the exposure levels of all 3 compounds were below the levels expected to result in acute hazard to workers, preventive practices such as wearing elbow-length vinyl gloves while mixing and loading concentrated formulations could reduce dermal exposure by 50 to 75% (22, 23). Other precautionary measures include keeping spray equipment in good condition, providing shower facilities and requiring workers to use them, encouraging workers to wear frequently laundered heavy cotton clothing, teaching the workers to have a healthy respect for all pesticides, and instructing workers what to do in case of accidental spills. The use of a closed system for handling concentrated formulations and loading spray machines would also eliminate a major source of pesticide contamination (13). Although, impermeable clothing provides excellent protection if properly used, covering skin already contaminated with pesticides may lead to greater absorption (18). The use of impermeable clothing may also increase absorption by workers applying fumigants.

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USE OF MILD STRAINS OF CITRUS TRISTEZA VIRUS (CTV) TO REESTABLISH COMMERCIAL PRODUCTION OF 'PERA' SWEET ORANGE IN SAO PAULO, BRAZIL

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Additional index words. sour orange, tolerant rootstocks, stem pitting.

Abstract. **'Pera' sweet orange (Citrus sinensis (L.) Osb.) is susceptible to CTV-induced decline when budded on sour orange (C. aurantium L.) and is also highly susceptible to CTV-induced stem pitting. Many 'Pera' groves were severely affected by stem pitting even though propagated on the decline-resistant Rangpur lime (C. reticulata var. austera hybrid), and by the late 1950's, planting of this important variety was discouraged in the State of Sao Paulo, Brazil. Subsequently, we found that trees experimentally infected with mild strains were protected against the severe, naturally-occurring strains. The stem-pitting effects of tristeza are now being satisfactorily controlled under commercial conditions by the use of mild strains. Approximately 10,000,000 trees of mild strain-protected 'Pera' sweet orange, the most important variety for Sao Paulo, are in nurseries, young groves, and producing orchards. Good protection also has been observed against the very severe Capao Bonito strain of CTV which occurs in the south of Sao Paulo. The protection of other citrus types with mild CTV strains, as well as the reutilization of the sour orange as a rootstock, is under investigation.**

'Pera' sweet orange (*Citrus sinensis* (L.) Osbeck), a late variety that presently represents 45% of the commercial plantings of the State of Sao Paulo, Brazil (6), always has been one of its most important round oranges (16). The fruit holds well on the tree without loss of quality, and also has good storing and shipping qualities.

Along with the other citrus budded on sour orange (*C. aurantium* L.), 'Pera' was nearly wiped out when the tristeza virus was introduced into Brazil. The observation that scions budded on sweet orange, Rangpur lime (*C.*

reticulata var. *austera* hybrid) and mandarin (*C. reticulata* Blanco) rootstock's were unaffected by CTV decline led to the use of tristeza-tolerant rootstocks for control (2). Initially, the shift from sour orange to tristeza-tolerant rootstocks was satisfactory. Within a few years, however, it was noted that many 'Pera' groves were severely affected by CTV-induced stem pitting, even though propagated on the decline-resistant Rangpur lime. Stunting, mineral-like deficiency patterns on the leaves and small fruit of no commercial value were additional symptoms of CTV infection on the affected plants (5). Growers claimed that 'Pera' trees stopped growing, or grew at a slower rate than trees of other varieties (17). CTV decline can cause dramatic nearly complete temporary loss of production. This loss, however, is restored by use of CTV-tolerant stocks. Stem pitting effects of CTV cause a less dramatic drop in production but the effect is continuous (Fig. 1). By the late 1950's, planting of this important variety was discouraged in the State of Sao Paulo and other late varieties, such as Valencia and Natal (a type of Valencia) were propagated to replace it in the market. However, because of its high fruit quality, this variety was preferred for both local and export markets, as well as for the then new processing industry; a gap difficult to be filled was left.

