Results from Preimmunization Tests to Control the Capão Bonito Strain of Tristeza

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ABSTRACT. Cross protection involving five sweet orange types was not effective when protected trees were challenged by tissue grafts. When preimmunized sweet orange scions were exposed to natural infection or challenged by aphid inoculation, the results were not clear cut. Protection was evident in most instances, but supposedly cross-protected trees showed Capão Bonito symptoms due to breakdown in protection or possibly failures in protective inoculation. Stem-pitting of young twigs was not present as a late symptom in Capão Bonito-infected plants. The best readings of protective effect were made by evaluating trunk-pitting on the rangpur lime rootstock at the budunion. Outstanding preimmunized trees and checks that did exceptionally well and were naturally preimmunized are being increased to confirm their protected condition and serve as a budwood supply source for the area.

Index words. Cross protection, rangpur lime, stem pitting.

A number of citrus tristeza virus (CTV) strains with different characteristics are known. One of those is the so-called Capão Bonito CTV strain, that was first brought to the attention of the first two authors in 1965 in Capão Bonito County, São Paulo, Brazil. This particular strain induced stunting, stem-pitting, and poor and sometimes misshapen fruit on practically all sweet orange scion varieties budded on sweet orange and on rangpur lime rootstocks. Most sweet orange citrus types previously had been considered tolerant to the commonly-occurring CTV strains in Brazil (3). These authors, indicated further that the Capão Bonito virus source may contain a component or components not present or associated with the other tristeza virus complexes. Initially it was considered that the Capão Bonito strain could be a new variant of CTV. Later, Rossetti (5) suggested that the Capão Bonito strain may have been introduced from Japan or from Southeast Asia.

In the same paper in which the occurrence of the Capão Bonito strain was described, the authors pointed out a number of approaches to control the virus besides quarantine measures. One of these was the preimmunization of virus-free, nucellar clones of sweet orange varieties with mild, common CTV isolates or complexes that would protect them against the invasion by the Capão Bonito strain. This was based on the good early results for the tristeza-sensitive scions Pera sweet orange, Galego lime and Ruby Red grapefruit recorded in a cross-protection field experiment at Campinas (2).

The idea was put into action, and an experiment was established in the Capão Bonito Expt. Sta. of the Instituto Agronômico. Preliminary results have been published (4).

MATERIALS AND METHODS

The nursery plants were prepared in 1965 in the Virus Department of the Instituto Agronômico at Campinas. For rootstocks, seedlings of rangpur lime were started in the greenhouse in 14 cm x 43 cm flats and later transplanted to 15 cm x 30 cm plastic bags. After these rootstocks were well established, they were placed outside the greenhouse. Regular sprays with pesticides were applied to avoid aphid breeding. As soon as the rootstocks reached the proper size, 10 CTV isolates (seven mild and three regular severe complexes), chosen from the earlier Pera sweet orange, Galego lime and Ruby Red grapefruit experiment (2), were bud-inoculated on the rootstocks, using five plants for each isolate. Two

months after the inoculation, material from five virus-free clonal lines of Lima, Baianinha, Hamlin and Valencia sweet orange were budded on the inoculated rootstocks, so that each isolate was on a single plant of the five clones of the four sweet oranges. Growth of the virus-carrying buds on the rootstocks was permitted to reach about 20 to 25 cm before being trimmed. In case of Pera sweet orange, which was also included in the experiment, buds were collected from seven Pera trees carrying the mild isolates from the earlier test (2) and these were budded on noninoculated, healthy rangpur lime rootstocks. The three severe CTV isolates were not used, since they are too severe for Pera sweet orange. When the scion growth of the plants attained the desirable size, they were topped and permitted to develop three or four branches. Following this, they were transported to the Capão Bonito Expt. Sta. 220 km south of Campinas. Late in 1966, they were transplanted to the field at a 5 m x 4 m spacing. Almost 290 plants were involved in the experiment.

