber ring, of 35 mm diameter in the centre of the middle plate, is placed over the leaf material on top of blotting paper which is continuously wetted. Rubber bands, are used to tightly seal the plates together. To acquire virus, 20–30 larvae were placed in each cage on systemically infected leaf pieces with comparable N-protein contents. The contents were determined from small disks, 5 mm in diameter, from the leaves to be used by ELISA. These leaves were cut into four or more pieces (approximately 2 × 2 cm) and randomly divided between the cages. Similarly sized groups of larvae were caged on non-infected plant material as blanks. After the acquisition access period, the larvae were transferred to Tashiro cages with virus free plant material of the same species as on which the virus was acquired and reared until adult emergence. Each adult was individually tested for its transmission efficiency on a leaf disk (13 mm in diameter) of *Petunia* × *hybrida* cv. 'Polo Blauw' in three successive inoculation access periods of 48 h as previously described (Wijkamp & Peters, 1993). After each inoculation access period, the leaf disks were incubated on water for three days at 27 ± 0.5°C for development of local lesions. The transmission efficiency was calculated as the percentage of infected leaf disks and statistically analysed by the paired *t*-test.

Transmission of tomato spotted wilt virus by different F. occidentalis *populations*

Newly hatched larvae were placed on infected *D. stramonium* leaf material for 24 h. After this acquisition access period, the thrips completed their larval and pupal development on non-infected *D. stramonium* leaves. Directly after becoming adult, the thrips were individually tested for their transmission efficiency. Each adult of ten *F. occidentalis* populations used in these tests was sampled, stored at −70°C and later assayed in cocktail-ELISA followed by enzyme amplification to determine the percentage of ELISA-positive thrips. Transmission experiments and ELISA were executed in two replicates in which 27–90 adults were used per population (fig. 1). All treatments performed in each replicate were done with all populations in the same conditions and on the same day, and the larvae acquired the virus from plants infected at the same date and kept after the acquisition period on leaf material of the same age.

Virus acquisition by first and second instar larvae of F. occidentalis *populations*

Age effects of virus acquisition on transmission were compared between newly hatched first instar (L1) larvae, 36-h-old larvae (being a mixture of L1s and second instars (L2s)), and L2 larvae (72-h-old) of the *F. occidentalis* populations IS2, NL3, and US2. These thrips were placed for a 4-h acquisition access period on tomato spotted wilt or impatiens necrotic spot virus infected leaf material of *N. benthamiana* plants. Before and after this acquisition access period, the thrips were kept on virus free *D. stramonium* plant material. The transmission efficiency was calculated by the number of infected petunia leaf disks (Wijkamp & Peters, 1993). In total, 44 to 113 thrips were used per treatment (fig. 2).

Virus acquisition and transmission by F. occidentalis *from and to different plant species*

Up to 4-h-old larvae of the populations IS2, NL3 and US2 were placed for 24 h on tomato spotted wilt virus infected leaf material of *D. stramonium* and *Impatiens* sp. The selected leaves had approximately the same virus content as determined by ELISA. After the acquisition access period, the thrips were maintained on leaves from non-infected plants of the species on which the virus was ingested. As they became adults, their ability to transmit this virus was individually tested in a 48 h inoculation access period on leaf disks of the species on which the larvae were maintained, and in two inoculation access periods of 48 h on petunia leaf disks. To randomize the effect of ageing of thrips on the transmission, they were placed in successive inoculation access periods on disks arranged in a Latin square design. Infection was demonstrated either by local lesions on petunia disks or by ELISA when disks of other plant species were used as they did not develop discernible symptoms. The transmission efficiency was expressed as the percentage of disks infected. The ELISA readings of the leaf disks infected by different *F. occidentalis* populations were compared. Leaf disks of non-infected plants of *D. stramonium* and *Impatiens* sp. plants served as controls. Results were obtained with 43, 33 and 61 adults on *D. stramonium* leaf disks and 20, 13 and 29 adults on *Impatiens* sp. disks from the populations IS2, NL3 and US2, respectively.

