OTHER VIRUS DISEASES OF CITRUS

Mechanical Transmission of a Virus that Produces Tatter Leaf Symptoms in *Citrus excelsa*

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Meyer lemon trees generally are infected with several viruses (*3, 7*). One of these was named tatter leaf virus (TLV) by Wallace and Drake (*7*), who subsequently reported that the symptoms produced in various citrus indicator plants and ascribed to TLV were actually produced by two viruses (*8*). One, for which the name tatter leaf was retained, has not been sap-transmitted, and produces symptoms only on *Citrus excelsa* (*8*). The second virus, named citrange stunt (CSV), produces symptoms in citranges and citremon plants. It is sap-transmissible to various non-citrus hosts, including cowpea and bean. In the period between the two reports, others used the name TLV to designate both TLV and CSV components, but Càtara and Wallace (*1*) reviewed the situation in detail and reported further evidence for CSV being the mechanically transmitted virus from Meyer lemon. They concluded that demonstration of mechanical transmission of TLV would require production of definitive symptoms in *C. excelsa*.

Garnsey and Whidden (unpublished) found that the agents causing CSV symptoms in citrange and herbaceous plants and TLV symptoms in *C. excelsa* are mechanically transmitted among citrus plants as contaminants on a knife blade.

This paper reports a virus, mechanically transmitted from citrus to herbaceous plants and back to citrus, that induced tatter leaf symptoms in *Citrus excelsa*.

MATERIALS AND METHODS

Virus source. The virus source for this test was an Etrog citron cutting inoculated with TLV and CSV by means of a knife contaminated by cutting a Meyer lemon plant infected with TLV and CSV, but not tristeza (*3*). Indexing tests showed that the inoculated citrus plant, although symptomless, contained viruses that induced typical CSV symptoms in citrange plants and herbaceous hosts, and induced typical TLV symptoms in *Citrus excelsa*.

Test plants. *Nicotiana clevelandii* Gray, Early Ramshorn cowpea, and Rusk citrange plants were grown from seed. Arizona 861 citron and *Citrus excelsa* plants were propagated as rooted cuttings. All plants were grown in a steam-sterilized potting soil, in clay pots or cans.

Test facilities. Plants were raised in a partially shaded, air-cooled greenhouse. Pests were carefully controlled, and all plants were watered and fertilized frequently. Some portions of the experiment were conducted in an air-conditioned chamber within the greenhouse. This chamber was constructed with clear plastic, on a greenhouse bench, and was maintained at 22 to 24° C.

Inoculation procedures. Inoculum for mechanical transmission tests was prepared by triturating young leaf tissue from donor plants in cold, neutral, .05 M potassium phosphate buffer with

a prechilled mortar and pestle. A ratio (W/V) of tissue to buffer of approximately 1:9 was used. Inoculations were made either by cutting stems of receptor plants with a razor blade freshly dipped in inoculum (knife-cut method) or by applying the inoculum with a sterile cotton swab to leaves predusted with 500-mesh carborundum (leaf-abrasion method). The razor blades were prewashed in xylene, soap solution, and water to remove a greasy protective coating. The cut stem area was wrapped with a self-adhesive rubber tape.

Graft inoculations were made by chip-bud, T-bud, or approach-graft procedures. Grafts were wrapped with vinyl plastic tape, and graft-inoculated plants were cut back after inoculation to force new growth.

RESULTS AND DISCUSSION

Young plants of *Nicotiana clevelandii* were mechanically inoculated, by the leaf-abrasion method, with inoculum prepared from tender new leaves of the citron source plant. This inoculum induced numerous necrotic local lesions on Early Ramshorn cowpea plants. The *N. clevelandii* plants were placed in the air-conditioned chamber for 16 days. Only a very faint mottle or blotching appeared on inoculated leaves. Inoculated leaves and young leaves formed after inoculation were harvested separately. A 2-gm sample of each was triturated in buffer as previously described. The two sources of inocula were applied in two ways to groups of five Arizona 861 citron receptor plants. Five citron plants were knife-cut inoculated (20 to 25 cuts per plant) and five were inoculated by the leaf-abrasion method (at least five young, tender leaves per plant). None of the citron plants developed symptoms.