Once in the field, in addition to being exposed to field infection by the oriental citrus aphid, *Toxoptera citricidus* Kirk., each stock-scion isolate combination in this experiment was handled as follows: 1) The first three plants in the row were kept without further treatment, exposed only to natural infection; 2) The fourth plant of each combination was super-inoculated with three buds carrying the challenging Capão Bonito strain. Every receptor plant got one bud from each of three infected sweet orange varieties (Piralima, Hamlin and Valencia sweet orange seedlings) showing severe Capão Bonito strain symptoms from an experiment described previously (1). The buds carrying the challenging virus were budded on the scaffold branches. These inoculations were carried out in 1969, or 3 yr after the experiment had been set into the field; and 3) The fifth plant in the row was super-inoculated with the Capão Bonito strain, by means of at least 50 *T. citricidus* confined in a cage. Aphids were collected from sweet orange trees in an old orchard in the Capão Bonito Expt. Sta., that showed typical decline symptoms induced by the virus. The inoculations were begun early in 1968, and continued until the fifth plant of each row had been infested by the viruliferous aphids, and finally, 4) Noninoculated, healthy combinations were the checks, and were exposed only to natural infection. The Pera sweet orange plot was planted in 1970, but followed the same pattern described above.

RESULTS AND DISCUSSION

Trees in this experiment were generally in average condition. A number of them died due to foot and root rot and also from infestation by a trunk borer.

The following results could be drawn from the experiment:

1) The cross-protection results were not clear-cut in many instances. In the same treatment (mild CTV preimmunized plants), there were cases of definite cross protection (fig. 1A) and trees in which no cross protection was observed, showing Capão Bonito tristeza symptoms similar to those shown by the control plants (fig. 1B). It is not known whether or not the symptoms of CB shown by preimmunized plants were due to breakdown in protection or failure of strain invasion of the protective isolate.

2) The best stem-pitting reading was in the rangpur lime rootstock trunk from the different treatments. Pera, Baianinha (fig. 1C and 1D) and Hamlin sweet orange were combinations on rangpur lime that gave the best stem-pitting readings in this order.

3) Pera sweet orange was the variety that consistently showed some stem pitting in its trunk. In a few cases this was also observed on Baianinha. In the other three varieties, no such symptoms were observed.

