

## Seven Isolates of Citrus Tatter Leaf Virus Induce Varying Levels of Xylem Tissue Abnormalities in Two Citrange Rootstocks

Liza B. Vela and Mani Skaria

**ABSTRACT.** Preliminary light microscope observations of a Citrus tatter leaf virus (CTLV)-infected and a healthy trifoliolate hybrid rootstock showed some abnormal variations in the width of the xylem tissue. The xylem tissue of the infected stem was narrower in width compared to the healthy. Cross sections of CTLV-infected and healthy stems showed that the anatomical structure of the vascular bundle tissue varied in thickness. The thickness of the xylem tissue from separate plants infected with five local CTLV isolates and healthy plants of Rusk and Carrizo citrange were measured. Analysis showed a significant difference ( $P < 0.05$ ) in the cross-section thickness of the xylem tissue between the control and two CTLV isolates in Carrizo citrange and three CTLV isolates in Rusk citrange. The other CTLV isolates showed no significant differences.

Citrus tatter leaf virus (CTLV) is a member of the genus *Capillovirus* and is now considered to be identical to *Apple stem grooving virus* (6). CTLV was first discovered in California by Wallace and Drake (10) in 1962 on Meyer lemon trees introduced from China. In the 1960s and 70s this disease was reported from other places such as Florida in 1964 (4), Texas in 1975 (9), Japan in 1976 (7), South Africa in 1977 (1), and Australia in 1979 (2). This was followed by several other reports from elsewhere in other parts of the world, including a report on the widespread occurrence of CTLV in China (11).

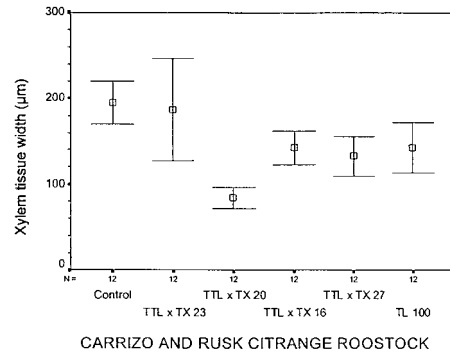
CTLV has flexuous filamentous virus particles of *ca.* 640 × 12 nm with obvious cross banding (6). CTLV is sap transmissible to at least 19 non-citrus hosts and is mechanically transmissible to citrus plants by knife blades or by leaf abrasion and by stem slashing (4, 8). The use of virus-free citrus budwood for propagation is the recommended management strategy for the control of CTLV infection in orchards. The majority of citrus in Texas is grown on sour orange rootstock, which is sensitive to *Citrus tristeza virus* (CTV). An imminent threat of CTV has prompted Texas to consider alternative rootstocks such as the trifoliolate orange hybrids, citranges, and Swingle citrumelo. Yet,

these rootstocks are sensitive to CTLV. The most common symptoms of CTLV on trifoliolate hybrids are chlorosis, tattering and ringspots on leaflets, distortion of the stem (zig-zag), and bud-union crease.

Herron and Skaria (5) conducted characterization studies on several CTLV isolates collected from Texas, including some that induced a bud union crease and ring spot on leaves. Preliminary observations of a CTLV-infected and a healthy trifoliolate hybrid rootstock showed some abnormal variations in the width of the xylem tissue. The xylem tissue of the infected stem was narrower in width compared to the healthy. This observation prompted us to measure and compare such variations, if any, in CTLV-sensitive rootstocks inoculated with CTLV isolates. In this study, we used healthy and infected Carrizo and Rusk citrange rootstocks for cross section preparation. Two healthy and two inoculated plants from each rootstock were used per CTLV isolate. The CTLV isolates used were the TL 100, TTL × TX 16, TTL × TX 20, TTL × TX 23 and TTL × TX 27. (TL 100 is generally known as a CTLV standard from California; however, it originated in Raymondville, TX). Samples for cross sections were taken from two to three inches below the growing tip of the rootstock. The diameter of the stem samples varied between 1.6 to 2.7

mm. From each stem, several free-hand cross sections were prepared for microscopy. Four to six of the best cross sections were stained with Toluidine Blue O (TBO) and three were selected for xylem tissue measurements. For each cross section, the width of the xylem tissue was measured at three sites and at each site three separate measurements were taken within a distance of 49.3 mm at 100 $\times$  and 48.8 mm at 200 $\times$  magnification. A one-way analysis of variance was performed on the healthy and CTLV infected stem tissue from five CTLV isolates to determine any significant differences between them using a program from SPSS, Inc.

Both Rusk and Carrizo citrange showed significant differences ( $P < 0.01$ ) ( $df = 1, 5$ ;  $F = 6.343$ ) between the control and CTLV isolates. Fig. 1 shows that isolate TTL  $\times$  TX 20 displayed the most significant difference between the control and the other



**Fig. 1. Comparative differences in the width of xylem tissue of Citrus tatter leaf virus-infected and healthy control citrange stems.**

CTLV isolates. Isolates TTL  $\times$  TX 16, TTL  $\times$  TX 27, and TL 100 also showed significant difference from the control. Further study of the above combinations is being prepared for electron microscopy to understand the alterations of the vascular bundle.

## LITERATURE CITED

1. da Graça, J. V.  
1977. Citrus tatter leaf virus in South African Meyer lemon. *Citrus Subtrop. Fruit J.* 529: 18.
2. Fraser, L. R. and P. Broadbent  
1979. Virus and related diseases of citrus in New South Wales. Dept. Agr. New South Wales, Australia. 52 pp.
3. Garnsey, S. M.  
1964. Detection of tatter leaf virus in Florida. *Proc. Fla. State Hort. Soc.* 77: 106-109.
4. Garnsey, S. M.  
1974. Mechanical transmission of a virus that produces tatter leaf symptoms in *Citrus excelsa*. In: *Proc. 6th Conf. IOCV*, 137-140. IOCV, Riverside, CA.
5. Herron, C. M. and M. Skaria.  
2000. Further studies on citrus tatter leaf virus in Texas. In: *Proc. 14th Conf. IOCV*, 185-194. IOCV, Riverside, CA.
6. Martelli, G. P., A. A. Agranovsky, M. Bar-Joseph, et al.  
2000. Genus *Capillovirus*. In: *Virus Taxonomy. 7th Report of the International Committee on Taxonomy of Viruses*, M. H. V. van Regenmortel et al. (eds.), 952-956. Academic Press, San Diego.
7. Miyakawa, T. and C. Matsui.  
1976. A bud-union abnormality of satsuma mandarin on *Poncirus trifoliata* rootstock in Japan. In: *Proc. 7th Conf. IOCV*, 125-131. IOCV, Riverside, CA.
8. Roistacher, C. N.  
1991. Tatterleaf. In: *Graft-Transmissible Diseases of Citrus, Handbook for Detection and Diagnosis*, C. N. Roistacher (ed.), 99-106. FAO, Rome.
9. Timmer, L. W.  
1975. Identification of citrange stunt virus from Meyer lemon in Texas. *J. Rio Grande Valley Hort. Soc.* 29: 65-69.
10. Wallace J. M. and R. J. Drake  
1962. Tatter leaf, a previously undescribed virus effect on citrus. *Plant Dis. Rept.* 46: 211-212.
11. Zhang, T. M., X. Y. Liang, and C. N. Roistacher  
1988. Occurrence and detection of citrus tatter leaf virus (CTLV) in Huangyan, Zhejiang Province, China. *Plant Dis.* 72: 543-545.