To estimate the amount of TSWV-N protein ingested, approximately 18 larvae of populations IS2, NL3 and US2 were randomly collected after an 8 h acquisition access period and stored at −70°C prior to ELISA. The ELISA readings were analysed using Duncan's multiple range test with a STATGRAPHICS 6.0 PLUS computer program (Schulman, 1992; Rijpkema, 1993).

Results

Variation in TSWV transmission efficiencies between different F. occidentalis *populations*

The efficiency of 14 populations of *F. occidentalis* (table 1), competent to transmit tomato spotted wilt virus, was compared (fig. 1a) using the paired t-test. Forty-eight percent of the adults of the reference strain NL3 transmitted this virus. Approximately similar, but slightly lower efficiencies, although not significantly different, were found for the populations collected in France (FR), Israel (IS1), Japan (JA) and New Zealand (NZ), and for the two other Dutch populations (NL1 and NL2). The American population US1 and a Dutch population (NL5) transmitted the virus at significantly lower efficiencies than the reference population NL3. The American population (US2) was a significantly poor transmitter. In contrast, the populations from Israel (IS2), Denmark (DK), Italy (IT) and The Netherlands (NL4) transmitted tomato spotted wilt virus with significantly higher efficiencies than NL3 (fig. 1a). IS2 was, with an efficiency of 75%, the most efficient transmitting population. These results indicated that the transmission efficiency did not only differ between thrips populations from different

geographic regions and hosts, but also between populations collected from the same region, i.e. Israel (IS1 and IS2) and the Netherlands (NL3, NL4 and NL5).

The percentage of ELISA-positive adults was always a factor of 1.2 to 2.7 higher than the number of transmitting thrips (fig. 1b). The transmission efficiency was weakly correlated (R = 0.7) with the virus content of the thrips. These results showed that transmission efficiency by adult thrips was the most useful parameter used to characterize *F. occidentalis* populations in tospovirus transmission instead of the time-consuming determination of percentage of ELISA-positive adults.

Acquisition of two different tospoviruses by first and second instar larvae of three F. occidentalis *populations*

Previous studies showed that only the first instar larvae of the *F. occidentalis* population NL3 could acquire tomato spotted wilt virus (van de Wetering *et al.*, 1996). To test whether this observation was a general phenomenon of *F. occidentalis*, the ability to acquire this virus was also determined for the first and second instar larvae of populations IS2 and US2. These populations were chosen because they differed strongly in their efficiency to transmit this virus (fig. 1a).

