A Comparison of Some Tristeza Isolates and a Cross-Protection Trial in Florida

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Florida is unique among tristeza-affected areas because a large proportion of its citrus trees on sour orange rootstock are known to be carrying the citrus tristeza virus (CTV) (Bridges and Youtsey, 1972; Cohen, 1956; Norman *et al.*, 1961); yet until recently, only a relatively small proportion of these trees have shown symptoms of tristeza decline. Some of these symptomless trees are known to have been infected for many years (Bridges and Youtsey, 1972; Norman *et al.*, 1961). The presence of mild and moderate strains of CTV in tristeza-intolerant trees, such as sweet orange on sour, may often serve to protect trees from damage by a later introduction of a more severe isolate of CTV (Giacometti and Araujo, 1965; Muller and Costa, 1972; Wallace and Drake, 1974). This is a report of efforts to determine whether the strains of CTV in certain symptomless trees in Florida actually do offer such protection.

MATERIALS AND METHODS

Two experiments are described, both involving Pineapple sweet orange grafted on sour orange rootstock. CTV isolates were introduced into plants as bark chips ("blind buds"). Aphid vectors were not used.

Experiment 1 was begun in 1959 to study the effect of tristeza strains on growth and performance of trees. A number of isolates of CTV were introduced into comparable six-tree groups of young virus-free plants. Plants were inoculated in the greenhouse and moved into the field in 1960. The following isolates were used: (1) CC2A—from a Valencia orange on sour orange near Winter Garden, Florida, not exhibiting tristeza symptoms for at least 10 years, having psorosis-like vein flecking and oak-leaf patterns on its spring flush but no bark scaling; (2) DD102BB—from a Valencia on sour orange tree southeast of Winter Garden which has remained symptomless for more than 20 years, except for being somewhat stunted; (3) BB4D—from a Temple orange on sour orange west of Winter Garden with symptoms of moderate tristeza decline; (4) Meyer lemon—from a tree near Fort Pierce carrying CTV, seedling yellows, and tatterleaf-citrange stunt viruses; and (5) self-inoculated controls.

Experiment 2 was designed to determine the extent to which isolates from symptomless tristeza-intolerant trees would protect inoculated trees against a subsequent introduction of a more injurious strain of tristeza virus. The plants used in Experiment 2 were prepared in January 1967 by inserting buds from a field-grown nucellar Pineapple orange seedling into sour orange seedlings in the greenhouse. Tissue from the Pineapple seedling, indexed for tristeza on Key lime seedlings at the time of budding, was negative for tristeza. Plants were divided into three groups of 12. Budlings in the first two groups were inoculated with two bark chips each in June 1967 with isolates CC2A and DD102BB, respectively. At the same time the third group was self-inoculated with 2 bark chips per plant from the parent nucellar Pineapple orange seedling. This seedling tree, free of tristeza in January 1967, had by June 1967 unexpectedly acquired a very mild isolate of CTV by natural infection. This isolate, designated T9R1, did not induce symptoms of tristeza when inoculated into tristeza-intolerant trees. Seventy days after

the initial inoculation, six budlings in each group of 12 were challenged-inoculated with one bark chip each of severe tristeza isolate T3. Inoculum bark chips were checked to be sure they were alive at least six weeks after insertion. Isolate T3, obtained from S. M. Garnsey, was originally described by Grant and Higgins (1957). Trees in this experiment were planted in the field in March 1970.

The T3 isolate of CTV induces stunting, strong vein clearing, and strong stem pitting in inoculated Key lime seedlings. This combination of symptoms was taken to indicate the presence of T3 CTV when plants in this experiment were indexed on Key lime seedlings since none of the other isolates induced such strong symptoms. Growth of trees was evaluated by measuring the trunk circumference. Yield was determined by counting fruits on each tree. Tree condition was evaluated visually on a scale of zero to 3 where zero meant a completely healthy tree and 3 referred to a tree which was almost dead.

RESULTS AND DISCUSSION

Strong differences in rate of growth of trees in the various inoculation groups of Experiment 1 appeared early and have continued. Table 1 lists average trunk circumference of trees in the different groups in 1967, seven years after trees were planted in the field. (Measurements made in 1967 are used because the close setting of these trees has inhibited later growth). Trees carrying isolates CC2A and DD102BB from symptomless donor trees are significantly smaller than the self-inoculated controls. Trees carrying isolate BB4D from the donor tree in decline are significantly smaller than those with DD102BB but not CC2A. Trees inoculated with Meyer lemon tissue were the most stunted in the experiment. The stunting may not have been induced by CTV alone since the Meyer lemon carried other viruses.

