Further Studies on Exocortis Disease of Citrus

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Exocortis, as a gum disease on Rangpur lime rootstocks, is known to have occurred in Brazil long ago (Moreira, 1946). Extensive losses due to citrus exocortis viroid (CEV) occurred in the 1950's, following the tristeza disaster, because replanted trees were mostly on exocortis-susceptible Rangpur lime (Moreira, 1955; Rossetti, 1955; Montenegro and Salibe, 1957; Salibe, 1961). Inarching declining trees with exocortis-tolerant rootstocks then became a common practice and the general use of CEV-free nucellar clones soon occurred. Currently, of the 150 million citrus trees in Brazil, about 90 per cent are on Rangpur lime, so exocortis, though now a rare disease, remains a permanent threat to the citrus industry. Some instances of CEV contamination on supposedly healthy nucellar trees have been found recently in commercial orchards, causing concern among growers. Mother trees used by growers are exocortis free and the seed transmission reported by Salibe and Moreira (1965*a*) is now considered to have been mechanical transmission. Spread of CEV must be occurring by mechanical means or by an inefficient insect vector. This paper reports the results of CEV inoculations in large citrus trees and noncitrus fruit trees, mechanical spread, and some relationships between Tahiti lime and CEV.

EXPERIMENTS AND RESULTS

Late infection. An experiment was conducted to determine the effect of late infection with CEV on the growth and production of large field trees. Exocortis infection due to cuts, pruning, or machine hedging is known to occur in the greenhouse (Garnsey, 1967) and field (Wutscher and Shull, 1975) and extensive spread of CEV might take place if an insect vector should appear, thus threatening an industry based almost entirely on a rootstock intolerant to CEV.

Trees of eight nucellar scion varieties, budded on Rangpur lime rootstock eight years old were used. Scions were: Dancy and Cravo tangerines, Murcott tangor; Hamlin, Barao, Baianinha Navel and Valencia oranges; and Tahiti lime. Inoculation was done in February 1974 by budding 10 young branches of each tree with a bud carrying a severe CEV strain. Four trees of each scion variety were inoculated at random and another four maintained as healthy controls. No visible difference between infected trees and healthy controls was observed in a five-year period, except for some occasional leaf yellowing of the inoculated Tahiti lime trees. No cracking, bark scaling, or gumming developed in the Rangpur lime rootstock of any tree. Comparisons of average tree growth and production in the five-year period, and of tree height in 1979, are shown in table 1.

The experimental trees will be followed for years to determine the incubation period of CEV in large trees and the extent of its detrimental effect on growth and production.

Indexing these trees for CEV as described by Calavan *et al.* (1964), revealed that all are infected with a severe strain of CEV.

Mechanical spread. A study was made to find out if some citrus varieties provide more highly infectious inoculum than others in mechanical transmission of CEV. If this is the case, it might explain the negative results in previous mechanical transmission experiments by Salibe (1961). Since the presence of viruses in the source plants might affect CEV transmission, this possibility was considered. Trees of four citrus varieties were selected as donor plants for the transmission experiment: a tree of Moro orange carrying CEV and psorosis and tristeza viruses; a tree of Salustiana orange carrying CEV plus a severe strain of tristeza virus; a tree of Eureka lemon infected only with CEV; and a plant of Etrog citron previously infected with CEV plus mild tristeza virus. All field trees of citrus in Brazil are infected by tristeza virus, except true lemons, trifoliate orange, and a few other citrus types having tissues resistant or highly intolerant to the virus.

Indicator plants were Etrog citron 60-13 and Rangpur lime, free from tristeza virus. Inoculations were made in the greenhouse in November and December 1975, using the technique described by Garnsey (1967). Twenty plants of each indicator variety were mechanically inoculated from each donor tree. Control plants were budded with blind buds from the same donor trees. Readings made up to one year after inoculation showed that most (90-95 per cent) of the mechanically inoculated Etrog citron plants developed mild or severe symptoms of CEV independently of donor variety and the presence of tristeza virus. However, the situation was different in Rangpur lime plants; only 45 per cent (9 of 20) plants with inoculum from Eureka lemon and 50 per cent (10 of 20) plants with inoculum from Etrog citron developed exocortis symptoms. None of the Rangpur lime plants mechanically inoculated from

TABLE 1

AVERAGE TRUNK SIZE AND GROWTH AND FRUIT PRODUCTION IN A 5-YEAR PERIOD AND TREE HEIGHT IN 1979 OF LARGE FIELD TREES ON RANGPUR LIME ROOTSTOCK INOCULATED LATE WITH CEV COMPARED WITH UNINOCULATED HEALTHY TREES

Scion varieties		Trunk circumference 1974 cm	Trunk circumference 1979 cm	Trunk growth 1974-1979 cm	Fruits no.	Tree height m
Murcott	I*	30.5	42.5	12.0	588	3.25
tangor	H*	33.0	46.0	13.0	570	3.70
Dancy	I	42.5	59.5	17.0	1,232	3.85
tangerine	H	43.5	60.0	16.5	1,268	3.42
Cravo	I	37.0	45.5	8.5	598	3.23
tangerine	H	35.8	48.0	12.2	625	3.40
Hamlin	I	45.3	58.0	12.7	908	4.18
orange	H	44.5	60.7	16.2	977	4.60
Barao	I	40.5	55.0	14.5	735	3.83
orange	H	38.0	48.5	10.5	930	3.85
Baianinha	I	38.8	51.3	12.5	560	3.60
navel orange	H	47.0	59.0	12.0	410	3.40
Valencia	I	33.7	42.0	8.3	567	2.83
orange	H	31.7	43.3	11.6	513	2.83
Tahiti	I	51.0	68.5	17.5	820	4.13
lime	H	62.3	75.0	12.7	1,014	4.33
AVERAGE	I	39.9	52.8	12.9	751	3.61
	H	42.0	55.1	13.1	788	3.69

* I = infected trees; H = healthy controls.

orange tree donors showed symptoms. All bud-inoculated plants developed typical exocortis symptoms beginning within 4 months. Two plants of Rangpur lime mechanically inoculated from the Moro orange tree developed psorosis leaf symptoms during the spring flush in September 1976.

