Further Studies on Citrus Tatter Leaf Virus in Texas

C. M. Herron and M. Skaria

ABSTRACT. Twenty-five citrus samples from 16 sites in the Lower Rio Grande Valley and Corpus Christi areas of Texas were indexed for Citrus tatter leaf virus (CTLV). Sixteen samples gave characteristic CTLV symptoms when biologically indexed onto citrumelo or citrange indicators. Six of the samples could also be readily detected by ELISA using the original donor tissue with CTLV and Apple stem grooving virus (ASGV) ELISA. No tissue samples reacted with Citrus tristeza virus antibodies. Indexing on limited herbaceous indicators proved unreliable under our conditions. Four of the samples with distinct CTLV symptoms gave bud union browning on citrange as early as 12 mo after grafting. Five samples were further indexed on a range of citrus indicators in 1997, the type and extent of symptoms observed are described.

Citrus tatter leaf virus (CTLV) is a filamentous virus of *ca.* 640 × 12 nm, and possesses a ssRNA of *Mr* 2.83×10^6 and a coat protein estimated at 27,000 Da (1, 26, 27, 28). Evidence based upon genome sequencing suggests that Apple stem grooving virus (ASGV), the type member of the *Capillovirus* genus (19), and CTLV are very closely related or even strains of the same virus (19, 26, 27). Antibodies raised against CTLV react with ASGV in Ouchterlony SDS double-diffusion tests and ELISA (15, 26).

CTLV is sap transmissible to at least 19 non-citrus hosts (9, 33, 36, 40) and is readily mechanically transmissible to citrus plants by knife blades or by leaf abrasion methods (9). There are no reports of seed or vector transmission, and very little is known about the role of latent and herbaceous hosts.

The only CTLV disease management strategy is the use of virus-free citrus budwood. Elimination of the virus via shoot-tip grafting has proved to be extremely difficult (25), although thermotherapy (35), the combination of thermotherapy and shoot-tip grafting (17) or thermotherapy and the use of the antiviral agent, ribavirin (14) have been used to eliminate the virus from various cultivars.

In the Lower Rio Grande Valley (LRGV) of Texas, the original introductions of Meyer lemon (13, 21) were noted as being incompatible with the commonly used sour orange rootstock, and later Citrus tristeza virus (CTV) was considered as the cause (29, 30). In 1930, a sour orange compatible Meyer lemon called the 'Rickert' strain became available (6), and this was found to be CTV- free (31). Wallace and Drake (38, 39) noted that many Meyer lemon trees latently carried what is now known as CTLV. Citrange stunt from a Meyer lemon source in the LRGV was also noted by Timmer (37). More recently, three budwood source trees in the LGRV, an Algerian tangerine, a Eureka lemon and a Meyer lemon were shown by biological indexing to be infected with CTLV (5).

The most devastating symptom caused by CTLV in commercial citrus is the bud union necrosis or abnormality which can develop when an infected symptomless cultivar is budded onto trifoliate orange, or trifoliate orange hybrid rootstocks (2, 8, 23, 24). Deep fluting of the rootstock trunk and an extended bud union crease can also develop. A general canopy chlorosis and stunting are normally associated with this symptom. In high winds, scions may sever completely at the bud union (34). However, some CTLV isolates do not cause a bud union disorder (24).

The recent movement of the efficient CTV vector, *Toxoptera citricida* Kirk. through Central America and the Caribbean into Florida (16, 32) has heightened concern amongst citrus scientists and growers in Texas. CTLV sensitive rootstocks such as citrange and citrumelo are 'CTV tolerant'. Commercial CTLV-infected scions grafted onto trifoliate could potentially suffer damage from CTLV. The objective of this study was to determine how widespread CTLV was in Texas and if available antisera could be used to detect any of the damaging CTLV from test tissue.

