Acquisition of *Tomato spotted wilt virus* by Adults of Two Thrips Species

F. M. de Assis Filho, C. M. Deom, and J. L. Sherwood

Department of Plant Pathology, The University of Georgia, Athens 30602.
Current address of F. M. de Assis Filho: AGDIA, Inc., 30380 County Road 6, Elkhart, IN 56514.
Accepted for publication 10 November 2003.

ABSTRACT

Assis Filho, F. M. de, Deom, C. M., and Sherwood, J. L. 2004. Acquisition of *Tomato spotted wilt virus* by adults of two thrips species. Phytopathology 94:333-336.

Only larval thrips that acquire *Tomato spotted wilt virus* (TSWV), or adults derived from such larvae, transmit the virus. Nonviruliferous adults can ingest virus particles while feeding on TSWV-infected plants, but such adult thrips have not been shown to transmit TSWV. Immunofluorescence microscopy was used to show that thrips 1, 5, 10, and 20 days after adult emergence (DAE) fed on TSWV-infected plants acquired TSWV with virus replication and accumulation occurring in both epithelial and muscle cells of *Frankliniella fusca* (tobacco thrips [TT]) and *F. occidentalis* (western flower thrips [WFT]), as indicated by immunodetection of the nonstructural (NSs) protein encoded by the small RNA and the nucleocapsid (N) protein, respectively. Adult WFT acquired TSWV more efficiently than TT. There was no significant effect of insect age on TSWV acquisition by TT. In contrast, acquisition by adult WFT at 1 and 5 DAE was higher than acquisition at 10 and 20 DAE. Subsequent transmission competence of adult cohorts was studied by vector transmission assays. All adult thrips tested that had an acquisition access period as an adult were unable to transmit the virus. These results indicate the susceptibility of adult TT and WFT to infection of midgut cells by TSWV and subsequent virus replication and confirm earlier studies that adult thrips that feed on virus-infected plants do not transmit the virus. The role of a tissue barrier in TSWV movement and infection from midgut muscle cells to the salivary glands is discussed.

Additional keywords: adult acquisition, immunolabel, thrips transmission.

Tomato spotted wilt virus (TSWV) is the type species of the genus *Tospovirus* in the family *Bunyaviridae* (23). The virus is transmitted by several thrips species in a propagative manner (7,18). The interaction of TSWV and thrips that leads to virus transmission has been intensively studied (reviewed in literature citations 16,18). It is generally accepted that only larval thrips that acquire TSWV or adults deriving from such larvae transmit the virus (13,15,19,22). Although Ullman et al. (19) initially reported TSWV in the cytoplasm of midgut epithelial cells of *Frankliniella occidentalis* (western flower thrips [WFT]) fed on TSWV-infected plant as adults, it was later determined that this likely was not TSWV that was observed (21). Cho et al. (3) used enzyme-linked immunosorbent assay (ELISA) to assay for TSWV in nonviruliferous thrips 8 days after being transferred from an infected to a healthy plant, and found that the virus persisted in thrips for a period too long to be attributed to the virus being limited to the gut lumen. In two recent independent reports of TSWV acquisition by adult thrips, virus acquisition, but not transmission, was observed (11,12). In both studies, virus infection was restricted to epithelial cells at midgut-1, and virus was not detected in the surrounding muscle fibers.

We have studied the dynamics of TSWV replication in the alimentary canal of *F. fusca* (tobacco thrips [TT]) and WFT at all developmental stages (larva, pre-pupa, pupa, and adult) upon acquisition at the first instar larva (2). In the present study, TSWV acquisition by TT and WFT fed on TSWV-infected plants as adults was investigated, and the dynamics of virus replication and accumulation within thrips infected as adults in conjunction with virus transmissibility were determined. A preliminary report has been published (1).

Corresponding author: J. L. Sherwood; E-mail address: sherwood@uga.edu

Publication no. P-2004-0130-01R

MATERIALS AND METHODS

Thrips colony. TT and WFT were reared and maintained at room temperature with 24 h light. Pods of green beans (*Phaseolus vulgaris* L.) were used as food and changed every 2 days. One week prior to virus acquisition access, the thrips were transferred to TSWV-free *Emilia sonchifolia* for acclimation. Initial and periodic confirmations of thrips species identity were done using morphological characters of adults (8).