TABLE 1
EXPERIMENT 1: INFLUENCE OF VARIOUS ISOLATES OF TRISTEZA VIRUS ON THE TRUNK CIRCUMFERENCE OF PINEAPPLE ORANGE TREES ON SOUR ORANGE ROOTSTOCK. TREES PLANTED IN 1960; MEASURED IN 1967

Tristeza virus isolate	Condition of tristeza source trees on sour orange rootstock	Average trunk circumference (cm)*
CC2A	symptomless	22.4 bc
DD102BB	symptomless	22.9 b
BB4D	moderate decline	17.8 c
Meyer lemon	(not on sour)	7.9 d
Control (self-inoculated)	—	29.2 a

*Averages followed by the same letter are not significantly different at the 95 per cent level, according to Duncan's multiple-range test.

Experiment 1 demonstrated that inoculum containing CTV isolates from symptomless trees could markedly reduce growth of inoculated trees. Isolate BB4D, obtained from a field tree in decline induced a greater reduction in tree size. Surprisingly most inoculated trees still appeared healthy. Only those trees carrying the Meyer lemon isolate appeared unthrifty in addition to being stunted.

Growth of trees in Experiment 2 which received only the original protective inoculum has been quite uniform (table 2). Although average tree size differs somewhat for the three groups, differences are not statistically significant. In contrast, there is considerable variability in size (as indicated by trunk circumference measurements) among replicate trees in the three groups which received the T3 challenge inoculum in addition to the original inoculation. Some trees are as

TABLE 2
EXPERIMENT 2: TRISTEZA CROSS-PROTECTION IN PINEAPPLE SWEET ORANGE TREES ON SOUR ORANGE ROOTSTOCK AS INDICATED BY DIFFERENCES IN TRUNK CIRCUMFERENCE. TREES INOCULATED IN GLASSHOUSE IN 1967; PLANTED IN FIELD 1970; MEASURED JULY 1975

	Trunk circumference (cm.)					
Original inoculum	CC2A		DD102BB		T9R1	
Challenge inoculum	T3	None	T3	None	T3	None
Rep. 1	29.5*	20.8	10.2	Dead	11.2	29.2
Rep. 2	Dead	30.0	34.3*	36.8	6.6	27.7
Rep. 3	9.4	25.9	27.7*†	21.8	37.6*	35.3
Rep. 4	9.9	29.5	17.5	27.7	10.2	30.0
Rep. 5	19.8*	22.9	19.8	32.0	11.2	38.4
Rep. 6	18.3	22.9	20.1	29.7	8.6	36.1
Mean‡	17.4 cd	25.3abc	21.6 bcd	29.6ab	14.2 d	32.8a

*Key lime indexing in 1972 did not show symptoms of T3.
†Tree which showed sudden decline in 1974 with simultaneous increase in severity of reaction on Key lime seedlings.
‡Averages followed by the same letter are not significantly different at the 95 per cent level, according to Duncan's multiple range test.

large as those which were not challenged, others are extremely stunted and still others are intermediate in size.

Table 2 shows that two of the original trees in the experiment have died. Both were dead by 1970 and their loss is attributed to cultural problems and is not believed to be related to the inoculations.

Trees were evaluated for tree condition in July 1975. All unchallenged trees were found to be thrifty in appearance and were rated zero. Many of the T3-inoculated trees were somewhat defoliated or showed slight dieback. Average rating for the challenge-inoculated trees which received the CC2A inoculum was 0.60; DD102BB inoculum - 0.42; T9R1 inoculum - 0.50. No trees were in severe decline.

Trees in Experiment 2 were indexed on Key lime seedlings in 1972. In general, experimental trees which had been challenged by inoculation with the T3 isolate and were stunted or intermediate in size showed characteristic symptoms associated with the T3 strain on the inoculated Key lime seedlings. Those plants which had been challenged by T3 but were in the same size range as corresponding unchallenged plants had symptoms of the original inoculum only. Unchallenged plants had mild CTV symptoms only.

One tree which had originally been inoculated with isolate DD102BB but was not stunted following challenge inoculation (replicate 3) showed symptoms of sudden tristeza decline in 1974. Reindexing on Key lime seedlings indicated that its reaction had changed from mild in 1972 to severe in 1974.

It is not possible to determine with certainty whether the sudden decline of this tree was due to field infection or to late release and movement of the T3 strain throughout the tree. Trees without challenge inoculum have been in the field for more than five years with no indication, to date, of tristeza decline. It seems, therefore, that release of T3 virus was a more likely cause of the decline than natural infection.