MATERIALS AND METHODS

Collection of test tissue. Twenty-five samples of budwood and leaves were collected from 13 sites from the Lower Rio Grande Valley and Corpus Christi areas of Texas during May and June, 1996. Sample tissues included Meyer lemon, Eureka lemon, Ponderosa lemon, variegated lemon, sweet lemon, Mexican lime, citremon, limequat, and lemonquat. The aim was to select as many different lime and lemon cultivars as possible as these were seen as the potential contamination source for grafting tools within the nurseries.

Indexing on citrus cultivars. The donor field samples were grafted to citrus receptors in two experiments. Experiment 1 was grafted on 6/26/96, to Troyer and Rusk citrange recipients. Experiment 2 donors were grafted onto Swingle citrumelo recipients on 6/30/96. Within each of these experiments, CTLV isolate TL-100 (obtained from C. N. Roistacher, UCR) was used as the positive control, being grafted onto Troyer and Rusk citrange recipients in experiment 1 and Swingle citrumelo in experiment 2. Another source of CTLV already identified (16-1) was also included as a test donor on Rusk citrange recipients in experiment 1. Nine of the donor graft tissues were found to be alive after the initial assessments were completed. These tissues were trained to grow under the same conditions, and the bark was peeled across the bud unions in 1997. Further indexing took place in 1997 with four of the donor samples from 1996 and two further sources of CTLV (39-13, 6-4), and six citrus recipients. Donor tissue in this case derived from the original donor tissue as in the previous experiments.

For each receptor/donor combination, six plants (three to a pot) were cultivated with two of the three plants per pot being inoculated, the third was left uninoculated, according to the methods outlined by Roistacher (33). Greenhouse conditions during testing ranged between 25.6° to 38.9°C and 34 to 100% relative humidity (1996), 36.7° to 17.8°C and 30 to 100% relative humidity (1997). The photosynthetic photon flux at plant level was a mean of 342 µmole. $m^{-2}s^{-1}$ (12 noon GMT, at 400-700 nm on 24/8/96) and a mean of 400 µmole. $m^{-2}s^{-1}$ (12 noon GMT at 400-700 nm on 24/3/97).

Citrus sources, inoculation and assessment. Troyer citrange, Rusk citrange, Swingle citrumelo, *Citrus excelsa* and rough lemon plants were raised from seed (33). Buds of C35 citrange from virus-free plants were propagated onto rough lemon for some tests. Two to six buds, blind buds, stem pieces or leaf pieces from each donor sample were graft inoculated onto pest-free, vigorously growing receptor seedlings of citrange or citrumelo, as outlined by Roistacher (33). Slash inoculation was used as an additional method for the lemonquat and limequat samples which failed to graft successfully. These hybrids are often noted for graft incompatibility with trifoliate orange hybrids. All grafting tools were dipped in sodium hypochlorite solution (1:4 v/v chlorox) between each grafting procedure. Visual assessments of all plants were carried out from 7 d after inoculation

and at regular intervals after this, three times per experiment. Foliar symptoms were detected using background lighting provided by Photo-ECT Sylvania 120V, 600W bulbs.

Indexing on herbaceous species. Cowpea (var. California Blackeye) and Red kidney bean (var. Shell bean) were raised from seed. Four seedlings were grown in one 1 gal pots. The seedlings were covered with small cages within the same greenhouse used for the citrus indexing in 1996. The plants were kept disease and pest free. *Chenopodium quinoa* L. was used in preliminary tests, but the plants bolted as soon as the first true leaves were formed due to the greenhouse conditions and therefore were not used. For each test sample two pots (four plants per pot) of cowpea and red kidney bean were used. One leaf on each of three test plants was inoculated per pot. The fourth plant per pot was left uninoculated. Plants of each species were also inoculated with test buffer on each test day. One pot of uninoculated plants of each species was kept with the test plants. The inoculum was very young citrus donor tissue titurated in a pre-chilled mortar with a pestle using cold 0.05 M potassium phosphate buffer, pH 7. A tissue:buffer ratio of 1:10 (w/v) was used. The resultant sap was then strained through two layers of sterile muslin. For some samples, dilutions of sap were made in the extraction buffer prior to inoculation. Primary leaves near to full expansion were dusted with carborundum and inoculated with a sterile muslin swab dipped into the inoculum. Test plants were then rinsed gently with tap water and shaded for 12 h, according to previous methods (9, 33). Care was taken to avoid cross contamination when handling more than one inoculum source. Plants were visually assessed for foliar symptoms daily for up to 14 d after inoculation.

