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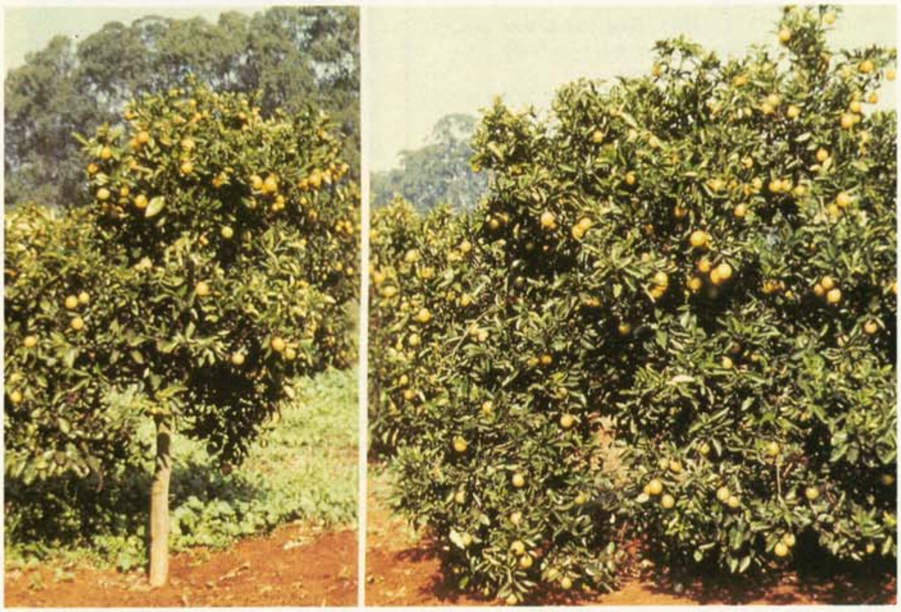
Tristeza Control by Cross Protection



Pera sweet orange on Rangpur lime rootstock. (Left) Tree naturally infected with severe strain of tristeza virus and (right) tree cross-protected by mild isolate of the virus. Both plants were exposed under comparable conditions.

Pera sweet orange fruits from (left) tree naturally infected with severe strain of tristeza virus and (right) tree cross-protected by mild isolate of the virus.

Less than two decades after tristeza was introduced from Africa into South America in the 1920s, the disease practically wiped out the citrus industry in areas of Argentina, Brazil, and Uruguay. Field observations and citrus rootstock trials (2,3,7,10) showed that the disease principally affected citrus combinations budded on sour orange rootstock (*Citrus aurantium* L.). Most sweet oranges (*C. sinensis* Osb.), mandarins, and tangerines (*C. reticulata* Blanco) that were budded on sweet orange, Rangpur lime (*C. limonia* Osb.), and mandarin were unaffected. This important finding led to use of tristeza-tolerant rootstock for basic control.

Meneghini's transmission tests of tristeza with the citrus oriental aphid (*Toxoptera citricidus* Kirk.) (6) and the tissue union transmission established for a similar disease in California (9) confirmed the viral nature of the disease that had been suspected by early investigators.

Alarm Prompts Joint Project

The rapid spread and heavy losses associated with tristeza in South America established the disease as a scourge of citrus and alarmed growers in other countries. Because citrus interests in California and Florida and U.S. agricultural agencies feared that tristeza might become widespread in the United States, Congress appropriated money to support cooperative work in Brazil by an American scientist. Information thus acquired would not only benefit Brazil but would also supply advance knowledge to American citrus growers. C. W. Bennett, the eminent plant virologist, arrived in Brazil late in 1946 and started a cooperative research project between the U.S. Department of Agriculture and the Instituto Agronômico, an agricultural research institution in the state of São Paulo.