It is not clear whether trees challenged by inoculation with isolate T3 and reaching the same size range as plants not challenged were truly fully cross-protected or whether there was an inoculum failure or variation in the inoculum. Whatever the mechanism, certain tristeza intolerant trees remained unaffected although they contained live tissue from a T3 infected source.

Yield on trees in experiment 2 was evaluated by counting fruit in July 1975. Figure 1 depicts yield observations. Of trees which received the T3 challenge inoculum, those first inoculated with isolate DD102BB were significantly more

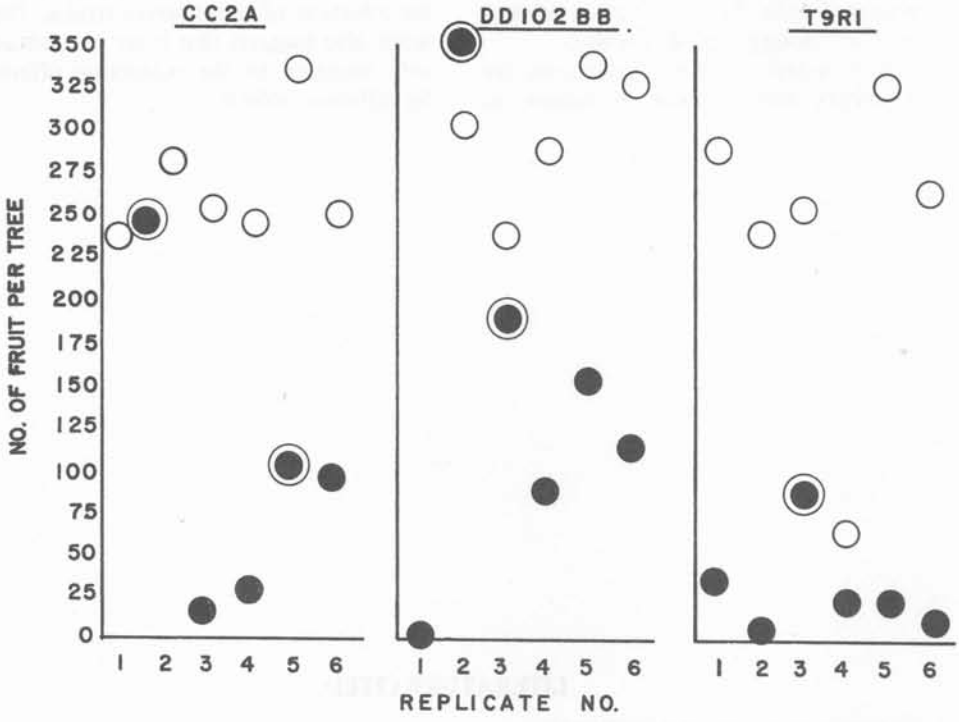

Fig. 1. Numbers of fruit on trees in Experiment 2. Count made in July, 1975. Open circles, trees which received original inoculum only; solid circles, trees which received original and T3 challenge inoculum but which did not produce typical T3 symptoms when indexed on Key lime seedlings in 1972.

productive than those which received the T9R1 inoculation. Those which received the CC2A original inoculum also showed some benefit. There was no significant difference in yield among tree-groups which received only single inoculations.

The absence of adequate controls such as uninoculated trees and trees carrying only the T3 isolate, makes interpretation of Experiment 2 more difficult and less precise. In general, however, uninoculated tristeza-free trees would probably be as large or larger than trees carrying the mildest CTV strain, in this case T9R1, would have a similar yield, and would be quite uniform in performance. Control trees carrying only the T3 strain would be as stunted, as low in yield, and at least as uniform in size as the five stunted trees in Experiment 2 first inoculated with T9R1 and then with isolate T3.

If cross protection had been complete in Experiment 2 all trees carrying the mild strains would have performed the same regardless of whether or not they were later challenged with CTV isolate T3. Obviously such perfect cross-protection was not obtained. The measure of the cross-protection value which was obtained is the extent to which trees challenged by the T3 inoculation are superior to the basic stunted plants which would have resulted from inoculation by T3 only. By this standard an appreciable degree of cross-protection was obtained from pre-inoculation with isolates CC2A and DD102BB. CTV isolate T9R1 appears to

have offered less protection. The cross-protection obtained appears even more impressive when it is recognized that the challenge method used, grafting of a bark chip, is probably quite severe as compared with the introduction of virus particles during aphid feeding.

If work reported here is representative of events which occur in nature in Florida, the widespread dissemination of mild isolates of CTV in symptomless Florida citrus trees has had definite value in moderating the damage which might otherwise have been done by the later introduction of more severe strains. This work also suggests that there is considerable variation in the protection offered by different isolates.

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