TSWV dynamics within the adult thrips. Thrips 1 to 2 days after emergence (DAE) from pupae as adults were given a 16-h acquisition access period (AAP) to TSWV-infected *E. sonchifolia*. Samples were taken immediately after the AAP, coined 0 hpa (hour postacquisition), and up to 35 days postacquisition (dpa) to investigate TSWV dynamics within the adult thrips. Both TSWV replication and accumulation were evaluated by immunofluorescence microscopy. The experiment was replicated three times for both TT and WFT.

Virus acquisition by adult thrips. A previously described TSWV isolate (14) maintained on *E. sonchifolia* was used. For virus acquisition, subpopulations of approximately 400 adult thrips were separately caged and given a 16-h AAP on detached leaves of TSWV-infected *E. sonchifolia* at 1, 5, 10, and 20 DAE. The insects were transferred to green bean pods after the AAP. Samples composed of 24 individuals were randomly taken at 10 to 14 days after AAP, and virus replication and accumulation in the midgut were evaluated by immunofluorescence assay. The experiment was conducted twice for each species, with five replications in each experiment. Statistical analyses were performed using analysis of variance (ANOVA) (SAS Institute, Cary, NC), with the percentage of labeled alimentary canals of each species

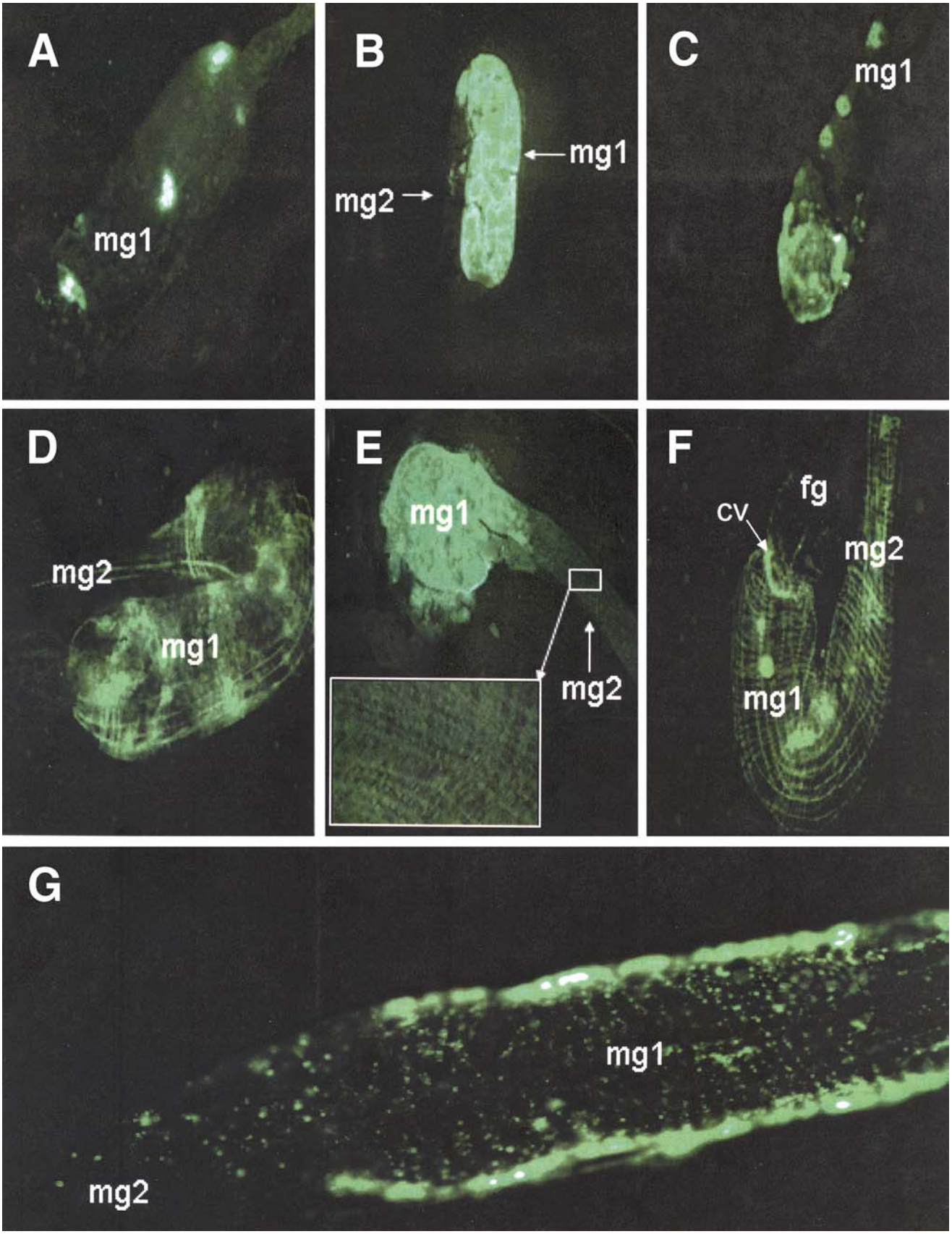

Fig. 1. Immunolocalization of *Tomato spotted wilt virus* infection in alimentary canal of two thrips that acquired the virus by feeding on infected plant at the adult stage. Antigen detection by immunofluorescence microscopy coupled with a monoclonal antibody **A to F,** against nonstructural protein or **G,** against the nucleocapsid protein. **A,** Alimentary canal from tobacco thrips (TT) 4 days postacquisition (dpa) showing bright green spots at midgut 1 (mg1). **B,** Alimentary canal from western flower thrips (WFT) 2 dpa showing extensive bright green label at mg1. **C,** Alimentary canal from WFT 2 dpa showing both bright spots and bright area at mg1. **D,** Alimentary canal from WFT 11 dpa showing both bright green and lattice pattern at mg1. **E,** Alimentary canal