Experiments by Bennett and one of us (Costa) (1) confirmed Meneghini's conclusion that tristeza was infectious and could be transmitted by the citrus oriental aphid. Transmission by budding

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A U.S.-Brazil Cooperative Success

and by dodder was also demonstrated. The possible reactions of different citrus types to tristeza based on virus increase in the plant and sensitivity of its phloem tissue (or rootstock, in the case of budded plants) to injury were discussed.

T. J. Grant, who followed Bennett as the U.S. scientist in the project, and Costa found that stem pitting was a reaction of some citrus types to tristeza infection and not a separate disease (4). In addition, infection with mild strains found in natural tristeza virus complexes in Brazil protected against injury by severe strains when cross-protected plants were exposed to natural infection (5). These findings paved the way for tristeza control by cross protection, or preimmunization.

The Most Promising Option

Initially, the shift from sour orange to tristeza-tolerant rootstocks was thought to be satisfactory for overall control. However, several important commercial citrus types permitted virus increase and had tissues sensitive to tristeza infection; these were injured even when budded on tristeza-tolerant rootstocks. This tendency was first noticed experimentally in the West Indian lime (*C. aurantifolia* (Christm.) Swing.) and grapefruit (*C. paradisi* Macf.) and later in orchards of Pera sweet orange, Brazil's most important citrus cultivar. To continue growing these citrus types, additional control measures had to be developed, and cross protection was considered the most promising option.

Although *Poncirus trifoliata* Raf. and other citrus types are resistant to tristeza virus, control by breeding has never been considered a solution. The possibility of recovering progeny with the qualities of the standard commercial citrus and the resistance of the other parent in the cross seems remote. Furthermore, the situation in Brazil appeared ideal for the cross protection approach because: 1) the disease was too widespread in citrus-producing areas to eradicate, 2) the existence of mild strains with their protective effect had been established, and 3) the protective effect probably could be maintained through successive propagations of the cross-protected clonal material without breaking down.

The Search for Mild Isolates

As a result of a visit of USDA scientists Claud L. Horn, Earl R. Glover, and Bert Lexen to the Instituto Agronômico early in 1959, a 5-year cooperative research project on tristeza control by cross protection was planned. The project, funded with proceeds from the sale of U.S. agricultural surpluses (P.L. 480) to Brazil, started in 1961 and aimed at controlling injury to citrus commercial types affected when budded on tristeza-tolerant rootstocks. Specifically dealt with were the Brazilian Pera sweet orange, the Galego lime (a type of West Indian lime), and the Ruby Red grapefruit.

Although a few mild isolates of the tristeza virus complex were available in 1961, others were collected to increase the chances of obtaining a desirable one for each citrus type being investigated. Many orchards of Pera sweet orange and Galego lime were surveyed but only a small number of Ruby Red grapefruit orchards, because this fruit is not commonly grown in the state of São Paulo. We looked for trees doing well in orchards that were uniformly and severely injured, 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 of the virus had been segregated and was offering good protection from severe infection predominant in neighboring trees.

On the basis of good growth and general appearance, 83 outstanding plants were selected: 53 Pera sweet orange, 23 Galego lime, and 7 Ruby Red grapefruit. Budwood material from these plants was established at Campinas in nursery rows of two tristeza-tolerant rootstocks (Rangpur lime and Caipira sweet orange) and on the intolerant sour orange rootstock.

Budding on the two tolerant rootstocks established the selected plants for further study and also indicated the presence of exocortis viroid in propagative material by the reaction of plants of the first scion-rootstock combination. This was true in two instances. The purpose of establishing sources of mild isolates on sour orange was to determine if some would be mild enough to permit the use of sour orange again as a rootstock. Although differences were noticed, most isolates were not sufficiently mild. One isolate, No. 50, showed some promise.