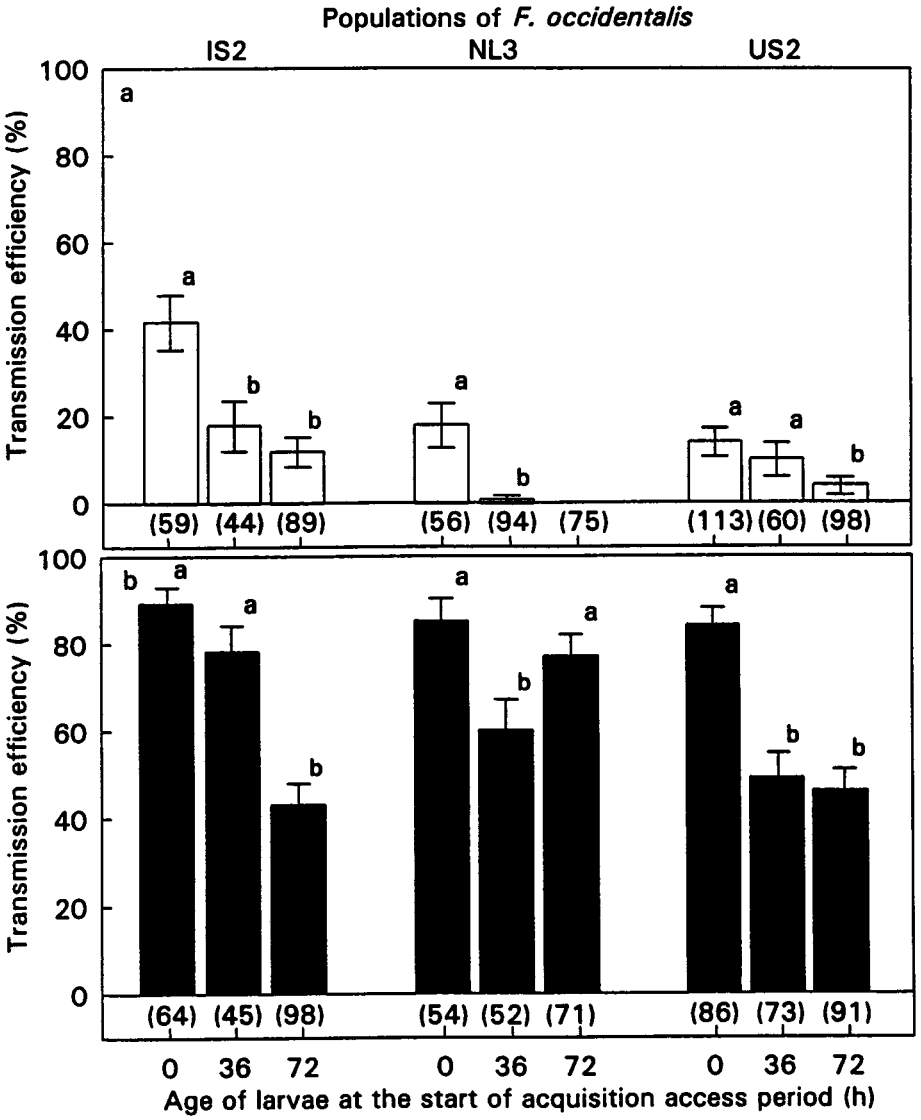

Fig. 2. (a) Tomato spotted wilt virus and (b) impatiens necrotic spot virus transmission after a 4 h acquisition access period by 0–4-h-old larvae (L1), 36 h (L1 +L2) and 72-h-old larvae (L2) of *Frankliniella occidentalis* populations from Israel (IS2), The Netherlands (NL3) and United States (US2) (means ± s.e.). Values in parentheses represent the number of thrips tested. Bars with a common letter are not significantly different (paired t-test, $P < 0.05$).

Table 2. Ingestion of tomato spotted wilt virus (TSWV) by newly hatched first instar larvae of three populations of *Frankliniella occidentalis* after 8 h exposure to virus infected *Datura stramonium* or *Impatiens* sp. plants.

Populations of *F. occidentalis*	Plant species					
	Datura stramonium			*Impatiens* sp.		
	n[1]	ELISA positive[2]	ELISA readings[3]	n[1]	ELISA positive[2]	ELISA readings[3]
IS2	18	18	0.126 ± 0.044[4a]	18	15	0.093 ± 0.022[4a]
NL3	15	14	0.146 ± 0.023[a]	17	7	0.072 ± 0.019[a]
US2	17	17	0.112 ± 0.019[a]	19	9	0.031 ± 0.002[a]

[1]Number of thrips tested by ELISA; [2]number of thrips showing a virus positive reaction in ELISA at 490 nm; [3]ELISA readings, at 405 nm, denoted as the average ± s.e.; [4]ELISA readings within a column followed by the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$).

Adults of the NL3 population failed to transmit when the virus was offered to 72-h-old second instars (fig. 2a), confirming earlier results (van de Wetering *et al*., 1996). In the present study, ingestion of virus by 72-h-old second instars of the populations IS2 and US2 resulted in a transmission efficiency of 12 and 4% by adults, respectively, showing that transmission efficiencies were considerably lower than when first instar larvae ingested this virus.

To study whether tospovirus acquisition like that of tomato spotted wilt virus is restricted to first instar larvae, another species, impatiens necrotic spot virus (INSV), was included in these experiments. This virus was significantly ($P > 0.05$) more efficiently transmitted by *F. occidentalis* than tomato spotted wilt virus (TSWV) (fig. 2b), confirming previous studies (Wijkamp & Peters, 1993; Wijkamp *et al*., 1995). With both viruses, transmission efficiencies decreased for the three populations tested with the age at which the larvae ingested virus. Adults of all three populations transmitted impatiens necrotic spot virus at efficiencies of approximately 85%, after ingesting the virus as newly hatched larvae (fig. 2b). The 72-h-old second instars of all three populations were able to acquire this virus, including the NL3 population, and as adults transmitted this virus with efficiencies ranging between 43% (IS2) and 77% (NL3).

