

## Factors determining vector competence and specificity for transmission of *Tomato spotted wilt virus*

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The competence of a *Frankliniella occidentalis* and a *Thrips tabaci* population to transmit *Tomato spotted wilt virus* (TSWV) was analysed. Adults of the *F. occidentalis* population transmitted this virus efficiently, whereas those of the thelytokous *T. tabaci* population failed to transmit. TSWV replicated in the midgut of the larvae of both populations after ingestion of virus; however, lower amounts accumulated in *T. tabaci* larvae than in *F. occidentalis* larvae. The virus was almost undetectable in *T. tabaci* adults, whereas high titres were readily detected in the *F. occidentalis* adults. The first infections in *F. occidentalis* larvae were detected by immunocytochemical studies in midgut epithelial and subsequently in midgut muscle cells, the ligaments, and finally in the salivary glands. The infections were weaker in the midgut epithelial and muscle cells of *T. tabaci* larvae, followed by an almost complete absence of any infection in the ligaments, and a complete absence in the salivary glands. Studies by electron microscopy revealed the budding of some virus particles from the basal membrane of midgut epithelial cells of *F. occidentalis* larvae into the extracellular space of the basal labyrinth. Enveloped virus particles were also seen in midgut muscle cells of *F. occidentalis* larvae. They were not discerned in epithelial and muscle cells of *T. tabaci* larvae and adults. This study showed that the rate of virus replication in the midgut and the extent of virus migration from the midgut to the visceral muscle cells and the salivary glands are probably crucial factors in the determination of vector competence.

### Introduction

*Tomato spotted wilt virus* (TSWV) is the type species of the genus *Tospovirus* within the *Bunyaviridae*. This virus family contains many animal-infecting viruses, including important human viruses (Regenmortel *et al.*, 2000). These viruses are mainly transmitted by mosquitoes and ticks (Calisher, 1996). Members of the genus *Tospovirus*, one of the five *Bunyaviridae* genera, are transmitted by some thrips species, of which most are also known as important pests in various agricultural and horticultural crops (Goldbach & Peters, 1994). The tospoviruses are transmitted by their vectors in a propagative–circulative way (Wijkamp *et al.*, 1993; Ullman *et al.*, 1993).

TSWV has a unique vector relationship with thrips in that the virus is acquired by the larvae but not by adults, while the

ability to acquire virus rapidly declines during larval development (van de Wetering *et al.*, 1996; Nagata *et al.*, 1999). After a temperature-dependent latent period, larvae at the end of their second stage and adults are able to transmit the virus (Wijkamp *et al.*, 1993). Until now, only eight thrips species of the genera *Thrips* and *Frankliniella* have been reported as vectors of tospoviruses (Mound, 1996; Webb *et al.*, 1998).

Interspecific as well as intraspecific differences were found in vector competence in a study using four tospoviruses and four thrips species of six distinct populations (Wijkamp *et al.*, 1995). Of the four viruses studied, TSWV was transmitted by all four thrips species, while *Impatiens necrotic spot virus* was transmitted solely by *F. occidentalis* with a high efficiency. Distinct differences were found in the transmission of TSWV by four *T. tabaci* populations studied. Three thelytokous populations, which propagate parthenogenetically and produce a progeny of only females (Moritz, 1997), did not transmit TSWV. An arrhenotokous population transmitted the virus with low efficiency (Wijkamp *et al.*, 1995). Thrips of these populations lay two different types of eggs. Fertilized eggs, which are diploid, produce females; unfertilized eggs give only

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males (Moritz, 1997). These results suggested the existence of several factors regulating the vector competence to transmit TSWV. Analysis of these factors may provide keys to explain the differences in transmission competence of the various vector species and the differential transmission of the various TSWV isolates by its vectors.

The amount of TSWV present in the adult thrips body is probably one of the main factors that affects vector competence and efficiency (Wijkamp *et al.*, 1995; van de Wetering *et al.*, 1996). Virus accumulation, most likely, depends on the invasion and rate of replication in thrips tissues, presumably the midgut and salivary glands. Besides the accumulation of virus in these tissues, barriers, which permit or prevent virus translocation, may also play an important role (Nagata *et al.*, 1999). The existence of such barriers may explain the failure of thelytokous *T. tabaci* populations to transmit TSWV, as the primary cell cultures of these populations support replication of this virus (Nagata *et al.*, 1997). This observation suggests that thelytokous *T. tabaci* are susceptible to TSWV, but lack the ability to become transmitters.

Here, we report a comparative study performed to elucidate the stepwise development of the infection in the midgut and salivary glands of an efficiently transmitting *F. occidentalis* population and a non-transmitting thelytokous *T. tabaci* population.

