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Midgut infection by tomato spotted wilt virus and vector incompetence of *Frankliniella tritici*

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Abstract: The mechanism leading to vector competence of thrips species to transmit tomato spotted wilt virus (TSWV) is not well characterized. We investigated the interaction of TSWV and the non-vector species *Frankliniella tritici*. A monoclonal antibody to the non-structural protein (NSs) of the TSWV was used to detect TSWV replication within the thrips by immunofluorescence microscopy and enzyme-linked immonosorbent assay (ELISA). TSWV was acquired by *F. tritici*, replicated and moved within the alimentary canal of *F. tritici* similar to a known vector of TSWV, *Frankliniella occidentalis*. However, virus was not found in the salivary glands of *F. tritici*, which is a prerequisite to virus transmission. Thus, movement to the salivary glands may determine vector incompetence of *F. tritici*.

Key words: ELISA, fluorescence microscopy, thrips, transmission, TSWV

1 Introduction

Tomato spotted wilt virus (TSWV) is the type species of the genus *Tospovirus*, family *Bunyaviridae* (Van Regenmortel et al., 2000). TSWV transmission by thrips was first reported in the late 1920s (Sakimura, 1962), and subsequently found to be transmitted by several thrips species in a propagative manner (Ullman et al., 1993; Wijkamp et al., 1993; Mound, 1996; Ullman, 1996; Webb et al., 1998). As the non-structural protein (NSs) of the TSWV is produced only when the TSWV gene is expressed, the serological detection of NSs has been used as a standard to determine TSWV replication (De Haan et al., 1990; Kormelink et al., 1991; Ullman et al., 1993).

The inability of certain thrips species, such as *Frankliniela tritici*, to transmit TSWV has also been known for sometime and was considered to indicate the inability of the thrips to acquire the virus (Sakimura, 1953, 1963). The lack of viral replication or movement in a non-vector was not considered as a basis for vector incompetence. The use of immunofluorescence microscopy and enzyme-linked immunosorbent assay (ELISA) to assay for NSs permits further study on what limits successful viral transmission. A preliminary report has been published on that count (Assis Filho et al., 2004).

2 Materials and Methods

Thrips were collected from canola (*Brassica campestris*) flowers at the North Florida Research and Education Center (Quincy, FL, USA) and individually identified based on morphological characters (Mound and Kibby, 1998; Frantz and Fasulo, 2003) before establishing a colony. Further identification was completed periodically on progeny in the thrips colony and on all individuals dissected for immunofluorescence. Representative voucher specimens are deposited in the USDA-ARS-CMAVE (Tallahassee, FL, USA). A colony was established as described before for *Frankliniella occidentalis* and *Frankliniella fusca* (Assis Filho et al., 2002), except at a temperature of 26 ± 1°C, relative humidity 65%, and 16 : 8 h light : dark period. The fecundicity of *F. tritici* was less compared to maintained populations of *F. occidentalis* and *F. fusca*, thus the number of individuals available for immunofluorescence was limited. This was likely due to the repeated manipulation during species identification to assure the work was conducted with *F. tritici*, as *F. occidentalis* and *F. bispinosa* are morphologically very similar to *F. tritici*. TSWV previously identified (Pappu et al., 1998), and used in our earlier work (Assis Filho et al., 2002, 2004) was maintained on *Emilia sonchifolia* by thrips transmission and used for virus acquisition. First instar larvae, up to 24 h old, were given a 24 h acquisition access period (AAP) as previously described (Assis Filho et al., 2002). Immunofluorescence technique (Nagata et al., 1999) was used as described (Assis Filho et al., 2002) to evaluate TSWV replication within the larvae and adults reared from larvae following the AAP, using a monoclonal antibody to the NSs protein (Bandla et al., 1994).

To determine if *F. tritici* were naturally infected with TSWV, insects were collected from flowers of tomatoes and bell peppers at the USDA-ARS Research Center, Tallahassee. Adult thrips were identified up to the species level as described above and then assayed by antigen-coated plate (ACP) ELISA to detect TSWV NSs protein (Agdia Inc., Elkhart, IN). Representative voucher specimens are deposited at the USDA-ARS-CMAVE. All samples were run in duplicate. The positive threshold for ELISA was the optical density mean + 3 times the standard deviation of the optical density of known TSWV-free thrips.

3 Results and Discussion

Tomato spotted wilt virus replication was detected by immunofluorescence in both the larvae and adults of *F. tritici* as indicated by a specific label of the alimentary canal, characterized by bright label of epithelial cells and a lattice pattern label of muscle fibre cells (fig. 1). A total of 114 individuals were assayed, with the specific label present in seven individuals. No label was observed in the salivary glands, ligaments, tubular salivary glands, hindgut, or Malpighian tubules (data not shown). The label pattern observed is similar to the one observed in *F. occidentalis*, a known vector of TSWV, run as positive controls during this experiment and as published in our earlier works (Assis Filho et al., 2002, 2004).

A total of 259 field-collected insects were individually tested by ACP-ELISA for TSWV detection. Four (1.5%) individuals tested positive. Thus, a small percentage of *F. tritici* were found to have acquired TSWV in the field.

Detection of NSs protein indicates TSWV replication and this study shows that *F. tritici* acquired the virus by feeding on TSWV-infected plants either as a first larval instar under experimental conditions or naturally under field conditions. Therefore, the previous assumption that the absence of transmission was due to the absence of acquisition is not necessarily justified.

