Continued Attempts Over a 22-Year Period to Separate Components of The Citrus Tatter Leaf-Citrange Stunt Virus Complex

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ABSTRACT. The relationship of citrus tatter leaf virus (CTLV) and citrange stunt virus (CStV) has been in question for many years. Two separate viruses were proposed by Wallace and Drake (9) based on data which shows that when an old-line Meyer lemon infected with CTLV is used to inoculate *Citrus excelsa* (CE) indicator seedlings, typical CTLV symptoms appear in the initial flush of growth. However, in subsequent flushes of growth, symptomless "recovered" shoots may sometimes appear. When this latter "recovered" tissue is used to inoculate CE and citrange indicator plants, strong CTLV symptoms occur in citrange but no symptoms appear in the CE. When virus-free Meyer lemon plants, or sour orange seedlings were graft-inoculated with "recovered" CE tissue and held over a long period, back-indexing of these plants to CE and citrange resulted in CTLV symptoms appearing in the new growth of citrange; whereas CE did not develop symptoms until after 2 to 5 yr thus indicating a temporary separation of the two virus components (5). This separation and reappearance of symptoms was also achieved using CTLV from infected and symptomatic Mexican lime (ML) seedlings by passage through a rough lemon holding plant. If however, tissue from symptomatic ML was used to inoculate Dweet tangor or sour orange, repeated sub-inoculations from these two holding plants over a 10-yr-period never resulted in symptoms in CE; whereas excellent symptoms always appeared in citrange, indicating a separation of the two components (6). This paper reports on results obtained over more than 20 yr showing that the citrange stunt component of the CTLV can be blocked or possibly eliminated in some hosts by some unknown host interaction.

The tatter leaf disease of citrus, induced by the citrus tatter leaf virus (CTLV) was first described by Wallace and Drake (8) as a transmissible disease found in old line Meyer lemon trees. When tissue from a Meyer lemon is inoculated into indicator seedlings of *Citrus excelsa* (CE), also called Kalpi lime, it induces a mottle plus a tearing-like or tattering of the margins of leaves, hence the name tatter leaf. The origin of this disease is almost certainly China where the virus is found to be well distributed (12). The disease is endemic in Japan, Taiwan and many countries of southeast Asia. Spread of this virus throughout other parts of the world was primarily by the distribution of the Meyer lemon named after plant explorer Frank Meyer who found the plant in Beijing, China in 1905 (2) and brought plants and budwood to the United States. Calavan et al. (1) first showed the destructive potential of this disease on citrange rootstock. When tatter leaf-infected Meyer lemon tissue was graft-inoculated to satsuma mandarin budded on Troyer citrange rootstock, severe stunting occurred with symptoms of deep fluting and pitting on the citrange rootstock accompanied by an intense bud-union crease and brown line at this crease. The presence of the tatter leaf disease limits the use of trifoliate orange or its hybrids, which are very valuable rootstocks for citrus wherever the disease is endemic, specifically China, Japan and Taiwan. For example, in Zhejiang Province, China, all citrus was shown to be infected with CTLV (13) and trifoliate orange could not be used as a rootstock. The Gou-tao sour orange, tolerant to the CTLV is universally used throughout the Province. This rootstock is subject to severe stem pitting and for many reasons (cold tolerance, fruit quality, tolerance to tristeza, etc.) it is not as desirable a rootstock as trifoliate.

CTLV is highly mechanically transmissible, is difficult to eliminate from tissue by shoot tip graft-

ing but can be eliminated from budwood by thermotherapy. Wallace and Drake (9) proposed that the Meyer lemon contained another virus in addition to CTLV. They showed that when symptomless tissue from a recovered shoot of CE was graft-inoculated to seedlings of CE and to citrange or trifoliate hybrids, no symptoms were induced in the CE but the citranges or trifoliate hybrids always showed strong symptoms. Also, if the symptomless recovered shoot of CE was inoculated with virus-infected Meyer lemon tissue, the CE would show symptoms indicating no cross protection. They concluded that the virus which affected the citrange but did not affect the CE was a new virus and they called it the citrange stunt virus (CSV).

In attempts to test whether tatter leaf and citrus stunt were one or two separate viruses, Roistacher (5) showed that sub-inoculations from recovered CE to holding plants of virus-free Meyer lemon and sour orange seedlings induced a temporary separation of the two components which lasted from 2.5 to 5.5 yr. After this period of time, the tatter leaf component reappeared in both of these holding plants. These results suggested that there were not two separate viruses present but there were two components of one virus and the original name of 'tatter leaf' as proposed by Wallace and Drake (8) should be used as an umbrella for both components.

However, Roistacher (5) did show an apparent separation of the two components when budwood from infected Meyer lemon was first inoculated into ML which showed specific mild psorosis-like symptoms (Fig. 2). This reaction in ML had been reported and illustrated by Wallace (10) and is a valid symptom reaction of the tatter leaf virus in ML. When sub-inoculations were made from the symptomatic ML to Dweet tangor or sour orange and these were kept as holding plants, periodic inoculations to CE from these holding plants induced no symptoms in the CE over a period of 5½ yr. However, inoculations to citrange consistently showed a strong positive reaction. This suggested a possible continued separation of the two components in these two holding plants.

