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Title

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Journal

International Organization of Citrus Virologists Conference Proceedings
(1957-2010), 10(10)

ISSN

2313-5123

Author

Roistacher, C. N.

Publication Date

1988

DOI

10.5070/C54p63m962

Peer reviewed

Citrus Tatterleaf Virus: Further Evidence for a Single Virus Complex

C. N. Roistacher

ABSTRACT. Studies have continued over 11.5 yr attempting to separate citrus tatterleaf virus (CTLV) from citrange stunt virus (CSV). Previous studies with buds taken from recovered shoots of *Citrus excelsa* infected with CTLV and graft-inoculated to sour orange seedlings or virus-free Meyer lemon as holding plants have shown that subsequent periodic bud inoculations from these holding plants back to *C. excelsa* and citrange induced no symptoms in the *C. excelsa* for 2.5 to 5 yr, whereas the citrange always reacted. However after 5 yr in the holding plants inoculations to *C. excelsa* and citrange showed positive symptoms in both indicators. The recovered *C. excelsa* apparently acted as a temporary filter plant for CTLV.

Studies were continued using Mexican lime as the filter host plant. Inoculation passage of the tatterleaf virus complex was made from three individual sources of Meyer lemon through Mexican lime and then from recovered Mexican lime into rough lemon, citron, Dweet tangor, and sour orange as holding plants. Periodic bud-inoculations from these holding plants back to *C. excelsa* and citrange were made continually over an 8- to 11.5-yr period with the following results: 1) Passages from symptomless rough lemon, citron, Dweet tangor or sour orange to citrange were always positive. 2) Passage of the virus complex via Mexican lime through rough lemon as the holding plant showed an incubation period of 3 to 4 yr before sub-inoculations to *C. excelsa* were positive. 3) Passage of the virus complex via Mexican lime through citron as the holding plant has shown no reaction in *C. excelsa* after a period of 8.5 yr. 4) Passage of the virus complex via Mexican lime through sour orange and Dweet tangor as the holding plants have shown, in general, no reaction in *C. excelsa* over an 11.5-yr period with one exception. Symptoms were observed in *C. excelsa* in one test at 10.5 yr.

Use of a single name i.e., the citrus tatterleaf virus, for this complex is suggested.

Citrus tatterleaf virus (CTLV) was first described and named by Wallace and Drake (11) in 1962. It was found when Meyer or Beijing lemon budwood was indexed to seedlings of *C. excelsa* and induced abnormal torn or tattered leaf symptoms. Leaf blotch or psorosis-like symptoms were also observed in young and mature leaves of *C. excelsa* and Mexican lime. Wallace and Drake (12) later described an apparent new virus associated with CTLV when they indexed Meyer lemon budwood to seedlings of citrange or citremon. The reactions in these hosts were strong blotchy spots, deformed leaves and stunted stems which showed severe pitting and grooving. They based the separation of CTLV from this new virus, which they called citrange stunt (CSV), on the observation that when symptomless, recovered shoots of *C. excelsa* were reinoculated with the original Meyer lemon tissue, typical tatterleaf symptoms appeared in the new growth of *C. excelsa* leaves. Recovered and symptomless tissue of

C. excelsa would not induce the tatterleaf reaction when indexed to *C. excelsa*, but did cause CSV when indexed to citrange or citremon.

Garnsey (2) could not separate CTLV and CSV by mechanical transmission. He found that citron tissue previously graft-inoculated with Meyer lemon budwood transmitted CTLV and CSV to herbaceous hosts and both components were mechanically transmitted back to citron.

Roistacher (8) demonstrated that when sour orange or Meyer lemon plants previously graft-inoculated with buds of recovered shoots of *C. excelsa* were periodically indexed to *C. excelsa* and citrange, only the citrange would show positive reaction for the first few years. However, after 5 yr both components were recovered from the sour orange and Meyer lemon holding plants. Thus, the existence of two separate viruses as proposed by Wallace and Drake (12) and predicated on the theory that recovered shoots of *C. excelsa* would filter the CTLV was not substan-

tiated. However, Roistacher (8) also showed that only the CSV component could be transmitted out of seedlings of rough lemon, citron, Dweet tangor or sour orange inoculated from the infected Mexican lime.

This paper reports on continued studies for over 11.5 yr to separate CTLV and CSV when Mexican lime is used as the intermediate filtering host plant.

