Effects of Tristeza Virus Infection on Growth and Yield of Three Citrus Varieties

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TRISTEZA VIRUS was first reported from Arizona by Carpenter (5) in 1956 in Meyer lemon. He infected other scion varieties experimentally under glasshouse conditions with the virus from Meyer lemon, but in a field survey failed to establish that it was being spread by insect vectors (6).

Discovery of the virus in mature trees of Clementine and Dancy tangerine and Marsh white grapefruit in 1962–63 prompted surveys and eradication measures (1, 2). Nearly all instances of disease were directly traceable to infected budwood sources introduced into Arizona prior to 1930. However, the presence of the virus in 2 sweet orange trees and in a single Kusaie lime tree, all of which were adjacent to infected Clementine trees in 3 widely separated areas, suggested that spread by insects might have occurred. This circumstantial evidence prompted trials to test for vectored spread and to develop specific information regarding the severity of Arizona strains of the virus in known susceptible stionic combinations under local field conditions. The latter objective seemed particularly important since nearly all diseased trees found had been propagated on rough lemon rootstock and showed only slight decline, which could not be attributed exclusively to tristeza.

Procedures and Results

EXPERIMENT 1.—In April 1964, 2-year-old balled nursery trees of old-line Clementine tangerine, old-line Campbell Valencia orange, and nucellar Tarocco orange, all budded on sour orange rootstock, were planted at the Citrus Branch Station at Tempe. Twelve trees of each variety were set in a 3-row plot, 1 variety per row. Rows were 8 feet apart with the 12 trees spaced at 5 feet within rows. Previous indexing of the Clementine test trees showed that these trees carried a mild strain of exocortis virus; otherwise all test trees were virus free except for the possibility of stubborn disease.

In April 1965, alternate pairs of trees within and across rows were double-bud-inoculated with a strain of tristeza virus from Clementine tangerine. This donor was free from all other viruses except possibly xyloporosis, for which no test was made. Observations were made for symptoms, effects on growth and yield, and insect transmission. Fruit were counted from each tree each year, trunks were measured 15 cm above bud unions at the beginning and at intervals during the experiment, and areas were calculated on the assumption that the tree trunks were circular. All trees were indexed on West Indian lime in July 1967 and February 1968.

Symptoms.—Chlorosis and bronzing of leaves of all inoculated trees of the 2 orange varieties were obvious in November 1965, only 7 months after inoculation, but Clementine tangerine showed neither symptom at this time. All inoculated trees, however, lost many leaves during the cold weather and rains of the fall and winter of 1965. In February 1966, all inoculated trees were markedly defoliated and chlorotic, including Clementine tangerine. Stunting was apparent in the inoculated trees of all varieties in June 1966, 14 months after inoculation. The degree of stunting was rated quantitatively on a scale of 0 (none) to 5 (very severe). Average numerical ratings for the 3 varieties were: Valencia, 3.0; Clementine, 2.8; Tarocco, 2.3. (Additional information on the stunting effect is in Table 2.)

In the fall of 1966 a striking difference was observed for zinc-deficiency symptoms in the nucellar Tarocco. This variety typically develops slight to moderate zinc-deficiency symptoms in noninfected trees. All inoculated trees developed very severe foliar symptoms of zinc deficiency. Neither of the other varieties appeared to lack this element until the fall of 1967 when a single inoculated Valencia tree developed slight symptoms.

An effort was made to induce collapse of infected trees. Water was withheld in August 1967, until moderate to severe wilt was observed in the control trees. Less wilt developed in the infected trees and collapse did not occur.

Fruit yields.—Inoculated trees responded with very heavy fruit set on Clementine and Valencia (80 per cent of the total yield) in the first year after inoculation and also set more fruit than controls (72 per cent of total) in the second harvest year as well (Table 1). The nucellar Tarocco orange did not fruit at all during the course of the experiment.

Tree growth.—Tristeza virus infection caused similar significant growth reductions in all varieties with none being more severely affected than another. Inoculation with tristeza virus produced significant reductions in trunk growth 34 months after inoculation and highly significant reductions in weight of trees at 38 months (Table 2). The differences between the noninoculated trees represent normal differences in vigor encountered among the 3 varieties.

Indexing.—The 36 test trees were each indexed for tristeza virus by double-budding to 4 West Indian lime seedlings to determine the presence of the virus in inoculated trees and to determine whether spread by insects had occurred. Tests initiated in July 1967 (27 months after inoculation, showed the presence of tristeza virus in inoculated trees as follows: Clementine, 5/6; Valencia, 2/6; Tarocco, 5/6. All tests of control trees were negative. When all trees were re-indexed in February 1968 (34 months after inoculation) reactions were positive for all 18 inocu-

TABLE 1. EFFECTS OF TRISTEZA VIRUS INFECTION ON FRUIT YIELDS

Variety	Average yield in fruit per tree			
	1966		1967	
	Inoculated	Control	Inoculated	Control
Clementine tangerine (OL)	16[a]	12	64[a]	36
Valencia orange (OL)	38[b]	1	56[b]	9
Tarocco orange (N)				

a. Differences significant at 5 per cent level.
b. Differences significant at 1 per cent level.