Serology. CTLV and ASGV: Tissues from the indexing experiments were subject to DAS ELISA. Tissues from the citrus indexing experiments were tested beginning from approximately 3 mo after inoculation in 1996 and 1997. Three antisera were obtained from different sources and were used in replicated parallel assays. The CTLV polyclonal primary antibody and monoclonal conjugate (CTLV-J) were used as supplied and recommended by A. Kawai, Yokohama Plant Protection Station, Nakaku, Japan (15). Two antisera were derived from antibodies raised against ASGV. A crude ASGV antiserum was obtained from A. N. Adams, Horticulture Research International, East Malling, Kent, UK. This was purified by ammonium sulphate precipitation and conjugated to alkaline phosphatase (3,4) and referred to as ASGV-A. Duplicate assays on serially diluted coating and conjugated antibodies estimated the optimum dilutions for further DAS assays. The ASGV-L antisera were purchased as a kit from Loewe Biochemica, Spatzenlohweg 16, Germany and the kit instructions were followed except all volumes were halved, and the extraction buffer recommended was replaced with that used for the CTLV-J. Very young citrus leaf tissue was used for all tests. Extraction buffer and sap from virus-free citrus tissue in duplicate were included in every microplate as controls. The ASGV kit contained an ASGV positive and negative control derived from *C. quinoa,* additionally, healthy apple leaf tissue was used as a control in some of the tests.

ELISA procedures and buffers are outlined according to Clark and Adams (4), within kit instructions or where stated differently within the text. Volumes added to each well were 110 µl for coating antibody then 100 µl at every other step. Coating antibodies were used at 4.4 µg/ml for ASGV-A, and at 2.5 µg/ml for CTLV-J in coating buffer (0.05 M sodium carbonate, pH 9.6). Each tis-

sue sample was titurated in extraction buffer (PBST plus 2% polyvinyl pyrrolidone MW 40,000 at 1:20 w/v), and added to duplicate microplate wells. Alkaline phosphatase conjugates for CTLV-J and ASGV-A were diluted with extraction buffer (CTLV-J 1:400 or 1:800, ASGV-A 1:200). The plates were incubated overnight at 4°C or for 4 h at 37°C then washed three times with PBST between all stages. The substrate, ρ-nitrophenol phosphate was used at 1.0 mg/ml in substrate buffer (10% diethanolamine adjusted to pH 9.8 with 37% hydrochloric acid) in all tests. Assessments were read visually from 15 min after the addition of substrate. The hydrolyzed enzyme substrate extinction values were read at 405 nm with a Bio-Tec EIA reader three times. Samples that were twice the optical density of the healthy controls and gave a consistent positive visual score were considered positive.

CTV. Mid-rib tissue from fully expanded citrus leaves was ground in extraction buffer (1:10 w/v) as described for CTLV and used in DASI CTV ELISA (10). This system used a polyclonal CTV IgG, CREC 28 (1 μg/ml) raised in rabbit, a secondary antibody, G604-10 (used at 1:6,000) raised in goat, an antigoat alkaline phosphatase conjugate (Sigma A-4187 used at 1:30,000). The primary and secondary antibodies were supplied by R. F. Lee, Citrus Research and Education Center, University of Florida, Lake Alfred. Conditions and all procedures were as for CTLV-J, except that the substrate buffer used was the CTLV-J extraction buffer plus ovalbumin. Tissues for CTV testing were taken from all recipient and donor tissues twice during the indexing.