## Methods

**Transmission of TSWV and amplification ELISA.** An *F. occidentalis* (NL-03; van de Wetering *et al.*, 1996) and a thelytokous *T. tabaci* (Wijkamp *et al.*, 1995; Nagata *et al.*, 1997) population were reared in glass jars at 25 °C on bean pods or on pieces of leek leaves. New-born larvae, less than 4 h old, were allowed to acquire virus for 16 h on *Datura stramonium* leaves infected with the TSWV isolate BR-01 (de Ávila *et al.*, 1993). After this acquisition access period, these thrips were reared on healthy *D. stramonium* leaves until they were sampled for the transmission and/or immunohistochemical studies.

The transmission capacity of each newly emerged adult was tested using the local lesion assay on petunia leaf discs (Wijkamp & Peters, 1993). They were individually placed on a leaf disc in an Eppendorf tube for 2 days at 25 °C. The discs were then transferred to a 24-well plate and incubated for 3 days on water for the development of local lesions.

Accumulation of virus in thrips was measured by ELISA targeting the nucleocapsid (N) protein. The reaction was amplified as previously described (Wijkamp *et al.*, 1995). Groups of five larvae, sampled at 0, 2, 4, 12, 24, 48, 72 h post-acquisition (p.a.), of prepupae, and of 2-day-old adults were triturated with 100 µl sample extraction buffer (Wijkamp *et al.*, 1995). Half of these extracts were used in ELISA and the other half stored to repeat the ELISA when necessary.

**Midgut preparation and whole mount immunofluorescent staining (WMIS).** Dissected midguts and/or salivary glands from larvae and adults were fixed with cold acetone on object glasses at -20 °C (Nagata *et al.*, 1999) and incubated for 1 h in PBS pH 7.2. This buffer contained 10% BSA to block non-specific reactions. The organs were incubated with polyclonal antibodies to the N protein (2 µg/ml) raised in a rabbit. To eliminate aspecific reactions the antisera were pre-absorbed with extracts from uninfected thrips for 2 h in 10% BSA-PBS (Nagata *et*

*al.*, 1999). Following this incubation the preparations were overlaid with 10 µg/ml pig anti-rabbit FITC conjugate (Nordic) in 10% BSA-PBS for 1 h. After washing, the preparations were mounted in CitiFluor (Agar Scientific) and studied by fluorescence microscopy (Leica, Laborlux 5).

**Immunohistochemical studies of thrips.** Thrips bodies were fixed, after amputating the legs and antenna, in Bouin's Hollande sublimate (Smid, 1998) and incubated under vacuum (Nagata *et al.*, 1999). After fixation, the specimens were embedded in Paraplast (Oxford Labware) and cut in 5 µm thick sections, which were mounted on object glasses. The sections were deparaffinized with xylene, rehydrated and incubated in PBS. After this treatment, the preparations were incubated with pre-absorbed N protein antibodies (1 µg/ml in PBS) for 2 h, washed with PBS and incubated with pig anti-rabbit antibodies conjugated with horseradish peroxidase (5 µg/ml in PBS containing 10% normal serum) for 1 h (Dako). After washing, the sections were incubated for 5 min with a substrate solution of 0.05% (w/v) diaminobenzidine (DAB) and 0.01% (v/v) hydrogen peroxide in 50 mM Tris-HCl pH 7.6. Some sections were stained with Mayer's haematoxylin (Sigma) to visualize the organs in detail and localize them correctly in the immunostained tissue. The samples were then mounted with DPX (Fluka) after dehydration and studied by light microscopy.

**Electron microscopy.** Whole thrips specimens or dissected midguts were fixed in a 3% paraformaldehyde-2% glutaraldehyde solution for 30 min in a microwave oven with water flow, dehydrated in a series of 50-100% ethanol solutions, and embedded in LR-Gold (London Resin). The resin was polymerized by UV light irradiation at -20 °C. The 60-70 nm thick slices were incubated for 2 h with 0.8 or 2.0 µg/ml pre-absorbed N or viral glycoprotein (G) antibodies, respectively, washed with 30 droplets of PBS, and incubated with gold-conjugated protein A for 1 h. After washing with PBS, the sections were post-fixed in 1% glutaraldehyde, contrasted with uranyl acetate and lead citrate, and studied with an electron microscope (Philips CM 12).

## Results

### Accumulation and transmission of TSWV by the *F. occidentalis* and *T. tabaci* populations studied

After an acquisition access period of 16 h, first instar larvae of *F. occidentalis* and *T. tabaci* were transferred onto healthy *D. stramonium* leaves. The virus titre was determined by ELISA in a sample of five thrips immediately after acquisition and at various intervals post-acquisition (p.a.). This assay was repeated twice, giving similar results in both repeats. The amount of virus ingested by the *F. occidentalis* larvae decreased within the first 2-4 h p.a. (Fig. 1). This decrease in virus titre is most likely caused by partial digestion of virus in the intestinal tract and by the excretion of virus from the intestinal tract together with the faeces. After this decrease, the ELISA values rapidly increased between 4 and 12 h p.a. Although a small decrease was observed in the larvae 24 h p.a. and a larger one in the prepupal stage, the amount of virus remained high in all stages, including the adult stage (Fig. 1). The increase in virus titre after an initial decrease supports previous conclusions that the virus multiplies in thrips (Wijkamp *et al.*, 1993; Ullman *et al.*, 1993; Nagata *et al.*, 1997, 1999). The decline of the titre found at 24 h p.a. in the larvae and the prepupal stages may be