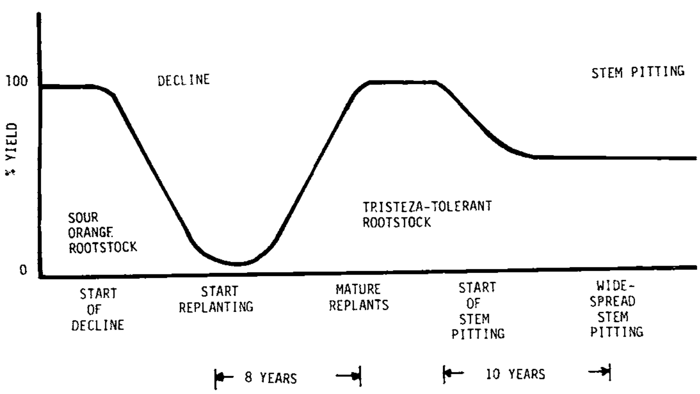

Fig. 1. Schematic representation of yield reduction in citrus due to tristeza-induced decline on sour orange rootstock and subsequent tristeza-induced stem pitting on tolerant rootstocks. Note continuing loss from stem pitting.

Materials and Methods

To continue growing 'Pera' sweet orange, additional control measures had to be developed, and cross protection or preimmunization was considered the most promising option.

The work already presented and discussed in more detail elsewhere (3, 10) started with a search for mild CTV isolates. We looked for trees doing well in orchards that were uniformly and severely injured by severe tristeza, and budwood material was taken from these outstanding trees for further study. If such trees were true to type, they probably originated from a bud in which a mild strain or complex was offering good protection from severe infection predominant in neighboring trees. On the basis of good growth and general appearance, some 80 outstanding plants were selected from 3 varieties under study. Budwood material from these plants was established at Campinas in nursery rows of tristeza-tolerant rootstocks and on the intolerant sour orange. Also the material was used to inoculate seedlings.

Among the citrus material carrying mild isolates that was collected and established, 45 were used to inoculate nucellar clones of 'Pera' sweet orange and also 'Galego' lime [*C. aurantifolia* (Christm.) Swing.] and 'Ruby Red' grapefruit (*C. paradisi* Macf.), in an experiment involving almost 2300 plants of the 3 scions; 5 plants representing the basic experimental unit. In addition to field exposure to natural superinfection by regular tristeza complexes, 2 of 5 plants of each virus-plant combination were challenge-inoculated with a severe virus isolate, one by budding and one by aphid vector. Noninoculated control plants and plants inoculated with severe isolates were also prepared.

Results and Discussion

From the originally selected mild isolates, 3 were entirely satisfactory for 'Pera' sweet orange. Plants preimmunized with these grew very well, had practically no symptoms of stem pitting, and produced good yields in the first crops, compared with plants from the same clones that either had not been preimmunized or had been deliberately inoculated with severe isolates. Plants preimmunized with many other selected mild isolates also performed better than the controls, but not as well as the best 3 isolates for 'Pera' sweet orange. A slight difference was noted between the different nucellar clones of 'Pera' sweet orange, with clone no. 2 being the best of the five evaluated. By 1968, these satisfactory experimental results made it possible for the best combinations of preimmunized 'Pera' sweet orange to be field tested by interested growers and nurserymen in different areas of Sao Paulo. The good performance of the preimmunized 'Pera' sweet orange, combined with the demand for nursery trees of this cultivar, led growers and nurserymen to propagate these plants as fast as possible (7, 9). At present, approximately 10,000,000 trees of preimmunized 'Pera' sweet orange are in nurseries, young groves, and producing orchards. The preimmunized 'Pera' clone is still the first in yield in its 8-year comparison with 12 other selected 'Pera' clones maintaining its former position (19). Currently, most of the 'Pera' budwood used in the State of Sao Paulo is derived from the original preimmunized material produced in the Virus Department of the Instituto Agronomico de Campinas.

Good protection also has been observed against the very severe Capao Bonito strains of CTV which occur in the southern part of Sao Paulo (14). Results show that preimmunized 'Pera' sweet orange exposed to the Capao Bonito tristeza variant in a 10-year period had a satisfactory performance, confirming previous results that selected mild tristeza isolates protect sweet orange plants against the Capao Bonito complex. Two orchards of preimmunized 'Pera' trees located in the Tatui and Angatuba counties of the southern region of the State of Sao Paulo where the occurrence of the Capao Bonito tristeza variant was recorded, were observed to perform well (13).

This work, practically the only one in the world on this commercial scale, definitely shows the potential of again growing the 'Pera' sweet orange by the use of material preimmunized with mild strains that protect the plants against subsequent natural infection by the severe isolates of the tristeza complex. In the period 1977-1979, the 'Pera' orange represented 55%, the 'Valencia' 4%, and the Natal 17% of all citrus nursery trees formed in the State of Sao Paulo, thus again attaining the position it had lost nearly 2 decades ago (J. Teofilo Sobr., unpublished data).