from TT 27 dpa showing extensive bright green label at mg1 and the lattice pattern at midgut 2 (mg2). Insert: higher magnification of mg2 region showing the lattice pattern label (muscle cells). **F,** Alimentary canal from WFT 16 dpa showing the lattice pattern at the whole midgut, cardiac valve (cv), and foregut (fg). **G**, Alimentary canal from WFT 10 dpa showing granular fluorescent label at midgut.

as the dependent variable. Differences referred to as significant are at $P < 0.05$. The experimental design was a split plot with species as the main plot, age as subplot, and five replications. The age effect was tested by one-way ANOVA.

Immunofluorescence assay. The whole-mount immunofluorescence staining technique (10) was used with modifications as described previously (2). Briefly, dissected alimentary canals were fixed with cold acetone; nonspecific binding sites were blocked with 5% nonfat dried milk. The primary antibody used was either a monoclonal anti-nonstructural (NSs) antibody for detection of virus replication or a monoclonal anti-nucleocapsid (N) antibody for detection of virus accumulation. The secondary antibody was conjugated to a fluorescent tag (Alexa Fluor 488 goat anti-mouse immunoglobulin G (IgG) conjugate, Molecular Probes, Eugene, OR), which allowed visualization with a fluorescence microscope (Model Labophot, Nikon Corp., Tokyo) equipped with a photometrics digital camera (Roper Scientific, Trenton, NJ). The following controls were used: alimentary canal from adult thrips fed on TSWV-free *E. sonchifolia*; no primary antibody; no secondary antibody; and a monoclonal primary antibody specific against an unrelated virus (*Peanut stripe mosaic virus*).

Virus transmission. A biological assay was used to determine if thrips fed on TSWV-infected plants as adults were able to transmit the virus. Groups of 10 to 50 individuals were confined to TSWV-free *E. sonchifolia* seedlings for an inoculation access period of 5 days. Three days later, ELISA was used to assay plants for infection. Virus-free control insects were similarly processed, except virus-free leaves were used as the feeding source.