RESULTS

CTLV-like symptoms were observed 4 to 8 wk after inoculation in the citrange or citrumelo foliage of 16 of the 25 samples in the 1996 experiments (Table 1). In Troyer and Rusk citrange, symptoms of notching of the leaflet margin, distortion, asymmetry, abnormal undulations or epinasty, associated with later branch epinasty were observed. Irregular chlorotic leaflet patches were associated with these symptoms when the plants were observed with strong background lighting (Fig. 1). Uninoculated plants did not produce these symptoms. TTL001 and TTL005 inoculated Troyer citrange plants produced small new shoots compared to the uninoculated plants. Rusk citrange generally showed greater severity of symptoms in the foliage and stems with TL-100 when compared to Troyer citrange. In Swingle citrumelo, symptoms in the TL-100 inoculated plants were first observed seven weeks after inoculation (and later for the test donors) as irregular vein clearing on one or two young leaflets. A marked irregular leaflet chlorosis was later associated with this isolate, this developed to give distinct chlorotic leaf patches when later observed. Comparing TL-100 in experiments 1 and 2, Swingle citrumelo gave the most distinct foliar symptoms.

The 1997 citrus indexing gave a more comprehensive confirmation of CTLV symptoms in six recipents for some of the donors tested. Under the different environmental conditions, the symptoms on all recipients were similar to that in 1996 but more distinct (Table 2). First symptoms were noted 8 wk after inoculation. Additional symptoms were observed with TL-100. These symptoms were distinct chlorotic leaf ringspots on the leaflets of Swingle citrumelo (Fig. 2). Swingle citrumelo gave the most distinct foliar symptoms for all the samples tested. Many leaflets of every plant inoculated were contorted, asymmetric and developed chlorotic patches. Rusk citrange and Troyer citrange

TABLE 1
SUMMARY OF 1996 CTLV INDEXING AND SEROLOGICAL TESTS

Host/Donor Source[z]	Citrus recipient symptoms[y]	ELISA[x]			
		Assay tissue	CLV-J	ASGV-L	ASGV-A
39-13	+ Carrizo	Etrog citron	+	+	—
6-4	+ Carrizo	citrange[w]	—	—	—
16-1	+ Carrizo	Etrog citron; Carrizo	+	+	—
TTL001	+ Troyer	Meyer lemon[v]	+	+	—
TTL002,4,5,7,11,12,13	+ Troyer	citrange	—	—	—
TTL003,6,8,9,10,14	— Troyer	citrange	—	—	—
TTL015,19	— Rusk	citrange	—	—	—
TTL016	+ Troyer	Meyer lemon	+	+	+
TTL017	+ Rusk	Eureka lemon	+	+	—
TTL018	+ Rusk	citrange	—	—	—
TTL020	+ Swingle	Meyer lemon	+	+	—
TTL021	— Swingle	citrumelo	—	—	—
TTL022	+ Swingle	citrumelo	—	—	—
TL-100	+[u]	[u]	+	+	+
Uninoculated	—[u]	[u]	—	—	—
Healthy Meyer	not tested	Meyer lemon	—	—	—
ASGV +	not tested	[t]	+	+	+
ASGV -	not tested	[t]	—	—	—

[z]Sources with the same results are grouped together in the same row.
[y]CTLV- like symptoms on indicator (named): + = symptoms; — = no symptoms.
[x]ELISA (OD_{405}) results: + = positive; — = negative.
[w]Citrange tissue was used in parallel tests in two earlier CTLV-J ELISA tests using lemon tissue and this sample was positive in both tests.
[v]Troyer citrange tissue also reacted with CTLV-J.
[u]Tissue used was from Carrizo citrange, Troyer citrange, Rusk citrange and Swingle citrumelo.
[t]Apple or *Chenopodium quinoa* tissue was used.

again gave more moderate foliar symptoms. Carrizo and C35 citrange gave the most indistinct foliar symptoms, however, some plants had only one leaflet contorted. Isolate 6-4 gave very small elongated chlorotic flecks on the leaves of *C. excelsa*. There was a distinct stunting of 6-4, TL-100, and 16-1 in inoculated *C. excelsa* compared with the uninoculated *C. excelsa* controls.