Roistacher (6) continued research on the emergence or separation of the tatter leaf and citrange stunt components. In passage from symptomless but tatter leaf-infected Meyer lemon source TL-102 to ML, and then from this symptomatic ML to rough lemon, the CE reactive component emerged in the rough lemon holding plant after 4.5 yr. However, if graft-transmissions were done from this symptomatic ML to Dweet tangor and sour orange, and sub-inoculations made from the Dweet and sour orange to indicator seedlings, again there was no reaction in CE even after 11.5 yr, but the citranges always showed strong positive reactions.

This paper reports continued attempts over a period totaling 22 yr to see if the separation of these two components would persist in their holding plants of Dweet tangor and sour orange.

MATERIALS AND METHODS

Figure 1 diagrammatically outlines this experiment, a continuation of the experiment reported by Roistacher (6) and extends the research for an additional 10 yr. In previous studies (5, 6), three sources of Meyer lemon were used and all reacted similarly. In this study we concentrate on one source, Meyer TL-102, a 5 yr old tree derived from a cutting of a Meyer lemon collected in 1958 by Dr. John Carpenter from the Dillon's 4-Winds Nursery in Mission San Jose, California. Comprehensive indexing tests were done prior to introducing this Meyer

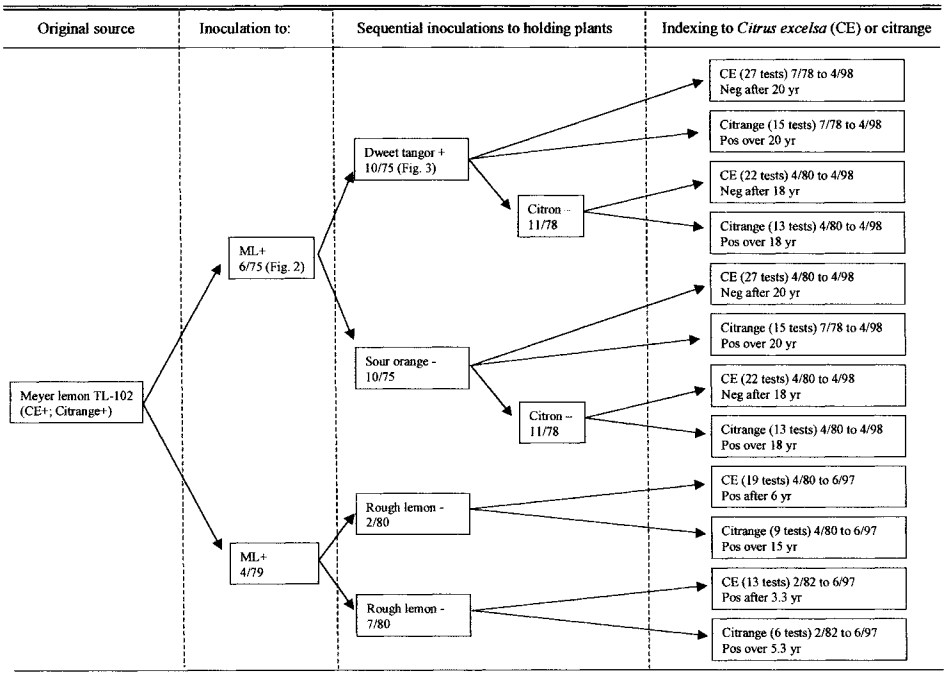

Fig. 1. Sequence of inoculations from Meyer Lemon TL-102 to Mexican lime and subsequently to Dweet tangor, sour orange, citron, and rough lemon and final reaction in *Citrus excelsa* and Troyer or Rusk citrange. + = Symptoms observed in holding plants; - = No symptoms observed in holding plants; Neg = Negative reaction; Pos = Positive reaction.

lemon as a candidate in our California Citrus Variety Improvement Program as V.I. 141. This index showed that this Meyer source was positive for tatter leaf virus in both CE and citrange indicator plants but was free of all other viruses and viroids. Continued indexing of TL-102 since 1972 had shown consistent positive reactions in both CE and citrange indicator plants.

Buds or blind buds from Meyer (TL-102) were inoculated into seedlings of ML in June, 1975. Distinct psorosis-like symptoms developed in many leaves of the ML (Fig. 2). In October, 1975, sub-inoculations were made from symptomatic ML to holding plants of Dweet tangor and sour orange. In July, 1980, inoculation were made from the symptomatic ML plants to rough lemon. A mild psorosis-like mottle was observed in the Dweet tangor (Fig. 3), but no symptoms were seen in the sour orange or rough lemon holding plants. A further sub-inoculation from symptomatic Dweet tangor was done to citron in November, 1978 and no symptoms were evident in the citron. These four holding plants (rough lemon, Dweet tangor, sour orange and citron) were used as inoculum sources to inoculate the indicators of CE and various citranges (Troyer, Carrizo or Rusk).