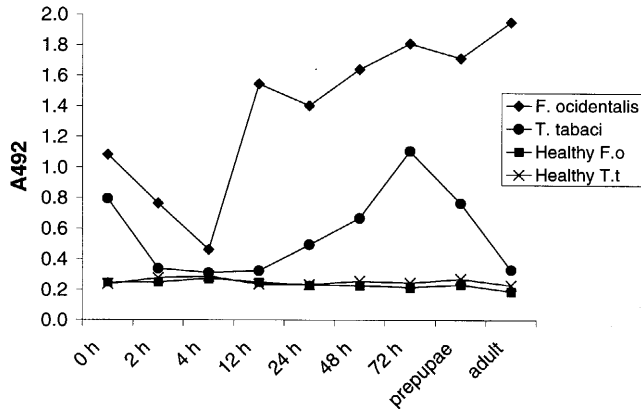


Fig. 1. Accumulation of TSWV in developing larvae, prepupae and adults of a transmitting *F. occidentalis* and a non-transmitting *T. tabaci* population as analysed by ELISA using antibodies to its N protein.

caused by histolysis of tissues containing infected cells during the moulting processes (Müller, 1926).

A deviating pattern of virus accumulation was found in the thelytokous *T. tabaci* population studied (Fig. 1). Like in *F. occidentalis*, the amount of virus also decreased in the first 2–4 h after ingestion and started to accumulate at a lower rate than in *F. occidentalis*. This observation shows that the virus also replicates in larvae of this *T. tabaci* population, though its adults are unable to transmit. The highest amount of virus was found before pupation at 72 h p.a. in these larvae. During the pupation, the virus decreased dramatically in amount and no resumption of virus accumulation could be detected in the *T. tabaci* adults. These results show that this *T. tabaci* population could support the multiplication of TSWV during the larval development, albeit with a lower efficiency than the larvae of

*F. occidentalis*, and that the infection was almost aborted during pupation.

Just after acquisition (0 h p.a.), a lower virus titre was found in *T. tabaci* than in *F. occidentalis*. This difference can be explained by the ingestion of smaller amounts of infected plant material, and thus virus, by *T. tabaci*, although its larvae produce larger feeding damage spots than those of *F. occidentalis*. A difference in the rate at which the virus is digested cannot be excluded.

Adults of the *F. occidentalis* population transmitted TSWV at a rate of 67.3% (206/306 individuals) and those of *T. tabaci* at 0% (0/456 individuals) in three independent experiments using the petunia leaf disc assay (Wijkamp & Peters, 1993).

#### Different levels of midgut infections in *F. occidentalis* and *T. tabaci* as determined by WMIS

Infection of the intestinal tract is the first discernible sign, which can lead to transmission of the virus by the vector. The midguts of larvae at 72 and 96 h p.a. (second stage larvae and prepupae) and of adults were analysed by WMIS (see Methods) for infection by TSWV since a dramatic difference in the accumulation of virus was detected by ELISA between late second instars and adults (Fig. 1). The midguts of these larvae and adults of *F. occidentalis* were found to be infected (Table 1). The infection was restricted to the midgut epithelial (Mg1) and midgut muscle cells of the anterior part of the midgut (Mg1 and Mg2) in larvae 72 and 96 h p.a. No infection could be discerned in the midgut epithelium of all adults, but was observed in the muscle cells of, almost, the entire midgut (Mg1, -2 and -3). These observations confirmed previous results that the virus is eliminated from epithelial cells, but not

**Table 1.** TSWV in the midgut regions of larvae (72 and 96 h p.a.) and 2-day-old adults of an efficiently transmitting *F. occidentalis* (Fo) and a non-transmitting *T. tabaci* (Tt) population

	Infected midgut regions as demonstrated by WMIS				
	None	Faint infection*	Mg1†	Mg2‡	Mg3§
Fo larvae, 72 h p.a.	0	1	12	7	0
Tt larvae, 72 h p.a.	8	4	9	6	0
Fo larvae, 96 h p.a.	0	1	13	12	4
Tt larvae, 96 h p.a.	4	4	14	8	0
Fo, 2 days, adults	0	0	1	13	23
Tt, 2 days, adults	32	51	6	0	0

\* Reaction was not discernible using a 100 × magnification but was visible with a 400 × fluorescent microscopy magnification.

† Infections restricted to the Mg1 region.

‡ Infections restricted to the Mg1 and Mg2 regions.

§ Infections occur in all three midgut regions.

