

Occurrence and Detection of Citrus Tatter Leaf Virus (CTLV) in Huangyan, Zhejiang Province, China

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ABSTRACT

Zhang, T. M., Liang, X. Y., and Roistacher, C. N. 1988. Occurrence and detection of citrus tatter leaf virus (CTLV) in Huangyan, Zhejiang Province, China. *Plant Disease* 72: 543-545.

Citrus tatter leaf virus (CTLV) was detected by graft transmission to indicator plants of Troyer and Carrizo citrange (*Poncirus trifoliata* (L.) Raf. × *Citrus sinensis* (L.) Osb.), and by mechanical transmission to cowpea (*Vigna unguiculata* (L.) Walp.). Graft transmission from infected plants induced characteristic CTLV symptoms of leaf blotch and leaf distortion on leaves of Troyer or Carrizo citrange, and mechanical transmission induced characteristic necrotic localized lesions on leaves of cowpea. Citrus tatter leaf virus was transmitted from all of 31 field trees representing the nine major cultivars growing on two rootstocks, *P. trifoliata* and Gou-tou-cheng (a sour orange hybrid) in the Huangyan district of Zhejiang Province, China. These nine cultivars were: Ben-di-zao (*Citrus succosa* Hort. ex Tan.), Mang-ju (*C. tardiferax* Hort. ex Tan.), Zhao-ju (*C. subcompressa*), Zhu-hong (*C. erythroa*), Ru-Ji (*C. kinokuni* Hort. ex Tan.), Bei-jing (Meyer lemon *C. limon* L. Burm. f. hybrid), Ponkan (*C. poonensis* Hort.), Okitsu-Wase mandarin (*C. reticulata* Blanco), and Liu sweet orange (*C. sinensis*). Results of indexing showed a correlation between positive CTLV symptoms on indicator plants and a yellowing, a decline, bud union ring, and bud union incompatibility of Ben-di-zao, Mang-ju, Zhu-hong, Liu orange, and Ponkan cultivars on trifoliolate rootstock. However, these same infected cultivars grafted onto Gou-tou-cheng 'sour orange' rootstock appear normal.

Wallace and Drake (9) first graft-transmitted a virus to various indicator plants from budwood of the Bei-jing (Meyer) lemon trees sent to California from Beijing, China, by F. Meyer in 1908. They named it citrus tatter leaf virus (CTLV), and described symptoms of blotchy spotting on leaves of Mexican lime (*Citrus aurantifolia* (Christ.) Swingle) and spotting, reduction in size, abnormal shape, and irregular and somewhat ragged margins in the leaves of Kalpi lime (*C. excelsa* Wester.), hence the name tatter leaf. Transmission of CTLV to citrange induced leaves that were distorted and strongly blotched. Wallace and Drake (10) reported that CTLV had not been found in citrus, other than in Meyer lemon.

Mechanical transmission of CTLV from citrus to herbaceous hosts has been done (7,12), and retransmissions from herbaceous hosts back to citrus has been demonstrated (3).

Calavan et al (2) reported that satsuma mandarin on Troyer citrange rootstock inoculated from buds of CTLV-infected Meyer lemon developed deep vertical corrugations in the citrange rootstock with a bud union crease or groove, and ultimately the trees declined. Miyakawa

(4) reported that CTLV was detected from field-grown trees of satsuma, Ponkan, and pummelo (*C. grandis* (L.) Osb.) cultivars in Japan. Bud union crease was reported on field trees of these cultivars on trifoliolate rootstock and could be experimentally induced by graft transmission. Su et al (8) reported that in

Taiwan, healthy looking orchard trees of Ponkan, Tankan (*C. tankan* Hag.), and Luchen sweet orange grafted on Sunki mandarin rootstocks were commonly infected with CTLV.

Wallace and Drake (11) suggested that two viruses (i.e., CTLV and citrange stunt virus) are present in Meyer lemon. However, recent investigations (5,6) indicate that CTLV and citrange stunt virus are probably part of one virus complex and the name tatter leaf should be used to designate both viruses.

In 1979, Broadbent et al reported a condition known as 'yellow ring' on Liu sweet orange on trifoliolate rootstock at the Guilin Citrus Institute in Guangxi Province, China (1). They suggested that CTLV might be responsible and recommended indexing for this virus. The junior author, visiting this same institute in 1981 and various citrus stations and groves in Zhejiang Province in 1986, observed this bud union ring on a number of scions budded on trifoliolate orange rootstock and also suggested indexing for CTLV. Declining trees with this stionic combination appeared similar to the tree shown in Figure 1.