METHODS AND MATERIALS

The Meyer lemon sources used as inoculum were: *MEY 100*—a Meyer lemon collected in Texas in 1958 by Dr. J. M. Wallace. When indexed it was found negative for tristeza, psorosis, concave gum, vein enation, exocortis and cachexia. *MEY 101*—a Meyer lemon collected in 1969 from Atkins nursery, Fallbrook, California. When indexed, it was found negative for tristeza, psorosis, concave gum, exocortis and cachexia. *MEY 102*—a Meyer lemon collected in 1962 by Dr. John Carpenter from the 4-Winds nursery, San Jose, California. When indexed it was found negative for tristeza, psorosis, concave gum, vein enation, exocortis and cachexia. Tissue from all three of the above sources of Meyer lemon consistently induced strong positive leaf symptoms in *C. excelsa* and Troyer or Rusk citrange indicator seedlings.

Expt. 1. When budwood from a 5-yr-old tree derived from a cutting of *MEY 102*, located at the South Coast Field Station in Orange County, California, was indexed, it induced severe blotchy psorosis-like symptoms in Mexican lime. Sub-inoculations from this infected Mexican lime to Dweet tangor and sour orange seedlings revealed very mild psorosis-like patterns in the leaves of Dweet tangor but no symptoms in leaves of sour orange. The inoculated Dweet tangor and sour orange were held and when budwood from these were periodically graft-inoculated to *C. excelsa* and Troyer citrange, only the citrange

showed symptoms over 6 yr as previously reported (8). This experiment was continued, and over an 11.5-yr period 23 inoculation tests were made at approximately 6- to 7-month intervals from these infected Dweet tangor and sour orange holding plants to *C. excelsa* and Troyer or Rusk citrange. The original holding plants of Dweet tangor and sour orange began to deteriorate after 1 yr and were subcultured as single buds on rough lemon seedlings. These budded plants have been maintained as holding plants and used as inoculum during the course of the experiment.

Expt. 2. Buds from the infected Dweet tangor and sour orange from Expt., 1 were graft-inoculated to citron seedlings as a third holding plant host. The citron was cut back periodically and new growth tissue was used to periodically inoculate *C. excelsa* and Troyer or Rusk citrange at 3 to 6-month intervals over 8.5 yr.

Expt. 3. Buds of each of the three Meyer lemon source plants (*MEY 100*, *MEY 101*, and *MEY 102*) kept as source trees in a virus bank at the Department of Plant Pathology Rubidoux facility were inoculated into three Mexican lime seedlings, respectively. After 12 months (test A), and again at 16 months (test B), buds from recovered shoots of the three Mexican limes showing no psorosis-like symptoms were graft-inoculated to rough lemon seedlings, as holding plants. Sub-inoculations were made from these holding plants to *C. excelsa* and Troyer or Rusk citrange indicator seedlings at approximately 6-month intervals for 8 yr.

All inoculated index plants were observed for symptoms for at least 6 months. Greenhouse temperatures were maintained at 26-29/19-20°C (maximum day/minimum night).

RESULTS

Expt. 1. The results of indexing to *C. excelsa* over 11.5 yr are shown in fig. 1 as Expt. 1. The sequence of graft inoculations was from *MEY 102*

Fig. 1. Expression of symptoms of tatterleaf virus in *Citrus excelsa* (CE) indicator seedlings after sequential passage by tissue graft from infected Meyer lemon (MEY) through Mexican lime (ML). Then from recovered Mexican lime to various symptomless holding plants of rough lemon (RO LE), Dweet tangor (DW), sour orange (SO) and citron, and from these holding plants to *C. excelsa* over a period of 11.5 yr. All parallel inoculations to Troyer or Rusk citrange were positive.

to Mexican lime, from recovered Mexican lime to Dweet tangor and sour orange as holding plants, and from the Dweet tangor and sour orange holding plants to *C. excelsa* and citrange indicator plants. No symptoms were evident in the *C. excelsa* indicator seedlings in 18 tests over 10 yr whereas parallel inoculations to citrange were always positive. However, in the 19th test after 10.5 yr, positive transmission was achieved in 6 of 22 inoculated *C. excelsa* indicator plants from both the Dweet tangor and sour orange holding plants. However, in four additional tests over a 1-yr period, symptoms of tatterleaf were not transmitted to the *C. excelsa* indicator plants.

