

## **Reduction in Yield of Galego Lime Avoided by Preimmunization with Mild Strains of Tristeza Virus**

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THE CULTIVATION of citrus varieties sensitive to tristeza virus is becoming increasingly difficult in regions where severe tristeza virus strains are prevalent. This is particularly true of Pera sweet orange, Galego lime, and grapefruit orchards in the state

of São Paulo, Brazil, where these varieties, especially the first two, are very important for local and export markets. Attempts to replace them with others have failed.

Tests to evaluate the effects of pre-immunizing trees of these varieties

with mild strains of tristeza virus and planting them in an area where they are exposed to infection by severe strains were started in 1962. Preliminary results have been published (1). Further results with Galego lime trees are presented in this paper.

### *Materials and Methods*

Detailed descriptions of materials and methods are given elsewhere (1). The experimental plantings are now (1969) 6 years old. Test plants have been exposed to natural infection in the field for 4½ years. In addition, 2 plants in each 5-plant row, the basic plot in the experiment, have been challenge-inoculated with severe stem-pitting isolates by budding and by vectors during the last 3½ years. Since there was no significant reaction to the challenge inoculations, data from the 2 plants that were challenge-inoculated have been combined with those from the other 3 trees in the basic plot and are presented in Tables 1 and 2.

Beginning in 1967, fruit from each tree were counted and weighed. Trees with less than 10 fruit were not harvested; moreover fruit less than 2.5–3 cm in diameter were not counted or weighed. The data presented in this paper are from Galego lime trees on Rangpur lime, Caipira sweet orange, and Cleopatra mandarin rootstocks. As previously reported, preimmunization was done with 45 mild isolates of tristeza virus collected from Pera sweet orange, grapefruit, and Galego lime sources. Of these isolates, only 8 from Galego lime adequately preimmunized Ga-

lego lime; only the 5 best isolates were considered for evaluation of the preimmunization effects.

### *Experimental Results*

SCION GROWTH.—The reactions of the preimmunized Galego scions to a given tristeza virus isolate were essentially independent of the rootstock on which they were grafted, though—as will be mentioned later—growth on Caipira sweet orange was better than that on Rangpur lime or Cleopatra mandarin. Therefore, the effects of a given virus isolate on the 3 different rootstocks have been combined (Table 1).

The data show that the mild isolates from Galego lime caused only mild symptoms and reduced growth only slightly, if at all, as compared with the controls exposed to natural infection (Fig. 1) and suggest that these mild isolates protected the plants from more severe strains that occur in the area. The mild isolates from grapefruit also seem to have offered protection from naturally occurring strains, but they caused more damage in Galego lime plants than did the mild strains from Galego lime. The strains from Pera orange may have protected the plants from infection by naturally occurring strains, but they caused about as much damage as did the naturally occurring strains. The stem-pitting isolates caused even more damage than the naturally occurring strains.

TRISTEZA AND STEM-PITTING SYMPTOMS.—All the control plants became naturally infected and responded with symptoms of various

degrees of severity according to the time they became infected and the strain of virus naturally transmitted to them. Some were infected early in the nursery with severe virus strains and died soon after transplanting. The rest became infected after transplanting. Most of them are now severely declined with symptoms almost as severe as those in plants experimentally inoculated with

the stem-pitting isolates. A few were apparently infected with a mild strain and show symptoms comparable to those in plants preimmunized with mild isolates from Galego lime.

YIELDS.—Data on yields of the Galego scions are grouped by virus sources in Table 2. Differences among the various groups were striking. Galego lime scions preimmunized with mild isolates from

TABLE 1. COMPARATIVE RATINGS OF SYMPTOMS, 4½ YEARS AFTER EXPOSURE TO FIELD INFECTION BY TRISTEZA VIRUS, IN GALEGO LIME PLANTS PREIMMUNIZED WITH MILD STRAINS OF TRISTEZA VIRUS AND IN THOSE NOT PREIMMUNIZED

Treatment	Average reaction of 30 or more plants per treatment		
	Tristeza <sup>a</sup>	Stem pitting <sup>a</sup>	Growth <sup>b</sup>
Controls exposed to natural infection	3.0	3.1	2.4
Inoculated from Galego lime (5 isolates)	1.0	1.1	4.7
Inoculated from grapefruit (2 isolates)	2.3	2.4	2.9
Inoculated from Pera orange (3 isolates)	3.1	3.5	2.5
Inoculated with stem-pitting strains <sup>c</sup>	3.5	3.9	1.7

a. Severity rated 0-5; 0, no symptoms; 5, most severe symptoms.

b. Extent of growth rated 0-5; 5 representing best growth.

c. Two isolates from Pera sweet orange, 1 from Galego lime, and 1 from grapefruit.