Bud union browning and creases were observed with isolates 39-13 (18 mo Fig. 3.), TTL016 (12 mo), TTL017 (14 mo), and TTL020 (14 mo) -the time of observation after inoculation is given in brackets. Buds from TTL001, TTL007, TTL014, TTL015, and TTL018 failed to produce any bud union abnormalities up to 18 mo after inoculation.

The inoculated herbaceous plants produced variable symptoms according to the test plant and between tests with the same donor in some cases. Small, 4 mm diameter, dark brown necrotic spots with chlorotic halos were visible seven days after inoculation in all Red kidney bean plants inoculated with isolate 39-13. All cowpea plants inoculated with sap from 39-13 showed no symptoms. TL-100 inoculated Red kidney bean plants gave a systemic vein necrosis 14 days after inoculation. Cowpeas inoculated with the same sap from TL-100 remained healthy. Sap from isolate 6-4 produced no symptoms in Red kidney bean.

In the ELISA tests, CTLV-J and ASGV-L antisera reacted with sap from TL-100, and six other isolates (Table 1). Most of the detectable test samples were tissues from Meyer lemon, Etrog citron or lemon, that is symptomless CTLV tissues. There

TABLE 2
SUMMARY OF 1997 CTLV BIOLOGICAL INDEXING

	Indicator[z]							
Donor ID	Carrizo	C35	Rusk	Troyer	Swingle	*C. excelsa*	Controls	Bud union plants/total tested #
TL-100	s	s	s, ld, lc	s, ld, e	s, ld, lc, e, r	s	0	nt
39-13	s, ld	s, ld	s, ld	s, ld	s, ld, lc, e	0	0	1/1
6-4	s, ld	nt	s, ld, lc, e	s, ld, lc, e	s, ld, lc, e	s, cf	0	nt
16-1	s, ld	nt	s, ld, lc, e	s, ld, lc, e	s, ld, lc, e	s	0	nt
TTL020	ld	s, ld	s, ld, lc, e	s, ld, lc, e	s, ld, lc,	nt	0	1/1
TTL016	s, ld, lc, e	s, ld, lc	s, ld, lc	s, ld, lc, e	s, ld, lc, e	nt	0	1/1

[z]nt = not tested. All symptoms are those compared to the uninoculated controls: 0 = no symptoms; s = plant stunted; ld = leaflet distortion or puckering; lc = chlorotic patches on leaflets; e = stem and branch epinasty; r = chlorotic ringspots on leaflets; cf = chlorotic flecks on leaves. # = number of plants with bud union crease/total number of plants in test.

Fig. 1. Irregular chlorotic leaflet patches induced by Citrus tatter leaf virus on Rusk citrange

were two notable exceptions to this; isolate 16-1, which had been inoculated onto the test Rusk citrange plant in 1994 and was detected by CTLV-J and ASGV-L but not by ASGV-A, then isolate TTL001 which was detected in Troyer citrange tissue by CTLV-J alone. Tissues from 10 samples which produced CTLV-like symptoms in citrus indicators were not detected by CTLV-J, all tissues came from citrange or citrumelo recipients in 1996. ASGV-A detected isolates TL-100, TTL0016 and the respective positive controls. None of the experimental tissues tested reacted with the CTV antibodies.

Fig. 2. Chlorotic ringspots induced by Citrus tatter leaf virus TL-100 on Swingle citrumelo.