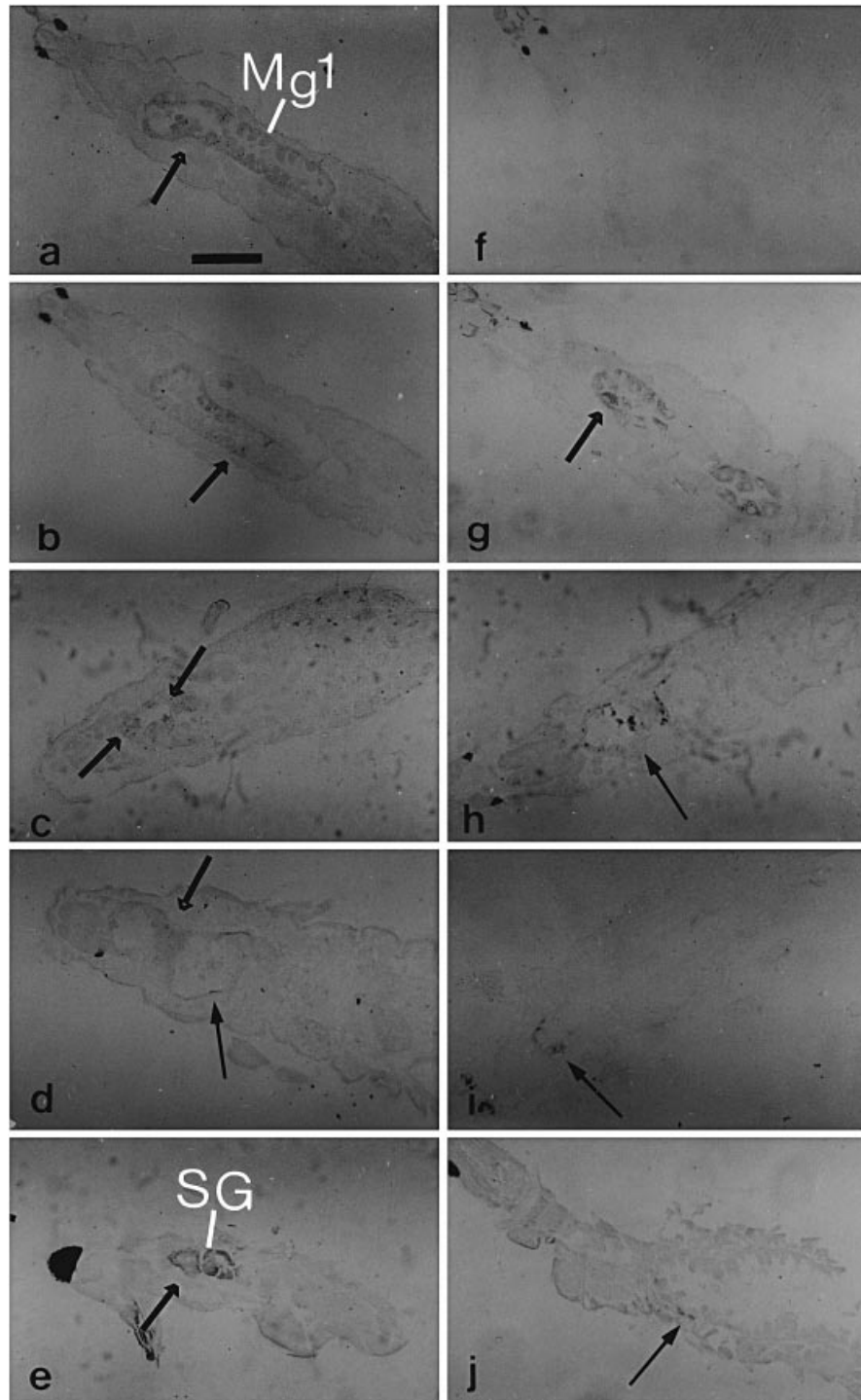


Fig. 2. Immunohistochemical detection of TSWV in tissues of thrips of a transmitting *F. occidentalis* and a non-transmitting *T. tabaci* population. Viral N protein was recognized by the dark colour caused by DAB precipitation. (a)–(e) *F. occidentalis* and (f)–(j) *T. tabaci*; (a, f) 24 h p.a.; (b, g) 48 h p.a.; (c, h) 96 h p.a.; (d, i) 144 h p.a.; (e, j) adult thrips. Arrows show the DAB precipitation. Mg1, Anterior midgut; SG, salivary glands. Bar, 100  $\mu$ m.

from muscle cells during the metamorphogenic pupation processes (Nagata *et al.*, 1999; front cover of this issue).