Field Tests of Mild Isolates

Among the citrus material carrying mild isolates that became established in the nursery, budwood from 45 was used to inoculate new virus-free citrus combinations of Pera sweet orange, Galego lime, and Ruby Red grapefruit that had been budded on three rootstocks: Rangpur lime, Caipira sweet orange, and Cleopatra mandarin. The five plants of each virus-scion-rootstock combination representing the basic experimental unit consisted of tops of five different nucellar clones of the citrus scion type concerned. The virus-free rootstocks were inoculated with the different isolates at the time of budding of the upper bud of the virus-free scion. Noninoculated control plants and plants inoculated with severe isolates were also prepared.

The nursery plants were later transplanted to field plots at spaces slightly closer than in a regular citrus orchard. Almost 2,300 plants of the three scions were involved. In addition to field exposure to natural superinfection by regular tristeza complexes, two of the five plants of each virus-plant combination were challenge-inoculated with a severe virus isolate, one by budding and one by aphid vector.

Early Results Show Promise

Data based on tristeza and stem pitting readings, observations on plant growth, and results of the first crops disclosed that of the 45 mild isolates originally selected, six were entirely satisfactory: three for Pera sweet orange, two for Galego lime, and one for Ruby Red grapefruit. Plants that had been cross-protected with these six were growing very well, had practically no symptoms of tristeza or stem pitting, and produced good yields compared with plants from the same clones that either had not been cross-protected or had been inoculated with severe isolates. Plants cross-protected with many other selected mild isolates also performed better than the controls but not as well as the six best. No

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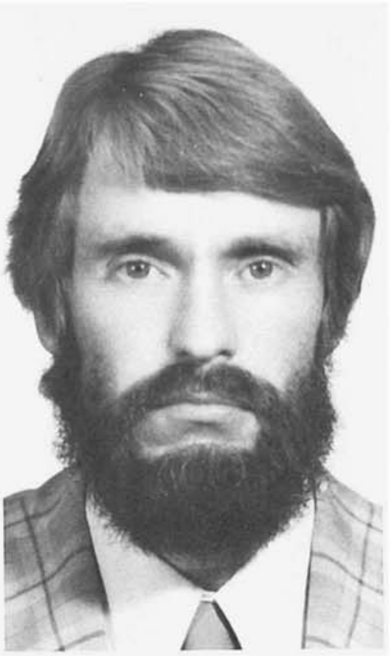

G. W. Müller

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differences in tristeza were attributable to the rootstocks.

Results of the challenge inoculations varied with the method of inoculation. Plants challenge-inoculated by tissue union showed symptoms, possibly from virus blending. No response to challenge inoculation by the vector was observed.

Galego lime reacted more severely to mild isolates from Pera sweet orange and Ruby Red grapefruit than to mild isolates from Galego. Similarly, the best isolates for Pera sweet orange were collected from Pera. A slight difference was noted among the reactions of the different nucellar clones of Pera sweet orange, with clone No. 2 being the best of the five.

By 1968, the best combinations of cross-protected Pera sweet orange and Galego lime plants were good enough to justify testing by interested growers and nurserymen in different areas of São Paulo. Cross-protected material for 50 plants was distributed to each for comparison with an equal number of plants from the growers' best sources.

Performance Creates Demand

The performance of cross-protected plants in the nursery and the results of early crops almost always surpassed those of the growers' best sources. An inquiry in 1974 among growers who received the first cross-protected samples revealed especially good results for Pera sweet orange, and the demand for propagating material was great.

The consensus was to wait a few years to obtain additional results from the original field test and from participating citrus growers before recommending large-scale propagation of the cross-protected Pera sweet orange and Galego lime. However, the good performance of the cross-protected Pera sweet orange combined with the demand for nursery trees of this cultivar and the desire "to beat the other fellow to the punch" led growers and nurserymen to propagate the plant as fast as possible. This occurred to a much lesser extent with the cross-protected Galego lime, which was in little demand even though its performance had been as satisfactory as that of Pera sweet orange.

By early 1977 (8), about 5 million cross-protected Pera sweet orange and Galego lime trees had been planted, with the sweet orange predominating. At present, the number of cross-protected Pera sweet orange trees exceeds 8 million.