Ingestion of tomato spotted wilt virus by first instar larvae of the IS2, NL3 and US2 populations was analysed after they had fed for 8 h on infected *D. stramonium* and *Impatiens* sp. leaf material (table 2). The results showed that ingestion of this virus did not differ significantly for the three *F. occidentalis* populations tested, according to Duncan's multiple range test ($P < 0.05$).

Effect of plant species on tomato spotted wilt virus acquisition and transmission

Datura stramonium and *Impatiens* sp. plants were used to test possible host plant effects on the acquisition and transmission of tomato spotted wilt virus by the *F. occidentalis* populations IS2, NL3 and US2. Each population transmitted the virus at an almost similar efficiency to the leaf disks of petunia, *D. stramonium* and *Impatiens* sp. ($P < 0.05$; fig. 3). Only, a significant difference (paired t-test; $P > 0.05$) was found in the transmission of tomato spotted wilt virus to *D. stramonium* and petunia leaf disks by US2 adults (fig. 3). Hence, it can be concluded that the efficiency at which the different *F. occidentalis* populations transmit tospovirus is not affected by the plant species used. In addition, the development of first instar larvae to adult did not differ between these three populations when they were reared on *D. stramonium* or *Impatiens* sp. plants (results not shown). The ELISA readings of the leaf disks infected by viruliferous adults of the populations IS2, NL3, and US2, however, differed considerably (Duncan's multiple range test, $P > 0.05$; table 3). Leaf disks of *D. stramonium* inoculated by viruliferous adults of IS2 had considerably higher ELISA readings than those inoculated by NL3 adults. In addition, infected disks of *D. stramonium* and *Impatiens* sp. by thrips of US2 population had significantly lower ELISA readings, implying a lower virus content, than disks infected by adults of the IS2 and NL3 population.

Discussion

These studies demonstrated that *F. occidentalis* populations, collected at fourteen locations from different crops, were competent to transmit tomato spotted wilt virus. However, the transmission efficiency, expressed as the percentage of transmitting adults of these populations, ranged from 18% (population US2) to 75% (population IS2) (figs 1a, 2a,b). The constant efficiency by which the NL3 reference population transmitted TSWV over a period of six years (Wijkamp & Peters, 1993; Wijkamp *et al*., 1993, 1995; van de Wetering *et al*., 1996), shows that the tospovirus transmission efficiency is a stable character. Since the other

Table 3. The tomato spotted wilt virus content of *Datura stramonium* and *Impatiens* sp. leaf disks as determined by enzyme-linked immunosorbent assay (ELISA) after inoculation by adults of three *Frankliniella occidentalis* populations.

Populations of *F. occidentalis*	Plant species	
	Datura stramonium	*Impatiens* sp.
IS2	(29)[1] 0.658 ± 0.087[2a]	(15)[1] 1.108 ± 0.061[2a]
NL3	(18) 0.384 ± 0.091[b]	(6) 1.035 ± 0.071[a]
US2	(19) 0.061 ± 0.008[c]	(8) 0.698 ± 0.113[b]

[1]Number of disks tested is indicated in parentheses; [2]ELISA readings at 405 nm denoted as the average ± s.e. Readings within a column followed by the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$).