The analysis showed that the virus occurred in much lower amounts in midgut tissues of *T. tabaci* larvae than in those of

*F. occidentalis*. The midgut of eight out of 27 *T. tabaci* larvae at 72 h p.a., four out of 30 at 96 h p.a. and 32 out of 89 adults did not show any signal of infection in the midgut (Table 1). These infections not only differed in the proportion of infected

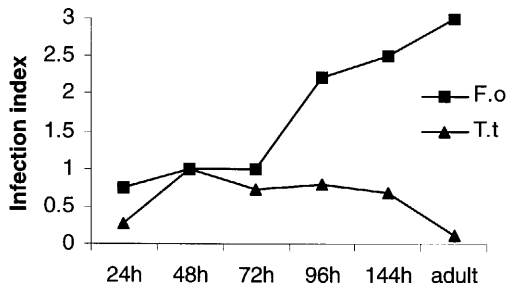


Fig. 3. Accumulation of TSWV infection in tissues during the development of thrips of a transmitting *F. occidentalis* (F.o) and a non-transmitting *T. tabaci* (T.t) population as evaluated by an infection index (0, no infection; 1, infection restricted to the midgut; 2, infection of the midgut and foregut; 3, infection of the midgut, foregut and salivary glands).

individuals, but also in the intensity of infection. The largest differences were observed among the adults of both species. *F. occidentalis* showed strong signals in the muscle cells of the three midgut regions in 23 out of 37 specimens studied, while in most *T. tabaci* (51 out of 57) only faint infections, visible as a few small spots, were observed in their midgut muscle cells (Table 1).

No sign of infection was found by ELISA in any of the *T. tabaci* adults tested, while weak but positive reactions in midgut muscle cells were found in 57 out of 89 adults using WMIS. This technique appeared to be more sensitive than ELISA to detect infection of TSWV in thrips.

#### Development of TSWV infection in thrips of both populations

To monitor the stepwise infection in the midgut as well as in the salivary glands, thrips of both populations were collected at 24 (1st instar), 48, 72 (2nd instar), 96 (2nd instar or prepupae), 144 h (pupae) p.a. and in the adult stage, and cut in thin longitudinal sections. These sections were serologically analysed by targeting the N protein using the DAB substrate reaction, visualizing positive reactions as dark brown precipitates. These reactions were not observed in thrips of either of

the populations fed on healthy plants and studied at the same intervals.

Distinct infections were observed in the midgut epithelial cells of *F. occidentalis* 24 h p.a. (Fig. 2a). These infections were only noticed in the anterior midgut (Mg1), and remained in most cases restricted in this region during the whole larval development of this thrips species (Fig. 2a, b). After pupation, no infections could be discerned in the midgut epithelium of adults, showing that multiplication was not resumed in this renewed tissue as reported earlier. Infections in the midgut of adults were only noticed in the visceral muscle cells, mostly in the whole midgut region (Fig. 2c). Infections of the foregut were often clearly observed in adults of *F. occidentalis*.

The first infections in the salivary glands of *F. occidentalis* were seen at 96 h p.a. in most specimens. Since no infections were observed in these glands in larvae of 72 h p.a., the first migration of virus to the salivary glands possibly takes place around 72 h p.a. Analysis of adult transmitters revealed heavy infections in the salivary glands (Fig. 2e).

Infection of the larvae and adults of the *T. tabaci* population was less pronounced (Fig. 2f–j) than in those of *F. occidentalis*, especially in larvae at 24 h p.a. and in adults. The infection became more prominent in the midgut of larvae at 48 (Fig. 2b) and 72 h p.a. Since *T. tabaci* moulted to prepupae almost 1 day earlier (72–96 h p.a.) than *F. occidentalis* (96–144 h p.a.), elimination of epithelial cells with the virus by histolysis consequently occurred 1 day earlier. No infection could be found in the salivary glands before pupation, in the pupal stages or in adults in any of the *T. tabaci* thrips studied.

The development of the infection in thrips of both populations was evaluated by an infectivity index, in which 0 stands for no infection, 1 for an infection restricted to the midgut, 2 for infection of the midgut and foregut, and 3 for infection of the midgut, foregut and salivary glands. The infection rate was indexed at 24, 48, 72, 96 and 144 h p.a. and in adults using eight to 25 thrips per interval. The average values obtained (Fig. 3) were almost equal to the tendency found by ELISA for the accumulation of virus in thrips of both

Table 2. The development of TSWV infection in the midgut (Mg), the ligaments (Lg) and the salivary glands (SG) in larvae 72 and 96 h p.a. and adults (2-day-old) of an efficiently transmitting *F. occidentalis* (Fo) and a non-transmitting *T. tabaci* (Tt) population

Tissue infected	Larvae 72 h p.a.		Larvae 96 h p.a.		Adults	
	Fo	Tt	Fo	Tt	Fo	Tt
None	0	8	0	NT	0	19
Mg only	0	8	1	NT	4	26
Mg and Lg, but not SG	2	0	5	NT	2	3
Mg, Lg and SG	2	0	5	NT	13	0
Mg and SG, but not Lg	0	0	0	NT	0	0