Fig. 3. Bud union gap between a Meyer lemon scion and Carrizo citrange rootstock, and rootstock browning induced by Citrus tatter leaf virus isolate 39-13.

DISCUSSION

The symptom expression in citrus indicator plants described in this study strongly suggests that CTLV is present in most of the citrus sampled in the LRGV of Texas. The CTLV symptoms reported here have been reported by workers elsewhere (7, 8, 12, 20, 22, 33).

From the 16 CTLV isolates, nine were tested for bud union disorders. Four of these nine isolates produced bud union gaps on citrange or citrumelo stocks as early as 12 mo after inoculation. These four CTLV isolates also were positive in ELISA

tests using CTLV and ASGV antibodies as early as 3 mo after inoculation using recipent tissue. Whilst most data collaborate previous conclusions that symptomless CTLV citrus tissue provides better detection of CTLV than sensitive CTLV tissue such as the trifoliate hybrids (11), there are exceptions. Of the remaining five CTLV isolates which did not produce bud union disorders, one was ELISA positive. TTL001 could be detected on Troyer citrange tissue using CTLV-J antibodies but at 14 mo after inoculation had not produced a bud union crease. All donor and recipient tissues were tested for CTV by ELISA. All samples failed to react with the CTV antibodies. This may indicate that the original source of CTLV could have been the 'Rickert' Meyer lemon (6, 31).

The herbaceous indexing was unreliable under the testing conditions. A systemic leaf vein necrosis in red kidney bean found in this study has been observed for ASGV inoculated Red kidney bean (18), but not reported for CTLV. Small dark brown necrotic lesions in cowpea have been reported as being diagnostic for CTLV detection by other workers (33) were not found in this work.

Since it seems CTLV can infect apple or pear and ASGV can infect citrus (27, personal observation) then these woody plants may be an important inoculum source. Plant nurseries in Texas may contain many citrus species, apple and pear, therefore, contamination may occur via grafting knives. From a phytosanitory standpoint this is important information, apart from the epidemiological and viral evolutionary implications. From this work, an ELISA-based test with available CTLV or ASGV antibodies provides the quickest and cheapest indication of CTLV in the field. However, there is still an urgent need to be able to distinguish consistently and rapidly between the damaging and non-damaging CTLV isolates, so that the extent of the current problem may be reliably estimated.

ACKNOWLEDGMENTS

Financial support for this project was from the USDA-APHIS-PPQ through a Co-operative Agricultural Pest Survey (CAPS) agreement to Dr. M. Skaria and Dr. J. V. French. Appreciation to Dr. R. F. Lee, Citrus Research and Education Center, University of Florida, USA and Dr. R. G. Hicks, School of Biology and Biochemistry, University of Bath, UK and the University of Bristol, UK for help with various aspects of the work. Many thanks to Dr. A. N. Adams and Dr. A. Kawai for kindly supplying antisera, and Nora Solís-Gracia for data compilation.

LITERATURE CITED

1. Bar-Joseph, M. and G. P. Martelli
   1991. Capilloviruses. Classification and nomenclature of viruses. Fifth report of the international committee on taxonomy of viruses. Arch. Virol. (Suppl. 2): 339-340.
2. Calavan, E. C., D. W. Christiansen, and C. N. Roistacher
   1963. Symptoms associated with tatterleaf virus infection of Troyer citrange rootstock. Plant Dis. Reptr. 47: 971-975.
3. Clark, M. F.
   1981. Immunosorbent assays in plant pathology. Ann. Rev. Phytopathol. 19: 83-106.
4. Clark, M. F. and A. N. Adams
   1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34: 475-484.
5. da Graça, J. V. and M. Skaria
   1996. Citrus tatterleaf virus in the Rio Grande Valley of South Texas. In: *Proc. 13th Conf. IOCV*, 198-200. IOCV, Riverside, CA.
6. Friend, W. H.
   1954. History of the Meyer lemon in the Valley. Proc. 8th Ann. Inst. Rio Grande Valley Hort. Soc.: 32-33.