All plants were grown at the Rubidoux glasshouse at the University of California indexing facility. Soils, fertilizers and plant care was based on the U.C. system of plant growth (7). Inoculated plants were held at a cool temperature regime of 24° to 27°C maximum day and 18° to 21°C minimum night. Positive and negative controls were always included in each test. Approximately 4 mo after inoculation, plants were

Fig. 2. Symptoms in Mexican lime leaf induced by the citrus tatter leaf virus inoculated into Mexican lime from tatter leaf infected 'Meyer" lemon. Control leaf on right.

Fig. 3. Mild mottle in the leaf of Dweet tangor inoculated with symptomatic tissue of Mexican lime (Fig. 1). Control leaf on right.

cut back to produce a second flush of new growth. Inoculated plants were observed for symptoms for 4 to 6 mo after inoculation. Eleven additional experiments, a continuation of the experiment of Roistacher (6), were conducted from January, 1987 through April, 1998, extending the period of testing of the holding plants from 12 to over 22 yr.

RESULTS AND DISCUSSION

Sequential passage from Meyer lemon TL-102 to Mexican lime and through rough lemon. As shown in Fig. 1, the tatter leaf component from Meyer TL-102 transmitted to ML and then to rough lemon as the holding plant was not expressed in the CE indicators until 3.3 to 6 yr. This indicated some form of slow incubation or replication in the rough lemon holding plants. This delayed reaction in the rough lemon holding plants also occurred with two other Meyer lemon source trees: Meyer TL-100 and Meyer TL-101 (6) where delay before symptom expression in CE was 4 and 5 yr respectively. Thus, both the tatter leaf and citrange stunt components of the CTLV complex were present in the symptomless rough lemon holding plants but the tatter leaf component was not expressed initially, and took a number of years to develop and emerge in the rough lemon. Continuing tests over the additional 10-yr period from 1987 to 1997 showed strong positive tatter leaf reaction in both the CE and citrange indicator plants, indicating both components were now present and fully active in the rough lemon holding plants. The reason for this delayed reaction is not known and could be due to: i) a the CE component replicates slowly or poorly in the initial symptomatic ML plants or in the rough lemon holding plant; ii) the CE component was initially in low concentration in these plants; or iii) some other mechanism which is not understood.

Sequential passage from Meyer lemon TL-102 to Mexican lime and through Dweet tangor or sour orange. As shown in Fig. 1, when Meyer lemon source TL-102 was inoculated to ML which showed a distinct leaf mottle (Fig. 2) and then sub-inoculated from the symptomatic ML to Dweet tangor seedlings, the Dweet tangor leaves also showed a mild but distinct psorosis-like mottle (Fig. 3). However, the inoculated sour orange holding plant

remained symptomless. Sub-inoculations from the Dweet tangor and sour orange holding plants to CE in 27 index tests to 184 plants over a 22-yr period showed no emergence of the tatter leaf component in either the Dweet tangor or the sour orange holding plants. However, sub-inoculations in 13 tests to 54 citrange indicator plants over this same period, there was consistent positive reactions in the citrange indicators. Positive controls derived from tissue of the original Meyer TL-102 source gave consistent positive reaction in all tests to both the CE and the citrange indicators. It is apparent that there was some form of replicative blockage of the CE tatter leaf component in the two holding plants of Dweet tangor and sour orange.

Sequential passage from Meyer lemon TL-102 to Mexican lime and then through Dweet tangor or sour orange to citron. As shown in Table 1, sub-inoculations in 22 tests to 169 indicator plants over a period of 18 yr, the tatter leaf component did not develop in the citron holding plant since there was no reaction in the inoculated CE indicator plants. However, citranges were consistently positive in all tests as were the positive controls of CE and citrange inoculated with Meyer source TL-102.

The reason why the tatter leaf component was blocked or possibly eliminated when it was first passed through ML and then through either Dweet tangor, sour orange or Etrog citron as holding plants, even after a holding period of over 22 yr in some of these respective hosts, is not clear or understood. In contrast, when CTLV present in three sources of Meyer lemon (TL-100. TL-101 and TL-102) were inoculated directly to sour orange without subsequent passage though ML, the tatter leaf component appeared in the sour orange holding plants after a period of 3 to 6 yr (6). This was also true if the holding plants were virus-free Meyer lemon plants (5). Also, when the initial passage from Meyer lemon was done first to ML and sequentially inoculated to rough lemon, the tatter leaf component would re-appear in the rough lemon after a few years.

Despite the blockage of the tatter leaf component by the Dweet tangor or sour orange holding plants inoculated with tissue of symptomatic ML we still believe that the CTLV is one virus containing strains or variants and not composed of two individual viruses as suggested by Wallace and Drake (9) and the name citrus tatter leaf virus should be retained as the formal name for the virus, and the name tatter leaf for this disease. Recent research indicates that CTLV is a Capillovirus (3) and is closely related to the apple stem grooving virus (11). It has also been sequenced (4). Perhaps further research at the molecular level may illuminate the nature of this virus and the reason for the blockage of the tatter leaf component when passed from symptomatic ML to Dweet tangor or sour orange.

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