In addition to the stem pitting in 'Pera' sweet orange, other sweet oranges show variable tolerance to tristeza virus strains in Brazil. One of the sweet orange varieties of potential value for Sao Paulo is the 'Westin' orange (6). Nucellar clones of this variety produced in the mid-1960's for the Sao Paulo Citrus Budwood Registration Program became naturally infected by a severe tristeza isolate, after being planted in the field, and budwood distribution had to be stopped because of stem pitting in the mother trees and in subpropagations (J. Teofilo Sobr, unpublished data).

An experiment set out in 1972 with several sweet oranges, is being carried out to determine if the less tolerant sweet orange varieties and even those considered highly tolerant could benefit from preimmunization with mild strains of CTV (12). Eight years after being set in the field, new nucellar clones (10 years old from seed) preimmunized with mild CTV isolates are growing better than new and old nucellar clones (20-30 years old from seed) infected with common tristeza virus complexes. The results of this experiment can be better evaluated when the trees reach bearing age, making it possible to compare quantity and quality of the fruit.

Still another use for the preimmunized 'Pera' budwood could be to rehabilitate tristeza-affected trees by topworking them with preimmunized budwood. It is expected that, in some cases, the mild isolates in the topworked budwood can suppress the severe tristeza isolate occurring in the affected tree, or at least blend with it producing somewhat milder symptoms (8). An experiment on this was initiated in 1975 in a 'Pera' orchard affected by CTV. The topwork treatments (replicated 4 times) included 'Pera,' 'Valencia,' and 'Natal' sweet orange as follows: a) budwood preimmunized separately with different mild tristeza isolates; b) budwood infected with severe tristeza isolates; c) tristeza-free budwood, and d) severe pruning with the canopy allowed to recover. A freeze killed a good number of plants, including nearly all of the 'Valencia' and 'Natal' sweet oranges, but the remaining trees recovered and are presently growing well. So far, some differences between the different treatments have been noticed, but they are not yet conclusive (Müller and Costa, unpublished data).

A type of decline of unknown cause similar to the young tree decline (YTD) of Florida is now starting to induce heavy losses in Sao Paulo orchards (15). Since this decline affects sweet orange scions grafted on Rangpur lime, it is of great concern to citrus producers, because Rangpur lime is the basic rootstock for all types of soils, presently accounting for nearly 85% of the rootstocks used in the State. In a search for alternative rootstocks, the good qualities of the sour orange are remembered and the interest to use it is high in spite of tristeza (11). Since sour orange is one of the most resistant rootstocks to YTD, (18) it could be very valuable if CTV damage could be controlled.

There are 3 approaches.

1. *Preimmunization of sour orange with mild isolates.* Mild tristeza isolates can be found in Florida that allow sweet on sour combinations to grow satisfactorily. Such a possibility seems remote under Brazilian conditions where millions of trees budded on sour orange were killed when the tristeza virus was introduced into the country. Not a single one survived, indicating that no segregation of mild strains occurred at that time under natural conditions or that mild strains were protective against CTV decline. In spite of this fact, some mild isolates for sour orange were found, but these were not good enough to permit the use of sour orange again as a rootstock.

2. *Sour orange types with more tristeza-tolerant tissues.* The possibility of finding sour orange types more tolerant to the tristeza virus always has been considered with great interest. More than a hundred selections of sour orange were tested at the Instituto Agronomico de Campinas, but none showed promising tolerance. More recently a Chinese sour orange, called Gou-tou, has been described as tristeza tolerant (1). It would be of interest to try this type under Brazilian conditions.

3. *Hybrids of sour orange with other tolerant citrus types.* Since it has not been possible to find a CTV-tolerant sour orange, a third possibility would be to obtain either artificial or natural hybrids with the good characteristics of the sour orange and the tristeza tolerance of the other parent. Among the citrus types best suited for these crosses are sweet oranges, mandarins, *Poncirus trifoliata* (L.) Raf. and its hybrids, and Rangpur lime.

In the near future, it seems that the best approach would be to use sour orange or sour orange hybrids with somewhat more tolerant tissues to tristeza, like the Smooth Seville orange (4), preimmunized with a mild isolate for the sour orange. It is possible that the combination of the two factors would permit the grower once again to use this excellent rootstock.

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