TABLE 2. EFFECTS OF TRISTEZA VIRUS INFECTION ON VEGETATIVE GROWTH

Variety	Trunk area (cm^2)		Tree weight (kg)		Weight reduction (%)
	Inoculated	Control	Inoculated	Control	
Clementine tangerine (OL)	6.8[a]	11.8	20.9[b]	67.1	69
Valencia orange (OL)	12.0[a]	18.6	37.8[b]	90.2	58
Tarocco orange (N)	16.1[a]	25.0	69.9[b]	155.1	55

a. Differences significant at 5 per cent level.
b. Differences significant at 1 per cent level.

lated trees and negative for the same number of controls. Vectored transmission of the virus had not occurred although each control tree was always adjacent to at least 2 infected trees. At this close planting (5 feet by 8 feet) the branches of adjacent trees within rows were interwoven during most of the test period.

EXPERIMENT 2.—During surveys for tristeza virus, 3 distinctive virus isolates had been recognized: that of tristeza alone, that of tristeza plus that of psorosis A, and those of the tristeza-seedling-yellows complex. The first 2 were obtained from different Clementine tangerine budlines and the last from a single Kusaie lime tree as well as from various Meyer lemon trees. In March 1964, these 3 isolates were tested for virulence by massive inoculation (10 buds from infected lime seedlings) into branches of 14-year-old, tristeza-free Clementine tangerine trees (OL) on the University Citrus Farm at Yuma. Each isolate was placed in 6 trees, of which 3 were on sour orange root and 3 on rough lemon root. Trees on the 2 rootstocks were in adjacent rows so that paired trees on different stocks were inoculated with each isolate. The 2-row plot was subdivided into 3 replicate subplots each containing 6 paired trees inoculated with the 3 isolates and 2 noninoculated controls. Indexing of the test trees prior to trial initiation showed them to be tristeza-virus-free, but 2 trees were infected with psorosis virus. Tests were not made for xyloporosis or exocortis viruses; none of the trees showed pitting at the bud union.

Results.—Inoculated trees on sour orange rootstock produced more blossoms 1 year after inoculation and were subject to earlier and heavier petal-drop than the controls or inoculated trees on rough lemon. Of the 3 isolates tested, the tristeza-psorosis inoculum caused earliest and heaviest petal-fall. In June 1965 (15 months after inoculation), all trees on sour orange root inoculated with tristeza virus alone or tristeza–seedling-yellows virus and 1 tree inoculated with tristeza and psorosis viruses showed severe leaf-bronzing and defoliation with tip dieback. Three trees on sour orange root collapsed within a 6-day period in May 1966, only 26 months after inoculation. Two of the collapsed trees received inoculum of tristeza-psorosis (the most common isolate found in Arizona) and 1 tree received tristeza inoculum only. Of the remaining 6 trees on sour stock, all showed decline symptoms and 1 with inoculum of tristeza–seedling-yellows was nearing collapse. The inoculated trees on rough lemon appeared unaffected.

Iodine–potassium iodide tests were performed in May 1966 on slivers of wood taken from both sides of the bud unions of all test trees. All trees on rough lemon stock and the controls on sour orange stock gave negative reactions, but 7/9 inoculated trees on sour orange stock showed positive starch depletion.

Tissue transfers to West Indian lime seedlings were also made at this time. Tristeza virus was recovered from 9/9 inoculated trees on sour orange rootstock but from only 4/9 of those on rough lemon although nearly all inoculum buds appeared to have been successfully grafted. This result is believed to have demonstrated retarded invasiveness and incomplete distribution of the virus in trees on rootstocks tolerant of tristeza virus.

SUPPLEMENTAL STUDY FOR POSSIBLE INSECT TRANSMISSION.—During surveys for tristeza virus in 1964, a 20-acre planting of 5-year-old Clementine tangerine trees growing on rough lemon rootstock was found to consist of approximately 10 per cent tristeza-virus-infected trees. Twenty diseased trees and 80 adjacent healthy trees have been indexed 5 times to date with no evidence of insect transmission although *Aphis gossypii* Glover was observed in great numbers on two occasions infesting black mustard (*Brassica nigra* [L.] Koch.), the predominant species of the weed cover crop.

Discussion

The studies described in this paper demonstrated that tristeza virus isolates commonly found in Arizona cause severe reactions in susceptible stionic combinations. These findings are particularly important to citrus growers of the Salt River Valley area where nearly 18,000 acres of trees are grown on susceptible sour orange stock. This information may seem of lesser value for the Yuma area where rough lemon is the prevalent stock for more than 31,000 acres of citrus; there are now, however, substantial plantings on alemow and Troyer citrange, both of which are intolerant of tristeza virus (3, 4).

Arizona growers fortunately have not experienced tristeza in epiphytotic form, and the hazard of such an occurrence has been nearly eliminated by prompt voluntary eradication of practically all known diseased trees and by the development of an effective citrus certification program. Also in effect is an extensive quarantine program whereby only budwood certified free from tristeza virus can be imported into Arizona. An educational program had advised growers of the danger of introducing uncertified budwood illegally. The fact that vectored tristeza has not yet been demonstrated is not considered justification for relaxing efforts to eliminate all tristeza inoculum sources.

Evidently the influence of an arid environment is no deterrent to tristeza, since 3 different cultivars reacted to inoculation with the same classic symptoms described from many other areas having milder climatic conditions.

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