Expt. 2. The sequence was the same as in Expt. 1, but with one added holding plant host, i.e., citron which was inoculated with buds from the Dweet tangor and sour orange of Expt. 1. Budwood taken from these two citrons was periodically inoculated to *C. excelsa* and citrange in 17 tests for 8.5 yr as shown in fig. 1 as Expt. 2. To date, no positive reaction has been observed in the inoculated *C. excelsa* indicator seedlings, whereas all parallel inoculations to citrange indicator seedlings were positive.

Expt. 3. In this experiment the entire inoculation sequence was repeated in two separate tests, 6 months apart, using inoculum from the three Meyer lemon bank plants sequentially inoculated to Mexican lime; then from recovered Mexican lime to rough lemon seedlings; and from these as holding plants to *C. excelsa* and citrange. Budwood from each of six rough lemon holding plants was periodically indexed to *C. excelsa* and citrange at approximately 6-month intervals for 8 yr.

The incubation period of CTLV in the rough lemon holding plants is shown in fig. 1 (Expt. 3). The first appearance of tatterleaf (TL) symptoms in *C. excelsa* for test A and test B, respectively, was 37 and 34 months for MEY 100; 56 and 48

months MEY 101; and 75 and 48 months for MEY 102. Again, as in Expt. 1 and 2, all parallel inoculations to citrange were consistently positive.

Positive controls were included for each index test. Inoculum used was from the original source plants of MEY 100, MEY 101, and MEY 102 held in a screenhouse. Nearly all inoculations to *C. excelsa* and citrange index plants from these primary source plants were positive for all tests.

Distribution of CTLV in rough lemon holding plants. When symptoms were first observed in the *C. excelsa* indicator plant previously inoculated with buds from the rough lemon holding plant after a 37-month incubation (Expt. 3), a test was made to determine the distribution of the virus within the rough lemon holding plant. The plant in test A (MEY 100 to Mexican lime to rough lemon) had been inoculated with buds of recovered Mexican lime in February 1980, and the first positive transmission from this holding plant to *C. excelsa* index plants was observed after a 37-month incubation. Transmission tests to study virus distribution in this holding plant were made 10 months later. Budwood was taken from all parts of this plant, carefully coded, and inoculated to *C. excelsa* seedlings by 2 bud grafts. Symptoms were observed within 8 to 12 weeks after inoculation. Fig. 2 shows that although CTLV was well distributed in this rough lemon holding plant it was not systemic. Numbers 1 to 12 in fig. 2 represent branches used as index inoculum 10 months after first symptoms were observed; 6 of the 12 branches indexed positive for CTLV and were found at all levels of the plant. The results of a second index test of new growth done 4 months after the first test are given as numbers 13 to 26 in fig. 2. The lower case letters a, b, c and d represent new growth from previously cut branches of the first test of numbers 1 to 12. Eight positives were obtained of 22

Fig. 2. Distribution of citrus tatterleaf virus in a rough lemon holding plant which had been inoculated with tissue from a recovered Mexican lime. The first evidence of a tatterleaf reaction in inoculated seedlings of *Citrus excelsa* occurred 37 months after the rough lemon was inoculated. Distribution indexing was performed at 10 months (No. 1-12) and at 14 months (No. 13-26) after the appearance of the first symptoms.

inoculations to *C. excelsa* and these were also distributed throughout the plant. In branch No. 3 (fig. 2) which was negative in the first test, regrowth was positive in the second test 4 months later. Conversely, branches which were positive in the first test were negative in 2 of 3 regrowth branches in the second test and branch 11, positive in the first test, was negative in 3 of 4 regrowth branches in the second test.

A third test was made to study the distribution of CTLV in a holding plant of rough lemon (plant B, Expt. 3—MEY 100 to Mexican lime to rough lemon). This holding plant had been

inoculated in July 1980 and was positive for CTLV after a 34-month incubation period. An index for distribution of CTLV was made as soon as symptoms were observed and when the new growth in the rough lemon holding plant had sufficiently matured. Fig. 3 shows that only one branch of 11 tested positive on *C. excelsa*, and this was from a low-growing branch.

Fig. 3. Distribution of citrus tatterleaf virus in a rough lemon holding plant which had been inoculated with tissue from a recovered Mexican lime. The first evidence of a tatterleaf reaction in seedlings of *Citrus excelsa* occurred 34 months after the rough lemon was inoculated. The distribution index was performed 10 weeks after the appearance of the first symptoms. Note that only one branch was infected at that time.