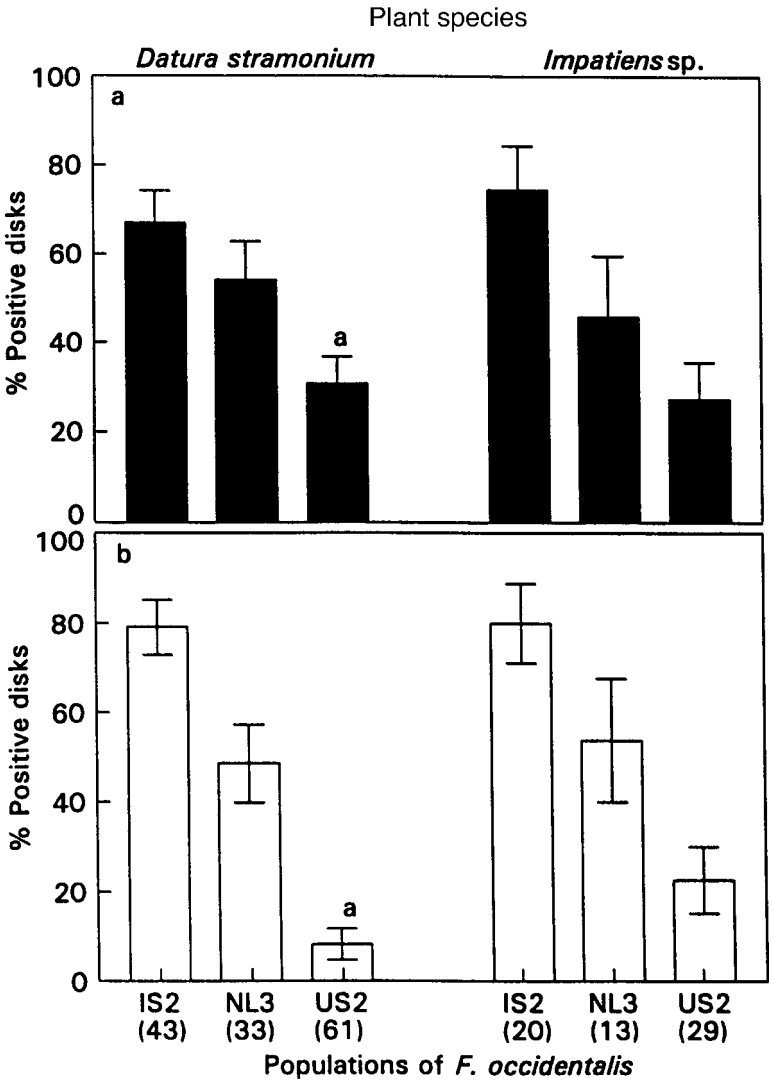

Fig. 3. Tomato spotted wilt virus transmission efficiency (± s.e.) after a 24 h-acquisition access period on TSWV-infected plant material of *Datura stramonium* or *Impatiens* sp. by different populations of *Frankliniella occidentalis*. After the acquisition access period, the thrips were reared on virus free leaf material of the same host species. Adult thrips were tested on the disks from the plant species on which they acquired TSWV (a) or on petunia leaf disks (b). Values in parentheses represent the number of thrips tested. The only significant difference between transmission to test plants is indicated with an 'a' (paired *t*-test, $P < 0.05$).

populations were reared under identical conditions for at least two-and-a-half years on bean pods, the transmission efficiencies found may reflect their original efficiencies. The different efficiencies are likely to be the result of a different development at the various locations.

As different *F. occidentalis* populations transmit tomato spotted wilt virus with distinct efficiencies, the question arises whether we can denote some of these populations as different 'biotypes'. Broadly speaking, the term biotype is an intraspecific category for a biological attribute, which refers to insect populations of similar genetic composition (Saxena & Barrion, 1987). Biotypes can be classified in, e.g. aggressiveness towards resistant or susceptible varieties of crop plants and/or disease vector capabilities, like the transmission ability of tospoviruses. According to the results described in this paper, we might classify some *F. occidentalis* populations, which transmit tospovirus with dissimilar efficiencies, as different biotypes. Examples of this are the populations IS2, NL3 and US2. Various *F. occidentalis* populations, used in this study, were analysed by de Kogel *et al.* (1997) for their performance on cucumber accessions with different levels of resistance. They concluded that the reproduction rate of each population could be used as a criterion for differentiating *F. occidentalis* populations.

Although these authors considered the NZ and NL1 populations as quite distinct, they did not significantly differ in TSWV transmission (fig. 1a). Future studies on the populations, including genetic analysis, are needed to describe the inter-population variation in tospovirus transmission as biotypic variation. Several studies on biotypic variation in insect species have been reported in relation to the transmission of other circulative plant viruses (Sylvester, 1980). Detailed studies with geminiviruses, transmitted by *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) showed that differences in transmission efficiency and virus specificity were mostly associated with differences in the epitope profiles of their coat proteins (McGrath & Harrison, 1995). Likewise, differences between *F. occidentalis* populations in transmitting two tospoviruses (fig. 2a,b) might be associated with distinct structural features of the surface (glyco)proteins of tospoviruses.

