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*Cross Protection from Tristeza in Different
Species of Citrus*

THE CITRUS BUDWOOD PROGRAM of the Brazilian Ministry of Agriculture has considered since 1959 the registration of trees, especially Pera orange, Galego lime, and grapefruit, that carry a mild strain of tristeza virus. The main scope of the present investigation is to determine how much protection the presence of such mild strains will give against severe strains of tristeza virus. The present paper reports the results thus far obtained with some of the mildest strains of the virus obtainable locally.

Apparently, Olson (4) was the first to report on the inhibition of symptoms of severe strains of tristeza virus by inoculating citrus plants with mild strains. Grant and Higgins (3) reported that Key lime plants inoculated with a very mild strain of tristeza virus had considerable protection from a severe strain of virus when challenged four months later. They also suggested, however, that presence of mixtures of strains of tristeza virus in plants is related to the failure of any one strain to become fully systemic. A mild strain of virus that does not become fully systemic is not likely to protect completely against a more severe strain.

Materials and Methods

Potted seedlings of the following species were inoculated: sour orange (*Citrus aurantium* L.), Eureka lemon [*C. limon* (L.) Burm. f.], Marsh seedless grapefruit (*C. paradisi* Macf.), Galego lime [*C. aurantifolia* (Christm.) Swing.], Pera sweet orange (*C. sinensis* Osbeck), Rio mandarin (*C. deliciosa* Tenore), and *C. webberii* Wester. The plants, which were six months old, were distributed into four sets for inoculation. The

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first set was inoculated with a very mild strain of tristeza virus, the second with a severe strain, the third set was inoculated first with the mild strain and four months later with the severe strain, and the fourth set was maintained uninoculated for healthy controls. The plants were kept under observation in a screenhouse.

The mild strain used for inoculation is the mildest of nine mild strains that were selected from the Rio citrus area. It causes very little pitting on Galego lime plants and only mild vein clearing. The severe strain was selected a few years ago by A. S. Costa and is considered the most severe strain ever known in Brazil. All the test plants were inoculated, for the second time, with the severe strain ten months after the first inoculation.

Six months after the first inoculation, all the plants were cut back to a height of 20 cm. As indication of the effect of the inoculations, the new growth that was produced was measured from the point at which the cut was made. The pits that developed in a 20-cm long portion of stem of this new growth were counted for each inoculated plant.

Results

Results of the inoculations are presented in Table 1 and will be summarized here.

SOUR ORANGE AND EUREKA LEMON.—The mild strain caused no reduction in growth and no stem pitting. The severe strain induced seedling yellows, with severe stunting and no recovery within 6 months from the first inoculation. Plants inoculated with the mild strain and later with the severe strain had symptoms similar to those induced by the mild strain alone; they were not reduced in growth and did not have seedling yellows.

GRAPEFRUIT.—Plants infected with the mild strain were not reduced in growth and developed about 17 pits in a 20-cm length of stem. Those infected with the severe strain were stunted, had smaller leaves that were mottled, developed numerous pits in the stem, and produced vigorous, apparently normal suckers. Plants inoculated with both viruses developed symptoms intermediate between those inoculated with the mild strain and those inoculated with the severe strain; they had small leaves with deficiency symptoms but no stem pitting.

GALEGO LIME.—The mild strain induced little or no stunting and little pitting; vein clearing developed in young leaves and persisted in mature leaves. The severe strain reduced growth, induced numerous pits and small cupped leaves; vein clearing developed on young leaves and

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TABLE 1. GROWTH IN CENTIMETERS AND STEM PITTING PER 20 CM OF SEVEN CITRUS SPECIES FOLLOWING INOCULATION WITH VERY MILD, SEVERE, AND MILD + SEVERE STRAINS OF TRISTEZA VIRUS

| Variety | Very Mild | | Severe | | Mild + Severe | | Control | |
|--------------------|-----------|----|--------|---|---------------|----|---------|----------------|
| | a | b | a | b | a | b | a | b |
| Sour orange | 73 | 0 | 28 | 0 | 81 | 0 | 81 | 0 ¹ |
| | 111 | 0 | 42 | 0 | 113 | 0 | 117 | 0 ² |
| Galego lime | 50 | 0 | 25 | 0 | 70 | 0 | 65 | 0 |
| | 120 | 77 | 31 | M | 67 | 43 | 134 | 0 |
| Eureka lemon | 57 | 0 | 27 | 0 | 67 | 0 | 46 | 0 |
| | 112 | 0 | 32 | 0 | 107 | 0 | 121 | 0 |
| Marsh grapefruit | 25 | 0 | 26 | 0 | 58 | 0 | 54 | 0 |
| | 96 | 17 | 44 | M | 70 | 0 | 129 | 0 |
| Pera sweet orange | 50 | 0 | 47 | 0 | 44 | 0 | 48 | 0 |
| | 88 | 0 | 96 | 0 | 72 | 0 | 97 | 0 |
| Rio mandarin | 24 | 0 | 33 | 0 | 27 | 0 | 29 | 0 |
| | 54 | 0 | 48 | 0 | 47 | 0 | 60 | 0 |
| <i>C. webberii</i> | 28 | 0 | 13 | 0 | 27 | 0 | 48 | 0 |
| | 40 | 0 | 26 | M | 36 | M | 74 | 0 |

¹ First reading—September, 1962.

² Second reading—July, 1963.

a=Growth in centimeters.

b=Number of pits per 20 cm of stem.

M=Massive stem pitting.

was present on half-mature leaves but did not persist on mature leaves. Symptoms on plants inoculated first with the mild strain and then with the severe strain were intermediate in severity.

PERA SWEET ORANGE AND RIO MANDARIN.—No symptoms developed on any of the plants; inoculated plants appeared to be similar to the healthy controls.

C. WEBBERII.—The plants of this species behaved very much like those of grapefruit and Galego lime.

Discussion and Conclusion

The severe strain that induces severe stem pitting and stunting in lime in Brazil is able to induce seedling yellows in sour orange and Eureka lemon and to produce stunting and extensive stem pitting in grapefruit. It does not, however, induce symptoms in Pera sweet orange. From this one might conclude either that a longer time is required for the virus

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to induce its effects in Pera sweet orange or that an entirely different strain of tristeza virus is involved in the stem pitting of Pera sweet orange. Either explanation would account for the fact that stem pitting on Pera sweet orange was not considered a problem until 12 years after stem pitting on lime and grapefruit was important (1, 2). Lime and grapefruit will show stunting in the nursery when they carry a severe strain of tristeza virus, whereas Pera sweet orange does not ordinarily show stunting or pitting until it is 5 years old or older. A third explanation for stem pitting in Pera orange, suggested by Grant, Moreira, and Salibe (2), is that it may be due to new or recombined mixtures of the tristeza virus complex.

Plants of Galego lime, grapefruit, and Pera sweet orange that originated from buds known to be carriers of a mild strain of tristeza virus were observed at the age of 3-8 years under field conditions where both the severe strain of tristeza virus and its aphid vector, *Toxoptera citricidus* (Kirk.), are known to be present. These plants were not severely diseased but rather exhibited symptoms intermediate between those characteristic of a mild strain of virus and those of a severe strain. Observations such as these, combined with the experimental results recorded above, suggest that plants of lime and grapefruit infected by a mild strain of tristeza virus are satisfactorily protected from the effects of a severe strain. Whether or not a similar conclusion can be reached for Pera sweet orange remains to be determined.

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