Protection Phenomena Against Tristeza in Trees Preinoculated with Vein Enation Virus

M. Koizumi and A. Sasaki

Citrus vein enation, described by Wallace and Drake (1953), has been recognized in many citrus-growing areas throughout the world. Tanaka and Yamada (1961) found the causal virus in apparently healthy citrus trees in Japan. McClean (1954) demonstrated that the brown citrus aphid, *Toxoptera citricida*, was a vector of citrus vein enation virus (CVEV). Thereafter, *Myzus persicae* (Wallace and Drake, 1959), and *Aphis gossypii* (Laird and Weathers, 1961) were recognized as vectors of CVEV. Thus, CVEV is probably widespread among citrus trees, but its economic importance has not been determined. In the present study, we discovered some trees in the field which were free from stem pitting and carried only CVEV, and trees preinoculated with CVEV were protected against citrus tristeza virus (CTV).

EXPERIMENTS AND RESULTS

Determination of CVEV-infected trees in the field. In the field at Kuchinotsu Branch, Fruit Tree Research Station, various citrus species, hybrids, and nucellar seedlings are being grown. *T. citricida* was frequently observed on the trees. About 1,000 trees (8-13-year-old seedlings), including hybrids and nucellar clones of sweet oranges, Natsudaidai, some shaddocks, grapefruit, Hassaku, and Hyuganatsu, were examined in 1975 for stem pitting or other tristeza symptoms. From 25-71 per cent of the trees were free from stem pitting depending on the parentage of the seedlings. Similar observations made 2-3 years later revealed that the percentage of trees free from stem pitting had dropped markedly in all cases, e.g., from 42 to 1 per cent in sweet orange, from 71 to 12 per cent in Natsudaidai, from 44 to 14 per cent in shaddock hybrids, and from 39 to 11 per cent in Hyuganatsu hybrids.

Scions from 20 of the trees free from stem pitting, which included five sweet orange trees, eight Natsudaidai trees, and seven Hyuganatsu hybrids, were budded onto four seedlings each of Mexican lime, sour orange, grapefruit, and Eureka lemon, and incubated for 3 months at 23-25°C in the greenhouse. Examination of the test plants showed that eight trees, including two sweet orange trees, four Natsudaidai trees, and two Hyuganatsu hybrids, were infected with CVEV only, five trees carried seedling yellows tristeza virus (CTV-SY), and seven trees were virus-free or carried a mild strain of CTV. The fluorescent antibody technique (Tsuchizaki *et al.*, 1978) confirmed that the CVEV-infected trees carried no CTV.

Protection in trees preinoculated against CTV with CVEV. Two seedlings each of the four test varieties were side-grafted with a scion carrying only CVEV from the trees mentioned above and then incubated at 23-25°C for 3 months. Buds collected from a nucellar sweet orange tree (No. 1513), which carried only severe CTV-SY were side-grafted 5 cm above the previous inoculation, incubated for 1 month, and the challenge inoculum and surrounding tissues were removed with a knife. All test plants were cut back to 25 cm when the challenge inoculum was removed. Test plants were observed for 3 months, then cut back and observed again.

Sour orange seedlings inoculated with CVEV developed small enations on the leaf veins. When challenged with CTV-SY, the sour orange seedlings preinoculated with five CVEV isolates were fully protected against it (fig. 1-A). Few vein enations were observed on the seedlings that were protected. Three other CVEV isolates, which caused many vein enations on sour orange and Mexican lime, provided incomplete

protection. Protection was also recognized on the preinoculated Mexican lime seedlings, but some showed severe vein clearing and stem pitting. There was no protection on the preinoculated grapefruit and lemon seedlings (fig. 1-B). Sour orange and grapefruit controls became severely stunted and yellowed when challenged with CTV-SY.

Another experiment was carried out using *Citrus junos* as a test plant and *T. citricida* as a vector. Ten 2-week-old nucellar seedlings of *C. junos* were inoculated with CVEV by feeding 15 aphids, which had fed for 48 hours on a Hassaku tree carrying only CVEV. After incubation for 3 months in a glasshouse, the preinoculated and control seedlings were exposed for 48 hours to 15 aphids which had fed previously for 48 hours on a dwarfed Hassaku tree (HS-841) infected with CTV. Observations at 1 and 7 months revealed that the seedlings preinoculated with CVEV were less affected by CTV (table 1.).