RESULTS

TSWV dynamics within the adult thrips. The dynamics of TSWV infection within the alimentary canal of thrips following acquisition at the adult stage were similar in both TT and WFT. The first sign of specific label was observed as soon as 0 hpa. The label was observed as bright green spots, extensive bright green areas, or a mixture of bright green spots and extensive bright green areas (Fig. 1A to C). At later days after AAP, the bright green label was not as evident and a nascent lattice pattern was observed with the bright green pattern (Fig. 1D). As soon as 4 dpa, the label observed was a bright green pattern in midgut-1 and a lattice pattern in midgut-2 and midgut-3 (Fig. 1E). As the infection front appeared to progress, the foregut and cardiac valve showed specific label characterized by the lattice pattern (Fig. 1F). No label was seen at the salivary glands or at the ligaments that connect those organs to the midgut.

Virus acquisition by adult thrips. A total of 2,040 insects fed for 16 h on either healthy or TSWV-infected leaves were used for immunofluorescence assay to evaluate TSWV acquisition by thrips fed on TSWV-infected plant as adults. From the subpopulation that fed on TSWV-infected plants, a total of 565 out of 960 alimentary canals dissected from TT and 750 out of 960 from WFT were specifically labeled, indicating virus acquisition. Hence, WFT acquired TSWV more efficiently than TT. Both the NSs (Fig. 1F) and the N (Fig. 1G) proteins were detected, showing similar distribution along the alimentary canal, characterized by initial label of midgut epithelial cells followed by muscle cells. The label by anti-N antibodies was granular in appearance in contrast to the lattice pattern characteristic of anti-NSs. No label was observed on the alimentary canal from thrips fed on TSWV-free *E. sonchifolia* as well as in the other controls. There was no significant effect of insect age on TSWV acquisition by TT. In contrast, WFT that acquired the virus at 1 and 5 DAE differed statistically ($P = 0.05$) from those that acquired the virus at 10 and 20 DAE (Fig. 2).

Virus transmission. Transmission competence of adult cohorts was studied using a vector transmission assay. Over 2,000 thrips were used in transmission assays. None of the thrips assayed caused infection on *E. sonchifolia*, indicating that all adult thrips tested that had an AAP as an adult were unable to transmit the virus. These insects had been randomly taken from groups that were positive for TSWV acquisition by immunofluorescence assay.

DISCUSSION

Thrips fed on TSWV-infected *E. sonchifolia* at 1, 5, 10, and 20 DAE as adults from pupae were able to acquire the virus as indicated by detection of both NSs and N proteins by immunofluorescence microscopy using the dissected alimentary canal. Despite virus acquisition, no transmission was observed when infected thrips fed on the experimental host *E. sonchifolia.* Most reports on the absence of TSWV acquisition by thrips fed on TSWV-infected plants as adults are based on indirect evidence inferred by the lack of transmission (6,15,17). The use of microscopy coupled with high-quality antibodies provided the means to evaluate acquisition uncoupled from transmission. This study shows that ingestion, acquisition, and transmission of TSWV by thrips are distinct processes. Ingestion refers to TSWV entry into the alimentary canal of a thrips along with the food, which may or may not lead to acquisition. Acquisition refers to virus infection of the thrips cells, and transmission refers to infection of a healthy host by TSWV inoculated by thrips.

The use of immunofluorescence microscopy coupled with two monoclonal antibodies specific against the TSWV-NSs protein and the N protein indicates virus replication and accumulation within the thrips alimentary canal, respectively (2,4,5,20). It was shown that TSWV acquisition occurs when adult thrips feed on TSWV-infected plants. There was no difference in the pattern of virus acquisition/accumulation in TT and WFT. The midgut epithelial cells were initially infected followed by muscle cells. Infection of midgut muscle cells following adult acquisition has not been reported previously. The virus dynamics differ from when acquisition takes place at the larval stage (2) by the absence of virus in the ligaments and the salivary glands. This distinction predicates transmission (9,11,22) and supports previous reports that the ligaments are the routes of TSWV from the midgut to the salivary glands (2,10).