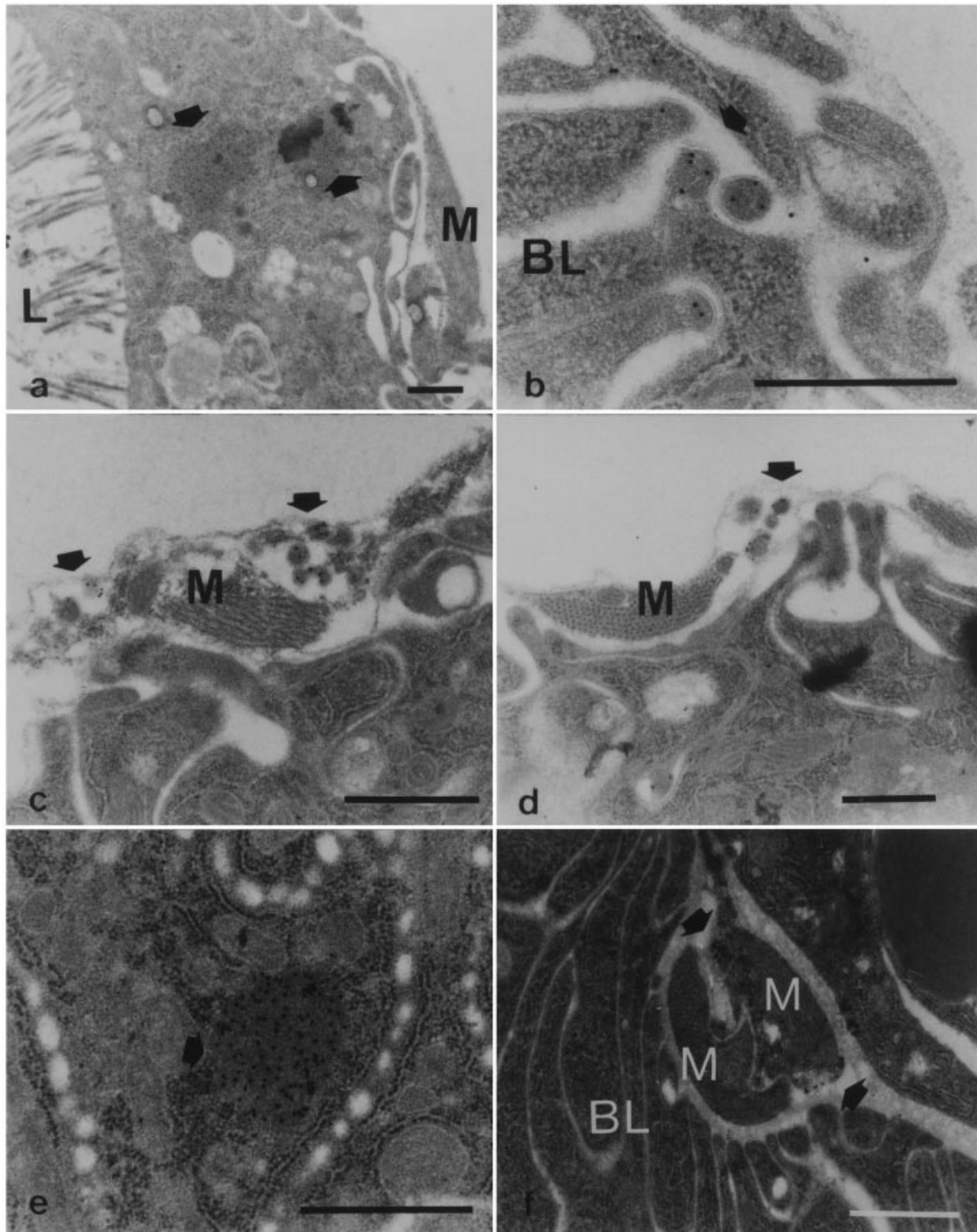


Fig. 4. Immunogold labelling of TSWV nucleocapsid (N) protein or glycoprotein (G) in the midgut of the larvae of a transmitting *F. occidentalis* and a non-transmitting *T. tabaci* population. (a)–(d) *F. occidentalis* at 72 h p.a.; (e, f) *T. tabaci* 72 h p.a. (a) Nucleocapsid aggregate in the cytoplasm of epithelium tissue (arrows). (b) Virus budding to the extracellular space of basal labyrinth (arrow), labelled with antibodies to N. (c, d) Enveloped virus particles labelled with antibodies to N (c) and particles labelled with IgG to G protein (d) (arrow) in the cytoplasm of midgut visceral muscle cells. (e) Nucleocapsid aggregates found in the cytoplasm of epithelium cells (arrow) labelled with antibodies to N. (f) N protein detected with N antibodies in the cytoplasm of midgut muscle tissue (arrow). M, Muscle; BL, basal labyrinth; H, haemocoel. Bar, 500 nm.

populations (Fig. 1). These results show that virus accumulation and the extent to which the tissues are infected are positively correlated. The distinct vector specificity cannot, therefore, be explained by a susceptibility of the different tissues, but by an impeded migration of the virus to the salivary glands in *T. tabaci*.