No Breakdown in Protection

Greenhouse and field tests have shown that protection given to citrus scions by inoculation with mild tristeza virus isolates is not broken down by super-inoculations with the vector or by field exposure to natural populations of the vector for long periods.

Large-scale propagation of cross-protected Pera sweet orange by budding has revealed almost no breakdown in protection in successive clonal generations. In a few instances, a small number (usually less than 1%) of the prepared cross-protected nursery plants have had stronger tristeza symptoms than expected. This is not considered a breakdown in protection but rather a consequence of virus blending resulting from propagating the cross-protected bud on rootstock naturally infected with the regular tristeza virus complex before budding.

A survey of rootstock nurseries in different regions of São Paulo before budding showed that a small percentage of plants are usually infected. The number of infected plants was smaller for Rangpur lime rootstock than for Pera sweet orange or Cleopatra mandarin. Because Rangpur lime is practically the only rootstock used in São Paulo, preparing virus-free rootstock for budding with cross-protected buds has not been a problem and preventive measures have not been necessary. If a shift to sweet orange or mandarin rootstock is made, however, control measures in the nursery will probably be needed to avoid tristeza infection.

If rootstock nursery infection becomes a problem in the production of cross-protected plants, the nursery can be located in a noncitrus area where the tristeza vector population is very low. This step, alone or combined with an aphid repellent mulch around the plants, gives adequate control.

Wider Use of the Method

Although tristeza control by cross protection was started to reduce losses of very sensitive citrus scion types, the method is also being considered for wider application. Initial tests on sweet orange cultivars sensitive to Capão Bonito strains of the virus have been successful. Most sweet orange and mandarin commercial cultivars are considered highly tolerant to regular tristeza infection, but even they may be benefited by mild isolates instead of the regular tristeza virus complex. This possibility is being investigated with cross-protected combinations of new nucellar clones of several tristeza-tolerant sweet orange types and with some old clones freed from tristeza by thermotherapy and then cross-protected with mild isolates.

Tristeza control by cross protection works well in Brazil, where the effect of the virus complex is usually rather severe, the efficient aphid vector is fairly dense, and the chance of challenge inoculation of the cross-protected material by natural exposure is high. Because protection has continued under these highly unfavorable conditions, this method of control in sensitive citrus combinations should work in other areas. Failures reported by some investigators in other countries should be considered temporary; new attempts should be made to secure protective mild isolates. If we had started our tests on the Pera sweet orange with 20 mild isolates instead of 45, the best accessions might have been missed.

Our experience in searching for protective mild isolates highlights a few important points. The investigators should: 1) start with a rather large number of mild isolates, 2) use field isolates collected in orchards of the scion concerned, 3) collect mild isolates from outstanding plants in orchards where almost all trees show strong symptoms, and 4) evaluate the protective value of the isolates by using the vector for transmission and not by tissue union alone.

Possible Future Considerations

Commercial orchards of cross-protected Pera sweet orange have been established for over a decade. Their performance has been highly satisfactory, and almost all new orchards of this cultivar are established with cross-protected material.

Planting large acreages of cross-protected citrus plants conceivably will modify the strain composition of the tristeza virus complex carried by vectors in these areas. So far, in Brazil, the chances of new complications seem small. The alternative would be to have an assorted population of strains, mostly severe and from different origins, but this would certainly increase the risk.

Some virus segregation almost certainly will occur in successive clonal generations of bud-propagated, cross-protected Pera plants when millions of trees are involved, but hopefully, alert growers or experts will be able to identify such segregants. The problem will be to differentiate improved clones resulting from virus segregation from those representing favorable bud mutations with less sensitive tissues.

When a complete crop change is made, any anomaly, insect, or disease appearing on the new crop seems ominous to the growers and is invariably blamed on the change. This has already happened in a few instances with the cross-protected Pera sweet orange in Brazil. Fortunately, this concern has been fairly easy to dispel.

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