The results presented here on TSWV acquisition by adult thrips do not have epidemiological implications because the thrips that acquire TSWV as adults cannot act as a vector nor can they be a source of virus to the progeny because TSWV is not transovarially transmitted. However, the detection of TSWV replica-

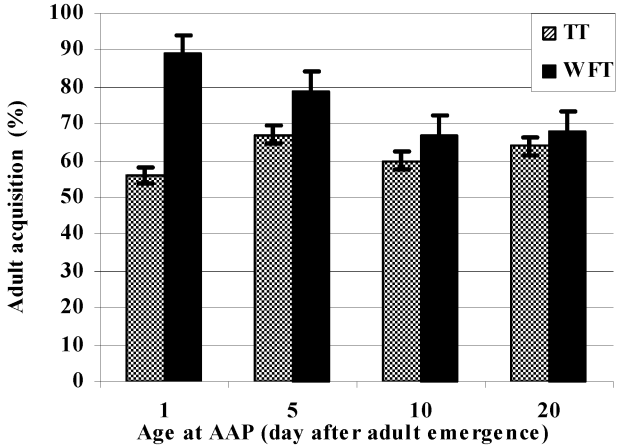

Fig. 2. *Tomato spotted wilt virus* acquisition by *Frankliniella fusca* (tobacco thrips [TT]) and *F. occidentalis* (western flower thrips [WFT]) determined by immunofluorescence microscopy. Means ± standard error are presented for five replications, with 24 alimentary canals per replication used to calculate percent acquisition ($n = 120$ per treatment). AAP = acquisition access period.

tion within the thrips that acquired the virus as an adult might need to be taken into consideration if abundance of viruliferous thrips in a crop inferred by detection of TSWV within the thrips is to be used as a parameter in a model to forecast epidemics. Finally, our observations indicate that the thrips midgut in adults does not constitute a barrier that prevents adult TT and WFT from acquiring TSWV. An event other than virus acquisition, such as a tissue barrier, possibly virus movement via the ligaments to salivary glands, apparently prevents TSWV transmission by TT and WFT that acquire the virus feeding on infected plants at the adult stage.

ACKNOWLEDGMENTS

This work was supported by the Georgia Agricultural Experiment Station and USDA-CSREES Special Grant 99-34412-7415 and the Peanut Collaborative Research Support Program of the United States Agency for International Development (Grant LAG-G-00-96-90013-00). We thank H. Scherm for assistance with the statistical analysis and for reviewing the manuscript.