DISCUSSION

The objective of these experiments was a further attempt to find out if tatterleaf and citrange stunt are two separate viruses or a single virus complex. The criterion, as proposed by Wallace and Drake (12), for calling citrange stunt a separate and distinct virus, i.e. the failure to transmit from recovered *C. excelsa*, was not fulfilled. In the previous report (8), where recovered shoots of *C. excelsa* obtained from three sources of Meyer lemon were inoculated into 11 holding plants, all of the holding plants ultimately transmitted positive to *C. excelsa* indicator plants. However, there was an incubation period of 30 to 60 months in these holding plants for the *C. excelsa*, symptom-inducing component before the first transmission. When inoculations are made from a *C. excelsa* showing symptoms of TL or from a recovered shoot of *C. excelsa* to *C. excelsa*, there appears to be a strong filtering or inhibitory action which prevents symptoms from being expressed in the inoculated *C. excelsa*; yet both the *C. excelsa* and the recovered *C. excelsa* will induce a reaction when inoculated to citrange index plants. However, if tissue from these citrange plants is sub-inoculated back to *C. excelsa*, positive TL symptoms will show in the *C. excelsa* (8, table 1). Mexican lime may act as a more potent filtering or inhibiting host than *C. excelsa*. In Expt. 1, where Mexican lime tissue was sub-inoculated to Dweet tangor and sour orange as holding plants, the component responsible for symptom reaction of *C. excelsa* was not evident for a period of 10 yr, but possibly became evident at 10.5 yr. Similarly in Expt. 3, where rough lemon was the holding plant, the component responsible for inducing symptoms in *C. excelsa* was not present, or perhaps, inhibited for 34 to 75 months for all of the six holding plants. However, in Expt. 2, where citron was the holding plant, symptoms of TL in *C. excelsa* have not as yet been induced from the cit-

ron tissue 8.5 yr after inoculation with infected-recovered Mexican lime tissue.

Other observations also suggest that CTLV may be a single virus complex rather than two separate viruses. All heat treatment tests of Meyer lemon budwood for elimination of CTLV from infected tissue failed to separate the complex into two components (4, 5). Also, shoot-tip grafting did not separate the components into separate viruses (6, 7). Garnsey (2) failed to separate the components by mechanical transmission in passage from infected citron to *C. excelsa* or Rusk citrange. Citrons were mechanically inoculated by leaf abrasions with inoculum from systemically infected *Nicotiana clelandii* leaves which had been in turn mechanically inoculated from infected citron plants. All purification attempts show a single type of flexuous rod particle (1, 3, 9) which appear similar. Attempts at purification of a "pure citrange stunt" from infected Troyer citrange inoculated from tissue in Experiment 1 also showed similar flexuous rods (Roistacher and Bar-Joseph, unpublished).

The behavior of this virus complex is comparable to that of psorosis-A and psorosis-B (10) where a sweet orange tree which is infected with psorosis may not develop the bark lesion component (psorosis-B) for up to 50 yr or longer, but can transmit this component to a very high percentage of progeny trees in which bark scaling will develop in 7 to 15 yr.

The uneven distribution of virus in the rough lemon holding plant shown in figs. 2 and 3 is somewhat similar to the uneven distribution shown by Garnsey and Jones (3) for citrange. This uneven distribution might be expected to decline with time.

The evidence presented in these studies and in previous studies (8), plus the lack of separation of the CTLV and CSV components by thermotherapy, shoot tip grafting, mechanical transmission and electron microscopy, all suggest that there are

not two separate and distinct viruses but, perhaps, one virus complex. Until such time as the components are permanently separated, purified, and Koch's postulates applied, the name citrus tatterleaf virus (CTLV) should be retained to designate this complex. Tatterleaf was first described by Wallace and Drake (11) and the symptoms induced in leaves of citrange are similar enough to that induced in *C. excelsa* i.e., tattered leaf margins and blotchy spots, to have this name cover both component responses.

ACKNOWLEDGMENTS

I wish to express my appreciation and thanks to Richard Blue, John Bash, Reg Wagner and Theresa Carson for their dedication and help in the inoculations and care of plants over this 11.5-yr period. Also to Mr. Ed Nauer, for his excellent supervision of the various horticultural aspects in the Citrus Variety Improvement Program where these studies were conducted. We also wish to express our appreciation to the California Citrus Advisory Board for their long and generous financial support.

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