Fig. 1. Yellowing of foliage, stunting, and decline of a 15-yr-old tree of Mang-ju mandarin on *Poncirus trifoliata* rootstock. The decline was shown to be due to infection with CTLV.

Although trifoliate orange is the predominant rootstock in most parts of China (1), this rootstock is not predominant in some provinces, especially in Zhejiang Province, where the Gou-tou-cheng (a sour orange hybrid) is the preferred and popular rootstock. Because CTLV was first found in the Bei-jing lemon exported from China at the turn of the century, and because of the observed incompatibility of sweet orange and mandarin on trifoliate orange rootstock within China and especially in Zhejiang Province, CTLV was suspected as the responsible agent for this incompatibility. Therefore, studies were initiated to index for the presence of CTLV in field trees in Zhejiang Province.

MATERIALS AND METHODS

Seedlings of Troyer and Carrizo citrange were used as indicators for graft transmission, and Blackeye cowpea was used as a receptor for mechanical transmission. All plants were grown and maintained in a glasshouse, and insects were carefully controlled by periodic spraying. Budwood was collected from 31 trees of nine cultivars (Ben-di-zao, Mang-ju, Zhao-ju, Zhu-hong, Okitsu-Wase, Ru-Ju, Ponkan mandarins, the Bei-jing [Meyer] lemon, and the Liu sweet orange). These represent the majority of local citrus varieties grown in the Huangyan district in Zhejiang Province. These cultivars were growing on trifoliate orange and Gou-tou-cheng 'sour orange' rootstocks. Budwood was collected from field trees and propagated in the greenhouse, or used directly for inoculation.

Three methods were used for indexing by graft transmission: 1) indicator seedlings of Troyer or Carrizo citrange were graft-inoculated with buds from budsticks of the above selected cultivars; 2) buds or scion pieces of indicator seedlings were grafted directly onto greenhouse-grown plants of the cultivars to be tested; and 3) seedlings of trifoliate orange, Gou-tou-cheng, or other varieties

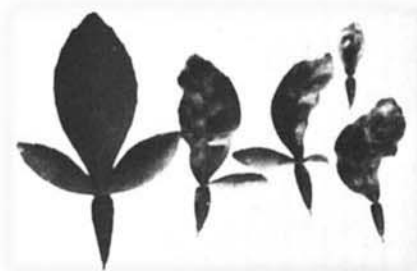


Fig. 2. Tatter leaf symptoms on leaves of Troyer citrange indicator plants. These symptoms are typical for Troyer or Carrizo citrange seedlings inoculated with tissue from field trees or from budded plants in the greenhouse. Symptoms were produced within 4 wk and most were evident within 8 wk. A normal leaf is on the left.

were inoculated with buds from test trees, and a bud of the citrange or *C. excelsa* indicator was simultaneously grafted above the inoculum buds for forcing.

Mechanical transmission was done by sap inoculation from symptomatic tissue or from leaves of plants that had been indexed by graft transmission and showed positive symptoms in indicators. Tissue was ground in a mortar and pestle with 1:5 dilution of 0.1 M phosphate buffer at pH 7.0. Cowpea leaves were dusted with 500-mesh Carborundum and the sap was applied by rubbing with a cotton swab.

RESULTS

All budwood taken from the 31 propagations made from field tree collections indexed positive for CTLV on 94 indicator plants of *C. excelsa*, Troyer, or Carrizo citrange. Leaves of Troyer or Carrizo citrange indicator plants showed typical blotch and distortion (Fig. 2). Where symptoms of tristeza were not overly severe, transmission to *C. excelsa* indicator plants induced leaves with the typical distorted and tattered margins plus strong leaf blotch. The chlorotic areas of the leaves on all indicator plants assumed a yellowish color, plants were stunted, leaves were small, and symptoms did not disappear as leaves matured. We demonstrated that all of the citrus clones tested (representing the nine major cultivars in the Huangyan district) were infected with CTLV.