### Pathway by which the salivary glands become infected

Infection of the salivary glands is a prerequisite to transmission of TSWV. However, the pathway by which the virus migrates from the midgut to the salivary glands in thrips remains to be elucidated. Two pathways of virus migration to salivary glands can be proposed. The virus may migrate from the midgut through the haemocoel to the salivary glands by a pathway as proposed for arboviruses in mosquito vectors (Hardy *et al.*, 1983; Hardy, 1988). The second pathway may involve a translocation of virus via a thin structure, designated ligament (Nagata *et al.*, 1999), which connects the midgut and salivary glands (Ullman *et al.*, 1989). Evidence for this pathway can be obtained by analysing the development of the infection in the midgut, the ligament and the salivary glands using WMIS. Since the first infection of the salivary glands was expected to occur late in the second larval stage, these tissues were studied in larvae at 72 and 96 h p.a. and in adults.

Although many thrips were dissected, only a small number of midgut–ligament–salivary gland complexes were obtained. The first, but weak, infections in the salivary glands of *F. occidentalis* were observed at 72 h p.a. These infections often consisted of small patches, visible as a small row of positive signals at the connection site between the ligaments and salivary glands. Infections in the ligaments always preceded those in the salivary glands (Table 2). Infections restricted to the ligament always preceded those in the salivary gland. Infections in salivary glands were never observed when infections could not be discerned in the ligaments (Table 2). These observations support the hypothesis that the salivary glands become infected by a process of virus migration in which the ligaments are involved.

Infected ligaments were found in three out of 48 *T. tabaci* adults and none of the 16 larvae studied. Infections were not encountered in any of the salivary glands of *T. tabaci* analysed. These results show that the incompetence of the studied *T. tabaci* population to transmit TSWV may be explained by a poor translocation of the virus from the midgut through the ligament and, hence, the failure to reach and infect the salivary glands.

### Electron microscopic observations on midgut cells

Infection of the midgut epithelium and muscle cells has immunohistologically been demonstrated by light microscopy in both thrips species. To elucidate the virus structures accumulating in the infected cells, ultra-thin sections of the midgut of second instar larvae 72 h p.a. and adults were

studied by electron microscopy. Gold-labelled antibodies to the viral N protein and glycoproteins (G1/G2) were used to follow the virus infection process.

In *F. occidentalis* larvae 72 h p.a., many large aggregates of nucleocapsids were observed in the cytoplasm of the epithelial cells of the Mg1 (Fig. 4a). In two out of four thrips examined, virus particle-like structures were only found in the extra-cellular spaces of the basal labyrinth of the Mg1 epithelium (Fig. 4b) and in the cytoplasm of visceral muscle cells in Mg1 (Fig. 4c, d). These structures were specifically labelled with antibodies to both the N protein (Fig. 4b, c) and the G1 and G2 proteins (Fig. 4d), and, hence, could be identified as genuine TSWV particles. They were roughly spherical and their diameter was estimated to be approximately 100 nm (Fig. 4b–d), similar to the size of TSWV particles seen in plants.

Particles in different phases of budding and maturation were observed in the same region (Fig. 4b), suggesting that TSWV may bud through plasma membranes into the haemocoel of the vector. Unlike other bunyaviruses (Matsuoka *et al.*, 1991; Jäntti *et al.*, 1997) and TSWV in plant cells (Kikkert *et al.*, 1999), maturation of TSWV particles has so far not been observed at the Golgi apparatus in midgut cells.

No enveloped virus particles, however, were seen in the midgut muscle cells of adults, while nucleocapsid aggregates were found in these cells in two out of three *F. occidentalis* adults studied. The assembly of enveloped virus particles seems therefore to be a transient event in the midgut basal labyrinth and midgut visceral muscle tissues of larvae. The lack of nucleocapsid aggregates in the cytoplasm of adult midgut epithelial cells confirms the results obtained by light microscopy.

In *T. tabaci*, nucleocapsid aggregates, although less abundant than in *F. occidentalis*, were the only virus structures found in the Mg1 epithelium (Fig. 4e) and visceral muscle cells (Fig. 4f) of larvae 72 h p.a. and in visceral muscle cells of adults. Enveloped virus particles were not observed in the midgut of these larvae and adults. From these observations, it may be concluded that the virus is less apt to escape to muscle cells or the haemocoel in *T. tabaci* than in *F. occidentalis*.

## Discussion

A comparative study was made on the accumulation and translocation of TSWV within the body of thrips of an efficiently transmitting *F. occidentalis* population, and of a non-transmitting *T. tabaci* population (Wijkamp *et al.*, 1995). Primary infections were established in the midgut epithelium within 1–2 days when larvae of both species had access to virus early in their development. This observation shows that the differences in vector competence cannot be qualitatively explained at the level of receptor-mediated virus entry into the midgut epithelial cells. The current study unequivocally shows that this tissue becomes infected in larvae of transmitting *F. occidentalis* as well as of non-transmitting *T. tabaci* populations.

Following the establishment of the infection, the virus accumulates at different rates in the midgut epithelium, reaching the highest levels in the *F. occidentalis* larvae.