7. Garnsey, S. M.
1964. Detection of tatter leaf virus of citrus in Florida. Proc. Fla. State Hort. Soc. 77: 106-109.
8. Garnsey, S. M.
1970. Viruses in Florida's 'Meyer' lemon trees and their effects on other citrus. Proc. Fla. State Hort. Soc. 83: 66-71.
9. Garnsey, S. M.
1974. Mechanical transmission of a virus that produces tatterleaf symptoms in Citrus excelsa. In: *Proc. 6th Conf. IOCV*, 137-140. IOCV, Gainesville.
10. Garnsey, S. M. and M. Cambra
1991. Enzyme-linked immunosorbent assay (ELISA) for citrus pathogens. In: *Graft Transmissible Diseases of Citrus - Handbook for Detection and Diagnosis*. C. N. Roistacher (ed.), 193-209. FAO, Rome.
11. Garnsey, S. M. and J. W. Jones
1968. Relationship of symptoms to the presence of tatterleaf virus in several citrus hosts. In: *Proc. 4th Conf. IOCV*, 206-212. IOCV, Gainesville.
12. Garnsey, S. M. and T. Miyakawa
1989. Tatterleaf and citrange stunt virus. In: *Compendium of Citrus Diseases*. J. O. Whiteside, S. M. Garnsey and L. W. Timmer (eds.), 47-48. APS Press, St. Paul. 80 pp.
13. Hodgson, R. W.
1967. Horticultural varieties of citrus. In: *The Citrus Industry Vol. 1*. W. Reuther, H. J. Webber and L. D. Batchelor (eds.), 431-591. Univ. Calif., Berkeley.
14. Iwanami, T. and H. Ieki
1994. Elimination of citrus tatterleaf virus from shoots of potted citrus plants by ribavirin. Ann. Phytopathol. Soc. Japan 60: 595-599.
15. Kawai, A., T. Tsukamoto, S. Namba, and T. Nishio
1995. Properties of the citrus tatter leaf virus (CTLV) and development of serological detection system of the virus. In: *Proc. 13th Conf. IOCV*, 339-342. IOCV, Riverside, CA.
16. Knapp, J. L., H. W. Browning, R. F. Lee, and P. A. Stansly
1996. Citrus tristeza virus relationship and management guidelines for Florida citrus. Citrus Ind. 77(3): 12-15
17. Koizumi, M.
1984. Elimination of tatterleaf citrange stunt virus from satsuma mandarin by shoot tip grafting following pre-heat treatment. In: *Proc. 9th Conf. IOCV*. 229-233. IOCV, Riverside, CA.
18. Lister, R. M.
1970. Apple stem grooving virus. CMI/AAB Descr. Plant Viruses No. 31.
19. Magome, H., N. Yoshokawa, T. Takahashi, T. Ito, and T. Miyakawa
1997. Molecular variability of the genomes of capilloviruses from apple, Japanese pear, European pear and citrus trees. Phytopathology 87: 389-396.
20. Marais, L. J. and R. F. Lee
1986. Citrange stunt virus associated with decline of Shamouti on Swingle citrumelo rootstock in South Africa. Plant. Dis. 70: 892 (Abstr.).
21. McKee, R.
1926. Chinese dwarf Meyer lemon introduced. In: *Yearbook of Agriculture*. United States Department of Agriculture. 218-221.
22. Miyakawa, T.
1980. Occurrence and varietal distribution of tatter leaf-citrange stunt virus and its affects on Japanese citrus. In: *Proc. 8th Conf. IOCV*, 220-224. Univ. Florida, Gainesville.
23. Miyakawa, T. and C. Matsui
1976. A bud-union abnormality on *Poncirus trifoliata* rootstock in Japan. In: *Proc. 7th Conf. IOCV*, 125-131. IOCV, Riverside, CA.
24. Miyakawa, T. and M. Tsuji
1988. The association of tatterleaf virus to bud-union crease of trees on trifoliate orange rootstock. In: *Proc. 10th Conf. IOCV*, 360-364. IOCV, Riverside, CA.
25. Navarro, L., E. L. Civerolo, J. Juarez, and S. M. Garnsey
1991. Improving therapy methods for citrus germplasm exchange. In: *Proc. 11th Conf. IOCV*, 400-408. IOCV, Riverside, CA.
26. Nishio, T., A. Kawai, T. Takahashi, S. Namba, and S. Yamashita
1989. Purification and properties of citrus tatter leaf virus. Ann. Phytopathol. Soc. Japan 55: 254-258.
27. Ohira, K., T. Ito, A. Kawai, S. Namba, T. Kusumi, and T. Tsuchizachi
1994. Nucleotide sequence of the 3'-terminal region of citrus tatter leaf virus RNA. Virus Genes 8: 169-172.
28. Ohira, K., S. Namba, M. Rozanov, T. Kusumi, and T. Tsuchizaki
1995. Complete sequence of an infectious full-length cDNA clone of citrus tatterleaf