Exocortis in Tahiti lime trees. Tahiti lime is highly valued and extensively grown in Brazil. It is estimated that over three million trees of this variety of citrus have been planted in the last 15 years. Five clones of Tahiti lime are commercially grown and are CEV infected, except for one clone named IAC-5 (Peruano). CEV is considered responsible for a bark disease on Tahiti lime trees (Salibe and Moreira, 1965*b*). Observations in commercial plantings of Tahiti lime with bark disease never showed bark-scaling symptoms on Rangpur lime rootstocks. For this reason, it was suspected that Tahiti lime tissues do not increase or maintain the more severe strains of CEV. This hypothesis was confirmed in an experiment at the "Presidente Medici" Experiment Station, in Botucatu, starting in January 1973.

Rangpur lime seedlings in the nursery were inoculated with blind buds (three buds per seedling) from a Hamlin orange tree known to be carrying a severe strain of CEV. Twenty days later these seedlings were budded with healthy Tahiti lime, IAC-5. Twelve of the 48 nursery trees obtained grew poorly and remained stunted in the nursery, being under 40 cm high 1 year after budding. Fifteen of the nursery trees that grew well were transplanted to the field. Five healthy uninoculated Tahiti lime trees from the same nursery were planted also, as controls. All CEV-infected trees, but none of the controls, developed bark disease symptoms in the branches. When tested for CEV on Etrog citron 60-13, all 15 inoculated trees were found to carry mild strains of CEV. Observations on the field trees, 6 years after planting, showed that inoculated plants were smaller than controls, but none had developed exocortis symptoms in their Rangpur lime rootstocks. Trees of Eureka lemon on Rangpur lime rootstock, of the same age and inoculated with buds from the same source, developed severe exocortis symptoms, but were less stunted than the Tahiti lime trees. Obviously, severe strains of CEV became established in Eureka lemon tissues but not in those of Tahiti lime.

Alternative hosts. Attempts were made to determine if certain fruit trees other than citrus could serve as alternative hosts for CEV. In the last 2 years, young seedlings or rooted cuttings were inoculated with CEV by budding with various infected citrus varieties. The experiment included loquat, annona, guava, papaya, pear, apple, pecan, persimmon, avocado, mango, and a few other local tropical fruits. Results were negative, except surprisingly, when seedlings of avocado, *Persea americana* Mill., were used. Citrus buds inserted into 2-3 months-old avocado seedlings remained alive for several months (up to 1 year). The avocado seedlings continue to grow and have developed yellowish areas in the bark near the site of budding, resembling symptoms on Rangpur lime seedlings infected with mild CEV. Percentage survival of citrus buds in avocado seedlings seems to vary with the species used, in the following descending order: citron (80), lemon (50), lime (50), orange (20), and tangerine (10). None of the citrus buds sprouted. Stems of Etrog citron infected with CEV topworked onto young avocado seedlings survived for several months but also failed to sprout. Bark rings of Etrog citron (3 cm diameter) used to replace similar bark rings of avocado seedlings also induced yellow blotching in the upper portion of the stem.

DISCUSSION AND CONCLUSIONS

The results obtained from the late infection experiment clearly show that CEV has a longer incubation period in large trees than in young plants, thereby diminishing the danger of sudden heavy losses to growers if rapid field spread of

CEV occurs. Growers could be advised to establish their orchards on more than one rootstock, but under Brazilian conditions, no other rootstock is equal to Rangpur lime.

The delayed effect of CEV inoculated into field trees should be considered for the production of dwarfed trees, as suggested by Samadi (1976). It should be investigated when inoculation with mild CEV is needed to obtain the desired stunting effect. Garnsey and Weathers (1972) have indicated that if trees on sensitive rootstocks are large when infection with CEV occurs subsequent stunting is less detrimental than to trees that are small when infected.

The results of the inoculum source study emphasized the importance of this factor in the mechanical transmission of CEV. All inoculum sources were effective for knife inoculations to citron but not to Rangpur lime, suggesting interrelationships between host donors, host receptors, and CEV. The presence of tristeza virus in host donors apparently did not affect transmission of CEV to citron, but may have prevented transmission to Rangpur lime. Inoculum from the two orange sources carrying tristeza failed to transmit CEV to the lime indicator. Garnsey and Weathers (1972) and Weathers *et al.* (1974) have pointed out that certain citrus varieties are poorer inoculum sources than others. Infected lemon trees apparently represent a better and more important source of inoculum for CEV spread in nurseries and orchards than some other citrus species.

Igwegbe (1967) found that calamondin is a poor host for CEV. My results indicate that Tahiti lime is also a poor host for CEV, for only mild strains of CEV remained in the Tahiti lime trees and caused no bark symptoms in Rangpur lime rootstocks. Tahiti lime, for this reason, may be useful in separating mild from severe strains of CEV. These results also suggest that severe strains of CEV are always accompanied by mild strains, as is known to occur with tristeza virus.

Avocado seedlings were found to be possible alternative hosts for CEV and to develop yellow blotching in the area of bud inoculation. This deserves further study.

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