Virus could not be detected in the midgut epithelium of the adults of either population after pupation. The loss of the virus in the midgut epithelial cells may result from the elimination of the virus with these cells in the renewal processes, which take place during pupation (Müller, 1926). However, virus could readily be detected in the muscle cells of all three midgut regions in *F. occidentalis* adults, and much less in these cells of the non-transmitting *T. tabaci*. This large difference in muscle cell infection suggests that the virus is either translocated at a high rate from the epithelial cells to the muscle cells in *F. occidentalis* or that the muscle cells of this population are highly permissive for TSWV.

The salivary glands of *F. occidentalis* thrips, which transmitted as second instars, became infected before pupation. No or only a limited infection could be detected in these glands of larvae and adults, which fail to transmit. The early infection in the larval salivary glands strongly indicates that the virus has to reach these glands before pupation to become transmitters. This view is supported by the observation that most viruliferous *F. occidentalis* adults were converted to transmitters before pupation when they acquire the virus early in the first stage of their larval development (Wijkamp & Peters, 1993). Those adults which acquired virus but did not transmit as larvae and also not as adults in the first days after emerging, did not become transmitters even after a long period of incubation. This observation implies that the virus is translocated at a low rate, if at all, to the salivary glands in *F. occidentalis* adults.

A complete virus particle seems to be essential to initiate an infection in the thrips cells, as shown in primary cell cultures (Nagata *et al.*, 1999). No infection occurs when these cultures are inoculated with nucleocapsid preparations. This failure has to be explained by the absence of cellular receptor binding sites for the N proteins at the cell surface. The presence of proteins in the thrips which bind to one of the G proteins has been shown (Bandla *et al.*, 1998; Kikkert *et al.*, 1998). They found a 50 and a 92 kDa protein, respectively. Recently, Medeiros *et al.* (2000) demonstrated that the 50 kDa protein is a candidate for TSWV entry in the midgut.

The efficient replication of TSWV in the midgut epithelial cells may result in a rather thorough infection of the muscle cells and a timely migration of the virus to the salivary glands. This successful replication seems to be one of the factors determining the vector competence. The lower rate of virus accumulation in the midguts of the non-competent *T. tabaci* population compared to *F. occidentalis* has to be explained by a lower rate of replication and a restricted translocation of the virus. This conclusion is supported by the limited replication of the virus in primary cell cultures derived from this *T. tabaci* population (Nagata *et al.*, 1999).

The restricted virus spread in the visceral muscle tissue of *T. tabaci* (Fig. 3) implies that the virus escapes less efficiently

from its midgut epithelium than in *F. occidentalis*. Assembly of virus particles at the plasmalemma of the midgut epithelial cells may be essential to enter midgut muscle cells as the acquisition of an envelope plays a fundamental role in virus release and re-entry into neighbouring cells. Since the midgut muscle cells of *T. tabaci* occasionally become infected, it is tempting to speculate that lower amounts of complete virus particles are assembled in the *T. tabaci* population than in *F. occidentalis*. To infect the muscle cells, the virus should pass the basal lamina, which is a thick extracellular matrix lying on the basal membrane of the midgut (Lerdthusnee *et al.*, 1995; Kaslow & Welburn, 1997). Still a mechanism to explain how the virus particle passes this matrix is not fully elucidated; it is well known that the basal lamina acts as physical barrier for virus circulation. The thickness of this layer may play a role as a barrier regulating the transmission efficiency, as shown for La Crosse virus (LaCV; Grimstad & Walker, 1991). Paulson *et al.* (1989) demonstrated that the transmission of LaCV by *Aedes triseriatus* was primarily controlled by a midgut escape barrier, which was partially overcome by introducing the virus directly in the haemocoel. This technique could not successfully be used in our study.

Virus infection was readily detected in the ligaments and salivary glands in the larvae and adults of competent *F. occidentalis*. The observation that infection in the ligament preceded the infection of the salivary glands, and that the salivary gland infection was always accompanied by ligament infection (Table 2) strongly suggests that virus migration to the salivary glands occurs through this tissue. TSWV had been thought to migrate, like other arboviruses, from the midgut to the salivary glands through the haemocoel, although TSWV particles have not yet been encountered in the haemolymph in any study (Ullman *et al.*, 1995). The failure of thrips to become viruliferous after injecting adults with infectious virus particles (data not shown) supports the idea that the virus in the haemolymph does not serve as source for infection of the salivary glands.

The results obtained show that vector competence is determined by the degree to which the salivary glands become infected. Partial or weak infections in salivary glands rarely lead to virus transmission (Nagata *et al.*, 1999). A heavy infection in the salivary glands and transmission (50% of male individuals) were observed for the males of an arrhenotokous *T. tabaci* population, while the salivary glands of its non-transmitting females were not infected (data not shown). This observation suggests that the absence of infection of the salivary glands of the poor transmitting population is due to the failure to deliver the virus to this organ before pupation.

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