DISCUSSION

Field observation and protection tests showed that CVEV is capable of protecting against tristeza. However, the protective ability varied with the CVEV isolate. Some isolates causing few enations provided good protection, whereas others causing abundant vein enations gave little protection. Since CVEV is easily transmitted by some aphids which transmit CTV, there may be some relationship between CVEV and CTV. The causal virus of citrus vein enation has not been definitely determined. However, Hooper and Schneider (1969) observed small, rod-shaped particles in the macerated tissues of CVEV-infected leaves. Clarification of the characteristics of CVEV is therefore urged.

TABLE 1
PROTECTION OF *C. JUNOS* SEEDLINGS PREINOCULATED WITH CVEV AGAINST HASSAKU DWARF-CTV INOCULATED BY *T. CITRICIDA*

Treatment	No. of trees used	Condition 1 month postchallenge		Condition 7 months postchallenge	
		Healthy	Vein clearing	Healthy	Stem pitting
Preinoculated with CVEV	10	7	3	6	4
Control	10	3	7	2	8

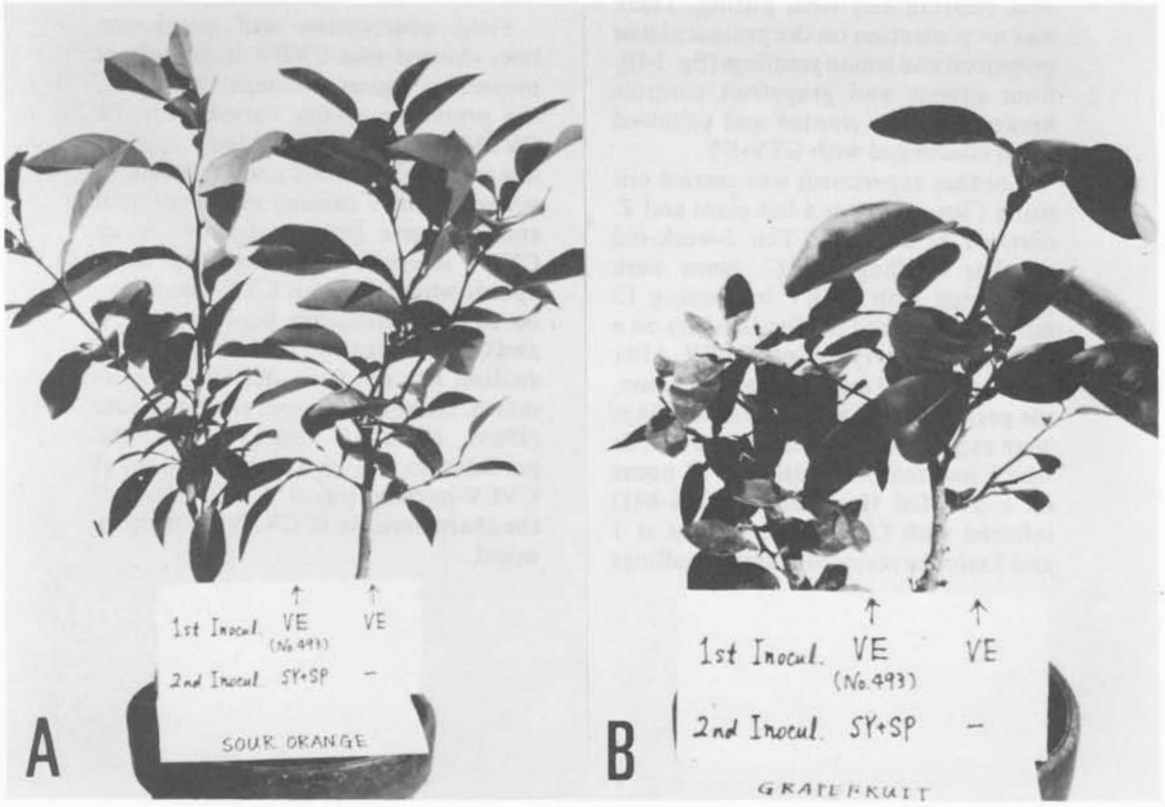

Fig. 1. Growth of the preinoculated sour orange (A) and grapefruit (B) seedlings with CVEV after challenge inoculation of CTV-SY (left plant) and without challenge inoculation (right plant).

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