Five months after inoculation, all 20 inoculated citron plants were indexed on Early Ramshorn cowpea by mechanical inoculation (*5*). Inocula from all 20 plants produced typical CSV local lesions on cowpea indicator plants. No

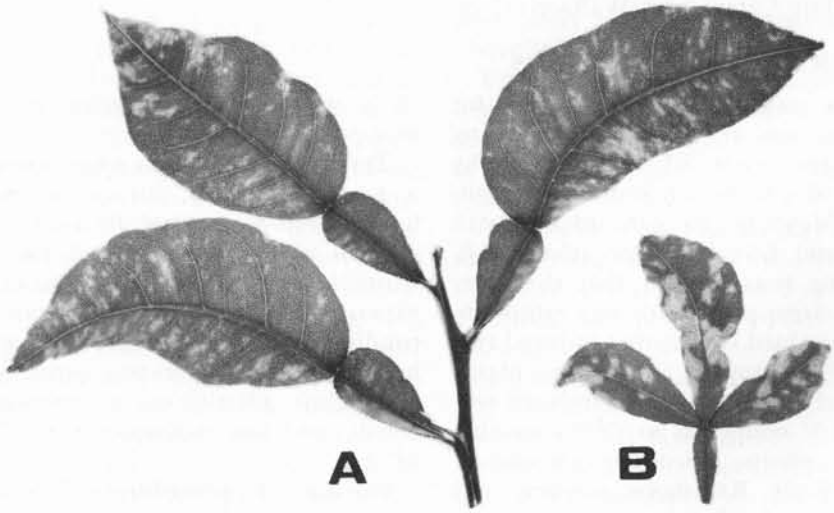

Fig. 1. Tatter leaf and citrange stunt symptoms caused by viruses mechanically transmitted from Etrog citron to *Nicotiana clevelandii* and back to Etrog citron, and thence, by grafting, to: *A, Citrus excelsa,* which shows typical TLV leaf symptoms; and *B,* Rusk citrange, which shows a typical CSV leaf pattern.

lesions were produced by inocula prepared from healthy citron plants.

Subsequently, the five citron plants inoculated by leaf-abrasion with inoculum from systemically infected *Nicotiana clevelandii* leaves were indexed on Rusk citrange and *Citrus excelsa* indicators by graft-inoculation. Typical TLV symptoms appeared in *C. excelsa* plants (fig. 1, A) inoculated from each of the five citrons, and typical CSV symptoms appeared in the Rusk citrange plants (fig. 1, B) inoculated from each source.

The other 15 citrons were approach-grafted to single *Citrus excelsa* plants, all of which developed typical TLV symptoms.

Judging from symptoms produced in *Citrus excelsa* plants, TLV apparently was transmitted mechanically from citrus to *Nicotiana clevelandii* and back to citrus. Several reasons could account for the successful transmissions in this test, when previous attempts had failed. The isolate of TLV used in this test may have differed from those used earlier, although most Meyer lemon plants grown in this country and carrying TLV were derived by vegetative propagation from plants introduced in 1908 (*3*). The choice of donor and receptor plants may have been significant. Fulton (*2*) found *N. clevelandii* to be a good increase plant for CSV (which he called TLV at the time). It probably is a good choice for TLV as well. Etrog citron plants are excellent receptor plants for mechanical transmission of citrus exocortis virus (*6*), and their use as an intermediate host was probably fortuitous. Although direct inoculations from *N. clevelandii* to Rusk citrange or *C. excelsa* plants were not attempted in this study, previous attempts to infect these plants by mechanical inoculation with other sources failed. Even graft inoculations to these indicators are not always successful. An intermediate host has been employed previously in transmission studies on TLV (CSV) by leaf-piece grafting (*4*).

These results remove one criterion for distinguishing TLV from CSV, but neither resolve the relationship between the diseases nor explain the "filtering" effect of *Citrus excelsa* on TLV (*8*). Now that mechanical transmission of both components has been demonstrated, attempts should be made to get an isolate of TLV that is free of CSV.

CONCLUSIONS

A virus that produces tatter leaf symptoms in *Citrus excelsa* plants was transmitted mechanically from Etrog citron to *Nicotiana clevelandii* and back to citron. Presence of virus in citron plants was determined by graft transmission to *C. excelsa*. The virus was transmitted by both the leaf-abrasion and knife-cut methods of inoculation.

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