The typical yellowing and decline



Fig. 3. Bud union yellow-brown ring on Liu-cheng orange on *Poncirus trifoliata* rootstock found at the Wenzhou Subtropical Experiment Station, Zhejiang Province. Identical symptoms were found in the same stionic combination at the Kwelin Citrus Experiment Station, Guangxi Province. Symptoms are typical for infection by CTLV.

observed in field trees of Liu sweet orange, Ben-di-zao, Mang-ju, and Ponkan mandarins grafted on trifoliate orange rootstock (Figs. 1 and 3) were observed in the glasshouse when these cultivars were grafted on *Poncirus trifoliata* rootstock, or when a virus-free Ponkan obtained from a seedling was graft-inoculated and then budded to *P. trifoliata*. Figure 4 shows the typical bud union brown band and crease of Ponkan on *P. trifoliata* six months after inoculation. These symptoms are the same as reported by Miyakawa (4) with inoculated satsuma mandarin grafted on trifoliate orange rootstock. However, no symptoms were evident when these cultivars were grafted on Gou-tou-cheng 'sour orange' (Fig. 4). Although infected scions budded on Gou-tou-cheng 'sour orange' rootstock show no bud union crease, our indexing tests confirm that CTLV is present and cultivars on this rootstock are symptomless carriers.

All of the three inoculation techniques used were successful in inducing symptoms of CTLV in indicator plants. Symptoms were evident as early as four weeks and most were positive within eight weeks after inoculation. Troyer citrange appeared superior to Carrizo citrange as an indicator, and symptoms were more prevalent during periods of cooler temperatures. Mechanical transmission to the primary leaves of cowpea produced red local lesions 3-4 days after inoculation. Necrosis was more prevalent on plants inoculated in the cooler months from October through May. Inocula prepared directly from leaves of field trees or from symptomatic tissue from indicator plants were effective in inducing symptoms in cowpea. Positive transmissions from symptomatic tissue of Troyer citrange to cowpea were 35 out of 56 plants inoculated. Positive transmissions from leaves of Ben-di-zao and Bei-jing lemon to cowpea were 26 out of 52 and 21 out of 56, respectively. Transmissions from cowpea showing

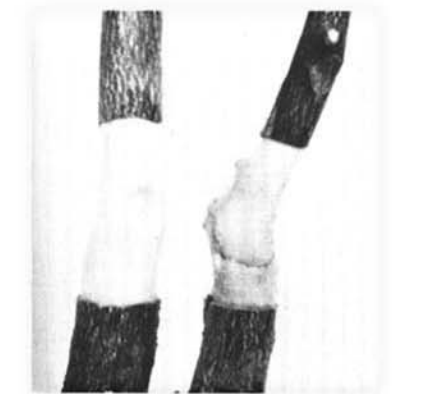


Fig. 4. Bud union brown band induced by CTLV in Ponkan mandarin on *Poncirus trifoliata* rootstock 6 mo after grafting. No symptoms are evident when Ponkan is grafted on Gou-tou-cheng 'sour orange' (left).

lesions to cowpea were 20 out of 72. Flower petals were an effective source of inoculum for transmitting CTLV to cowpea, but the numbers of red local lesions induced were lower than those obtained when extracts from young leaf tissue were used.

DISCUSSION

This is the first report of indexing for tatter leaf virus in China and shows that the problem of bud union crease or 'yellow ring' is associated with CTLV infection.

The tatter leaf disease of citrus was first reported to be present in trees of the Beijing or Meyer lemon imported into the United States as budwood from China. Miyakawa (*personal communication*) reported that the citrus cultivars Ben-di-zao, Man-ju, and Ponkan, introduced to Japan from the Huangyan district in 1975, were all found to be infected with CTLV. These reports suggest that CTLV has been present in China for many years. The disease may be quite widespread throughout China. The observed decline and yellowing, coupled with 'yellow ring' of Liu orange on trifoliolate rootstocks reported in Guangxi Province (1), is probably caused by CTLV. The report of poor performance of *P. trifoliata* as a rootstock in Guangdong Province (1) also suggests presence of CTLV. The

preference for the Gou-tou-cheng 'sour orange' as a rootstock in Zhejiang Province was probably the result of selection by growers and research personnel because of the incompatibility of existing scions with trifoliolate rootstock induced by CTLV.

We now show that CTLV is a serious problem in our commercial orchards in the Huangyan district, especially on those trees on trifoliolate rootstock. It now appears that perhaps all of the major citrus cultivars in the Huangyan district carry CTLV, including the nine major cultivars tested in this report. Some of these native cultivars have been growing in this district for about 1,700 years and it is reasonable to conclude that CTLV must have been present for a long time in the Huangyan district. The need for an indexing and certification program within China to prevent distribution of CTLV or other virus or viruslike diseases in propagative budwood is apparent.

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