TABLE 2. COMPARATIVE YIELDS OF PREIMMUNIZED GALEGO LIME PLANTS 4½ YEARS AFTER EXPOSURE TO FIELD INFECTION BY TRISTEZA VIRUS

Treatment	Average of 30 or more plants							
	No. of fruit				Weight in kg.			
	1967	1968	1969	Total	1967	1968	1969	Total
Controls exposed to natural infection	38	151	133	322	0.9	2.4	3.5	6.8
Inoculated from Galego lime (5 isolates)	43	440	743	1226	1.1	12.3	20.6	34.0
Inoculated from grapefruit (2 isolates)	19	113	250	382	0.4	2.7	6.5	9.6
Inoculated from Pera orange (30 isolates)	0	19	41	60	0.0	0.4	1.0	1.4
Inoculated with stem-pitting virus (4 isolates)	0	0	25	25	0.0	0.0	0.5	0.5

Galego lime sources yielded about five times as much as the nonpre-immunized control plants. Of the scions preimmunized with the 5 best isolates of Galego lime, 2 (Nos. 40 and 141) yielded nearly 19 per cent more than the other 3 (Nos. 42, 50, and 128). Not only did the trees preimmunized with the 5 best mild isolates yield more, but they also bore larger fruit than did trees in the other treatments.

**INFLUENCE OF ROOTSTOCK.**—Pre-immunized Galego lime scions made better growth on Caipira sweet orange than on Rangpur lime or Cleopatra mandarin rootstocks, the last 2 being comparable. Yields were, however, different. Comparable scions on Rangpur lime in the first 3 years of harvest yielded about 20 per cent more than on Cleopatra

mandarin and 40 per cent more than on Caipira sweet orange rootstocks.

#### *Discussion and Conclusions*

The results with preimmunized Galego lime budlines are of major importance to growers in areas where severe stem-pitting strains of tristeza virus are prevalent. Contrary to a belief common among former Galego lime growers in São Paulo, it is possible by preimmunization to obtain vigorous high-yielding orchards of Galego lime. The Galego lime trees preimmunized with some of the best mild strains have grown satisfactorily for 6 years in an area where they have been exposed to infection with severe tristeza virus strains carried in surrounding trees, as was shown by



FIGURE 1. A 6-year-old Galego lime tree preimmunized with a mild tristeza virus isolate from a Galego lime tree photographed  $4\frac{1}{2}$  years after exposure to natural infection. B. Check tree of the same age naturally infected with a severe tristeza virus isolate.

the fact that control plants were naturally infected with such severe strains and declined severely.

The results provide evidence that the preimmunized plants were protected from severe strains of virus occurring naturally in the area. Further evidence for protection is given by the fact that plants preimmunized with mild strains of tristeza virus from Galego lime continued to make satisfactory growth and to bear good crops even when superinoculated with severe stem-pitting virus either by budding or by means of the vector. Thus far, no breakdown in protection has been noticed. We nevertheless consider it advisable to locate Galego lime nurseries and plantings of preimmunized trees far from citrus orchards that might be sources of severe stem-pitting viruses.

The difference in yield between the preimmunized trees and the controls—about fivefold in the first 3 harvests—is bound to increase as the trees mature. Since all trees represent new clones, they were late to come into bearing, and the early crops have been small. In addition,

the preimmunized trees are still growing vigorously, whereas the controls have practically ceased to grow and are dying out.

The preimmunization of Galego lime trees was initially made with 45 mild isolates from Pera sweet orange, grapefruit, and Galego lime sources (1). Of these, only 5 isolates, all from Galego lime sources, were really mild in Galego lime plants. This observation, together with the fact that 2 of the isolates were outstandingly mild, indicates the need for long-range experiments to determine the best mild isolates for preimmunization purposes. It also indicates, specifically for Galego lime clones, that there is a better chance of finding adequate mild isolates for preimmunization in orchards of the same variety.

Budwood of the best preimmunized Galego lime clones is already being distributed on a small scale to citrus growers in order to establish small plots in several areas of the state of São Paulo and in other parts of Brazil, thereby permitting evaluation of preimmunized Galego lime trees under different conditions.

#### *Literature Cited*

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