LITERATURE CITED

1. Assis Filho, F. M. de, Deom, C. M., and Sherwood, J. L. 2002. Replication of *Tomato spotted wilt virus* ingested into the alimentary canal of adult thrips. (Abstr.) Phytopathology 92(suppl.):S4.
2. Assis Filho, F. M. de, Naidu, R., Deom, C. M., and Sherwood, J. L. 2002. The dynamics of *Tomato spotted wilt virus* replication in the alimentary canal of two thrips species. Phytopathology 92:729-733.
3. Cho, J. J., Mau, R. F. L., German, T. L., Hartmann, R. W., Yudin, L. S., Gonsalves, D., and Provvidenti, R. 1989. A multidisciplinary approach to management of tomato spotted wilt virus in Hawaii. Plant Dis. 73:375-383.
4. De Hann, P., Wagemakers, L., Peters, D., and Goldbach, R. 1990. The S RNA segment of tomato spotted wilt virus has an ambisense character. J. Gen. Virol. 71:1001-1007.
5. Kormelink, R., Kitajima, E. W., de Haan, P., Zuidema, D., Peters, D., and Goldbach, R. 1991. The nonstructural protein (NSs) encoded by the ambisense S RNA segment of tomato spotted wilt virus is associated with fibrous structures in infected plant cells. Virology 181:459-468.
6. Linford, M. B. 1932. Transmission of the pineapple yellow-spot virus by *Thrips tabaci*. Phytopathology 22:301-324.
7. Mound, L. A. 1996. The Thysanoptera vector species of tospoviruses. Acta Hortic. 431:298-309.
8. Mound, L. A., and Kibby, G. 1998. Thysanoptera–An Identification Guide. 2nd ed. CABI, Wallingford, UK.
9. Nagata, T., Almeida, A. C. L., Resende, R. O., and Avila, A. C. 2002. The transmission specificity and efficiency of tospoviruses. Pages 45-46 in: Thrips and Tospovirus. Proc. Int. Symp. Thysanoptera, 7th. R. Marulo and L. Mound, ed. Australian National Insect Council, Canberra, Australia.
10. Nagata, T., Inoue-Nagata, A. K., Smid, H. M., Goldbach, R., and Peters, D. 1999. Tissue tropism related to vector competence of *Frankliniella occidentalis* for tomato spotted wilt tospovirus. J. Gen. Virol. 80:507-515.
11. Nagata, T., Inoue-Nagata, A. K., van Lent, J., Goldbach, R., and Peters, D. 2002. Factors determining vector competence and specificity for transmission of *Tomato spotted wilt virus.* J. Gen. Virol. 83:663-671.
12. Ohnishi, J., Knight, L. M., Hosokawa, D., Fujisawa, I., and Tsuda, S. 2001. Replication of *Tomato spotted wilt virus* by adults *Thrips setosus* is restricted to midgut epithelial cells. Phytopathology 91:1149-1155.
13. Paliwal, Y. C. 1974. Some properties and thrips transmission of tomato spotted wilt virus in Canada. Can. J. Bot. 52:1177-1182.
14. Pappu, H. R., Todd, J. W., Culbreath, A. K., Bandla, M. D., and Sherwood, J. L. 1998. First report on the multiplication of tomato spotted wilt tospovirus in tobacco thrips *Frankliniella fusca*. Plant Dis. 82:1282.
15. Sakimura, K. 1963. *Frankliniella fusca*, an additional vector for the tomato spotted wilt virus, with notes on *Thrips tabaci*, a thrips vector. Phytopathology 53:412-415.
16. Sherwood, J. L., German, T. L., Whitfield, A. E., Moyer, J., and Ullman, D. E. 2001. Tospoviruses. Pages 1034-1040 in: Encyclopedia of Plant Pathology. O. C. Maloy and T. D. Murray, eds. John Wiley & Sons, New York.
17. Smith, K. M. 1932. Studies on plant virus diseases. XI. Further experiments with a ringspot virus: Its identification with spotted wilt of the tomato. Ann. Appl. Biol. 19:305-330.
18. Ullman, D. E. 1996. Thrips and tospoviruses: Advances and future directions. Acta Hortic. 431:310-324.
19. Ullman, D. E., Cho, J. J., Mau, R. F. L., Westcot, D. M., and Custer, D. M. 1992. A midgut barrier to tomato spotted wilt virus acquisition by adult western flower thrips. Phytopathology 82:1333-1342.
20. Ullman, D. E., German, T. L., Sherwood, J. L., Westcot, D. M., and Cantone, F. A. 1993. *Tospovirus* replication in insect vector cells: Immunocytochemical evidence that the nonstructural protein encoded by S RNA of tomato spotted wilt tospovirus is present in thrips vector cells. Phytopathology 83:456-463.
21. Ullman, D. E., Sherwood, J. L., and German, T. L. 1997. Thrips as vector of plant pathogens. Pages 539-565 in: Thrips as Crop Pest. T. Lewis, ed. CABI, Wallingford, UK.
22. Van de Wetering, F., Goldbach, R., and Peters, D. 1996. Tomato spotted wilt tospovirus ingestion by first instar larvae of *Frankliniella occidentalis* is a prerequisite for transmission. Phytopathology 86:900-905.
23. Van Regenmortel, M. H. V., Fauquet, C. M., Bishop, D. H. L., Carstens, E. B., Estes, M.K., Lemon, S. M., Moniloff, J., Mayo, M. A., McGeoch, D. J., Pringle, C. R., and Wickner, R. B. 2000. Virus taxonomy. Seventh Report of the ICTV. Pages 599-621. Academic Press, New York.