virus: comparative sequence analysis of capillovirus genomes. J. Gen. Virol. 76: 2305-2309.

29. Olson, E. O.
1955. A survey for tristeza virus in Texas citrus. Proc. 9th Ann. Inst. Rio Grande Valley Hort. Soc.: 51-60.
30. Olson, E. O.
1956. Mild and severe strains of tristeza virus in Texas citrus. Phytopathology 46: 336-341.
31. Olson, E. O. and B. Sleeth
1954. Tristeza virus carried by some Meyer lemon trees in South Texas. Proc.8th Ann. Inst. Rio Grande Valley Hort. Soc.: 84-88.
32. Rocha-Peña, M. A., C. L. Niblett, R. F. Lee, F. M. Ochoa-Corona, R. Lastra, S. M. Garnsey, and R. K. Yokomi
1995. Citrus tristeza virus and its vector *Toxoptera citricida*-threats to citrus production in the Caribbean and Central and North America. Plant Dis. 79: 434-444.
33. Roistacher, C. N.
1991. Tatterleaf. In: *Graft Transmissible Diseases of Citrus - Handbook for Detection and Diagnosis*. C. N. Roistacher (ed.), 99-106. FAO, Rome.
34. Roistacher, C. N.
1995. A historical review of the major graft-transmissible diseases of citrus. FAO, Rome.
35. Roistacher, C. N., L. Navarro, and T. Murashige
1976. Recovery of citrus selections free of several viruses, exocortis viroid, and *Spiroplasma citri* by shoot-tip grafting in vitro. In: *Proc. 7th Conf. IOCV*, 186-193. IOCV, Riverside, CA.
36. Semancik, J. S. and L. G. Weathers
1965. Partial purification of a mechanically transmissible virus associated with tatterleaf of citrus. Phytopathology 55: 1354-1358.
37. Timmer, L. W.
1975. Identification of citrange stunt virus from Meyer lemon in Texas. J Rio Grande Valley Hort. Soc. 29: 65-69.
38. Wallace, J. M. and R. J. Drake
1962. Tatterleaf, a previously undescribed virus effect on citrus. Plant Dis. Rept. 46: 211-212.
39. Wallace, J. M. and R. J. Drake
1968. Citrange stunt and ringspot, two previously undescribed virus diseases of citrus. In: *Proc. 4th Conf. IOCV*, 177-180. Univ. Florida, Gainesville.
40. Zhang, T. M., X. Y. Liang, and C. N. Roistacher
1988. Occurrence and detection of citrus tatterleaf virus (CTLV) in Huangyan, Zhejiang province, China. Plant Dis. 72: 543-545.