Bedford *et al*. (1994) showed that differences in geminivirus transmission by distinct biotypes of the whitefly, *B. tabaci* (Bedford *et al*., 1992, 1993; Brown *et al*., 1992; Byrne & Devonshire, 1993; Perring *et al*., 1993a), which are also considered to be different species (Bartlett & Gawell, 1993; Bedford *et al*., 1993; Campbell *et al*., 1993; Perring *et al*., 1993a, b), were due to differences in virus distribution in the plant and feeding (ingestion) behaviour of the whiteflies. Since studies in this paper were executed with virus sources that contained similar virus contents and pieces of these leaves randomly divided between the Tashiro cages, the variation found in transmission efficiency between the different *F. occidentalis* populations cannot be attributed to differences in virus distribution or concentration. Also, the amount of tomato spotted wilt virus ingested by different *F. occidentalis* populations was comparable (table 2), ruling out an ingestion effect on the obtained transmission variation.

Differences in transmission efficiencies by *F. occidentalis* populations were greatly influenced by the age of larvae when feeding on infected material, and by the tospovirus species used (fig. 2a,b). First and second instar larvae of the *F. occidentalis* populations IS2 and US2 acquired both tomato spotted wilt virus as well as impatiens necrotic spot virus. Likewise, the latter virus was also transmitted when ingested by second instar larvae of the NL3 population, whereas these instars failed to acquire tomato spotted wilt virus. It can, therefore, be concluded that the ability of instars to acquire tospovirus depends on both the *F. occidentalis* population and the tospovirus species used.

The dissimilarities in acquisition of these viruses by the second instar larvae of the NL3 population may be explained for example by the presence of different receptors for both tospovirus species in this thrips species. However, variation in the transmission efficiencies may also be due to dissimilar midgut and salivary gland properties as shown for different populations of *Thrips tabaci*, affecting the replication rate of tospoviruses and their translocation in thrips (Nagata *et al*., 1999). Differences in the midgut receptors, the activity of digestive enzymes in the gut affecting the infectivity of the virus, and dissemination barriers are other factors which can be involved in regulating the acquisition and release of the virus by and from the gut cells. At the level of salivary glands, a barrier may exist in the infection of the salivary glands. Differences in the replication rate in these glands and release of virus in the saliva may be other factors affecting the transmission as described for other plant viruses, replicating in their vectors (Ammar, 1994). The study presented here does not allow discrimination between any of these factors.

Transmission of tomato spotted wilt virus by *F. occidentalis* populations was not affected by the plant species used as virus source and test plants, as shown for *D. stramonium* and *Impatiens* sp. (fig. 3a,b). However, the virus content in leaf disks of these species infected by viruliferous thrips differed. The disks infected by viruliferous IS2 adults, the most efficient transmitter, contained the highest amounts of virus, whereas considerably lower amounts were found in leaf disks infected by the most incompetent transmitter, US2 (table 3). These data suggest that the different *F. occidentalis* populations inject different amounts of virus into disks, resulting in different virus contents. Although not very likely, it cannot be ruled out that selection might occur during the virus multiplication in the vector, resulting in variants with different replication abilities in plants. Future research has to verify whether whole plants, as found for leaf disks, are also poorly infected by the *F. occidentalis* population US2, and efficiently by IS2.

In view of the distinct differences in tospovirus transmission efficiencies between *F. occidentalis* populations, improved understanding of tospovirus–thrips interactions needs detailed analysis not only at the organism but also at the population level. Also, general conclusions on tospovirus spread should also be based on the dynamics of these vector populations, to circumvent inaccurate statements. To date, tospovirus control methods are focused on early detection of tospovirus and thrips vectors, and on the prevention and elimination of virus introduction and spread in a crop. The variation in tospovirus transmission by different populations of one thrips species makes prediction of virus spread more difficult. In our view, the development of the integrated tospovirus control strategies should focus on the more efficient vector populations.

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