Tomato spotted wilt virus movement within a competent thrips vector has been well established (Assis Filho et al., 2002; Nagata et al., 2002a,b), but a similar study in a reported non-vector species has been lacking. TSWV invasion and replication in the salivary glands is required for successful virus transmission (Van de Wetering et al., 1996; Nagata et al., 1999, 2002a,b). The ligaments connecting the midgut and salivary glands have been identified as a route for virus movement to the salivary glands (Nagata et al., 1999; Assis Filho et al., 2002). The absence of TSWV detection in the ligaments and salivary glands reported here indicates that vector incompetence of *F. triciti* is likely determined by failure of virus to move into and replicate in the salivary glands. Absence of transmission in the presence of virus acquisition by other thrips species has been reported before (Wijkamp et al., 1996; Nagata et al., 2000; Ohnishi et al., 2001; Assis Filho et al., 2004).

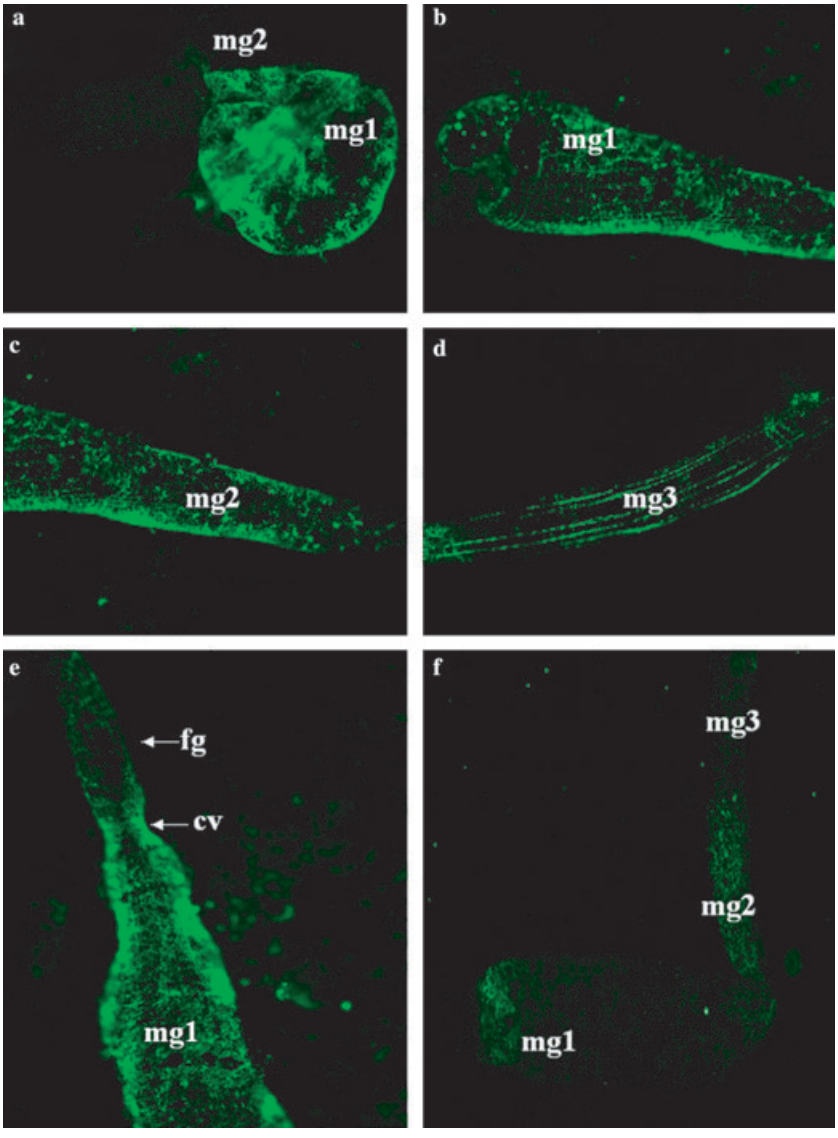

Fig. 1. *Immunolocalization of tomato spotted wilt virus infection in alimentary canal of* Frankliniella tritici *fluorescence microscopy coupled with a monoclonal antibody against the TSWV NSs protein. (a) Alimentary canal from second instar larva 5 days post-acquisition showing bright green label and the initial stage of a lattice pattern. Alimentary canal from adult 10 days post-acquisition at the larval stage showing the lattice pattern at mg1 (b), mg2 (c), and mg3 (d). (e) Alimentary canal from adult 12 days post-acquisition at the larval stage, showing label at mg1, cv and fg. (f) Alimentary canal from adult 10 days after feeding on TSWV-free* Emilia sonchifolia *at the larval stage, showing the absence of specific label at mg1, mg2 and mg3. Abbreviations: mg1, midgut 1; mg2, midgut 2; mg3, midgut 3; cv, cardiac valve; fg, foregut*

As *F. tritici* does not grow efficiently under laboratory conditions (Sakimura, 1953), it was impossible to establish a large colony to produce individuals for a study of magnitude similar to that previously completed on *F. occidentalis* and *F. fusca* (Assis Filho et al., 2002, 2004), in which over 3500 thrips were examined. However, the number of individuals was sufficient for species identification and to obtain progenies for virus acquisition. Similar to Sakimura's (1953) conclusion, our findings suggest that *F. tritici* does not transmit TSWV. Thus, *F. tritici* should still be considered as a non-vector thrips species for TSWV.

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