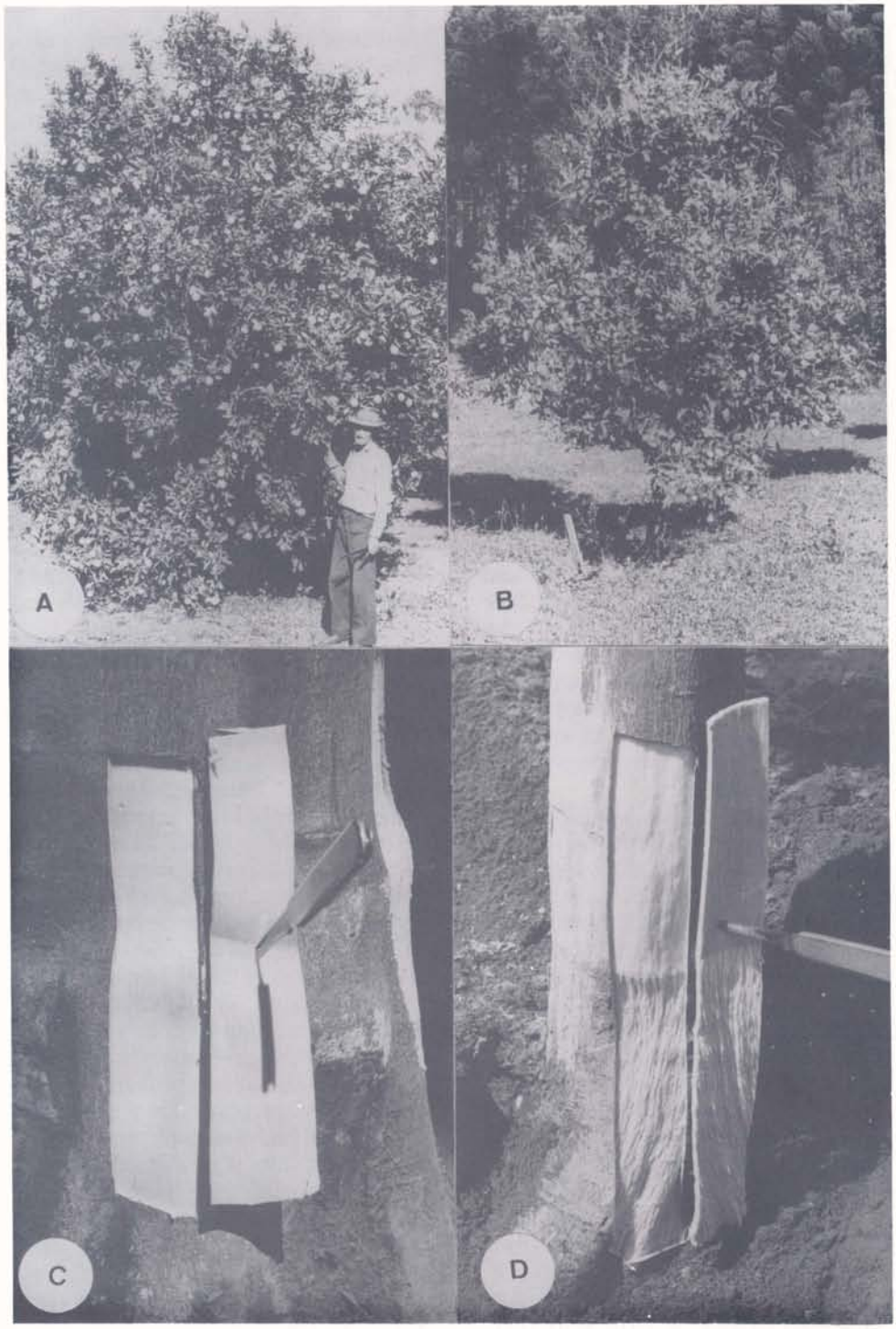

Fig. 1. Cross protection and the Capão Bonito (CB) strain of citrus tristeza virus (CTV). A. Baianinha sweet orange preimmunized and showing cross protection after 20 yr of field exposure. B. Control plant showing symptoms of the CB strain of tristeza. C. Trunk and inside of bark from tree in (A). D. Trunk and inside of bark from tree in (B).

4) Observations carried out for at least 15 yr in the experiment, showed stem pitting in the twigs of the canopies, mainly in Pera, followed by Baianinha, Hamlin and Lima. Valencia virtually did not show stem-pitting symptoms. In the last years of the experiment, there was no stem pitting in the newly formed wood.

5) In the case of challenge with the Capão Bonito strain by graft inoculation, a breakdown in protection was generally observed.

6) It was observed that the common severe CTV isolates induced a faint depressive growth effect on all varieties, when compared with the mild isolates. These differences, however, were not noticed on the yield.

7) The results on Baianinha sweet orange were those which mostly approached the expected model, with the preimmunizing mild isolates inducing the mildest symptoms, followed by the common severe. The Capão Bonito in general had a pronounced effect on growth and yield of the infected plants.

8) Lima and Pera sweet oranges did not behave as well as other scion varieties under the Capão Bonito environment, growing less than the others.

9) Some checks grew very well without severe symptoms, suggesting that they had been naturally infected by CTV isolates different from the Capão Bonito isolates present in the area.

10) Propagations will be made from the best plants (including checks) of the experiment in the experiment station itself. The purpose is to establish whether the protective effect will last. In case this is confirmed, these propagations may be used as bud sources for citrus groves in the Capão Bonito area.

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