

New Test Varieties for Exocortis Virus

LOSSES DUE TO exocortis virus in Brazil have been surpassed only by that caused by tristeza virus. More than ten million citrus trees are infected. Nearly 50 per cent of these are budded on Rangpur lime, a rootstock that is intolerant of exocortis virus. More than forty thousand trees have been inarched with tolerant rootstocks as a control measure and many diseased trees have been pulled out. The seriousness and extent of the problem justifies the exhaustive studies that are being carried out on the exocortis disease in Brazil. Much of the work has been undertaken since the development by Moreira (2) of the Rangpur lime test for exocortis virus. This test has been used in studies on exocortis since 1957 (3).

Several citrus varieties other than trifoliolate orange [*Poncirus trifoliata* (L.) Raf.], some of its hybrids, and Rangpur lime (*C. limonia* Osbeck) are susceptible and may show symptoms when infected. Calavan and Weathers (1) found symptoms resembling exocortis on certain citrus varieties and hybrids used as rootstocks in California. Salibe (3, 4) reported that 46 citrus varieties, used as scions in an experimental test, developed exocortis symptoms within 110 to 400 days after inoculation. Most of the affected varieties showed yellowing, splitting, shelling, and gum exudation in the bark of the branches.

This paper presents the results of studies carried out at the Limeira Citrus Experiment Station in a search for new test varieties to be used as fast and reliable indicators of exocortis virus. Those varieties previously reported to show early symptoms of exocortis were included in the studies.

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Materials, Methods, and Results

Thirty citrus varieties, including three of lemon [*C. limon* (L.) Burm. f.] (Acido, Harvey, and Kulu), four of citron (*C. medica* L.) (Comprida, Doce, Etrog, and Redonda), six of sweet lime (*C. limettioides* Tanaka) (Americana, Columbia, Dourada, Persia, Teheran, and Vermelha de Goias), two of trifoliolate orange (diploid and tetraploid), and 15 of mandarin-lime (Rangpur lime, Sweet Rangpur lime, Kusaie, Ling Ming, Pook Ling Ming, and other selections of Rangpur lime), were tried as indicators for exocortis. Nucellar lines were used.

In November, 1959, 180 Caipira sweet orange [*C. sinensis* (L.) Osbeck] seedlings, one year old in the nursery, were inoculated with exocortis virus. The source of inoculum was a Hamlin orange tree known to be carrying only exocortis and tristeza viruses. Three buds were used to inoculate each seedling. At the same time, these seedlings were budded immediately above the inoculating buds in groups of six, with a bud of one of the previously mentioned 30 citrus varieties. Twenty days later all seedlings were cut back and the various citrus varieties allowed to sprout. The young sprouts were periodically inspected for symptoms. They made vigorous growth because of the favorable summer conditions. Yellow blotching of the bark of the sprouts was the first exocortis symptom noticeable. The lemon varieties started to show symptoms within 110 to 180 days (Harvey—110, Acido—140, and Kulu—180), the citron varieties within 120 to 140 days (Etrog—120, Comprida and Redonda—130, and Doce—140), the sweet lime varieties within 120 to 140 days (Persia and Vermelha de Goias—140, and the others—120), the trifoliolate orange varieties within 140 to 150 days (tetraploid—140 and diploid—150), and the mandarin-lime varieties within 110 to 120 days (Ling Ming and Pook Ling Ming—110, and the others—120).

A last inspection, carried out 300 days after budding, revealed variations in the severity of symptoms produced by different varieties. Bark splitting with gum exudation occurred in the sprouts of mandarin-lime, sweet lime, Acido lemon, and Kulu lemon. Gum pockets in the bark of the sprouts were found on the trifoliolate orange plants. Harvey lemon and the varieties of citron exhibited twisting of branches, death of the peduncle of many leaves, dead areas in the place of insertion of the peduncle with gum exudation, and death of twigs. Severe bark scaling occurred in the plants of Rangpur India lime C 26-1, a selection sent to the authors by E. F. Frolich, California.

The varieties found to develop early and conspicuous exocortis symptoms (Harvey lemon, Etrog citron, and Ling Ming) were used as indicators in another series of tests. The Brazilian Limão Cravo selection of Rangpur lime and the diploid trifoliolate orange were also included in this test. Nucellar lines of these varieties were budded onto Cleopatra tangerine [*C. reshni* (Engl.) Hort. ex Tanaka] seedlings, one year old in the nursery. One-third of the seedlings, budded with each variety, was at the same time inoculated with a mild strain of exocortis virus and another third with a severe strain. The remaining third (5 seedlings with each top) was kept to serve as non-inoculated controls. The severe and mild strains were selected on the basis of the severity of symptoms they caused in trees of Hamlin orange on Rangpur lime rootstock (5). Budding was made in October, 1961. The seedlings were cut back 20 days later and the buds of the test varieties allowed to sprout. Periodical inspections revealed that all the varieties except the trifoliolate orange were reliable indicators of the mild and severe strains of exocortis virus used. There was a noticeable relationship between earliness in appearance and severity of symptoms and the strain of the virus used for inoculation. Plants inoculated with the severe strain started to develop symptoms in about 110 days whereas those inoculated with the mild strain showed symptoms only after 100 additional days. Symptoms on indicator plants with mild strains were less conspicuous 280 days after the beginning of the experiment than those induced by severe strains. Only small variations were observed in symptoms in the various varieties. However, the trifoliolate orange plants when inoculated with the mild strain showed some stunting but no yellowing or cracking of the bark of branches.

The inspection carried out in October, 1962, one year from budding, revealed that the Harvey lemon was the best indicator plant for the severe and mild strains of exocortis virus. All trees infected with the severe strain were dead at this time, and those with the mild strain were stunted and had yellow branches which showed cracking and gumming. The Etrog citron plants infected with the severe strain were stunted with twig dieback, but those with the mild strain were vigorous, showing only yellow areas in the bark of the branches. Rangpur lime and Ling Ming plants were less affected than others by the strains of virus but a conspicuous difference could be seen among different treatments.

The relative degree of stunting of the different scion varieties when infected with mild and severe strains of exocortis virus is indicated by the data of Table 1.

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TABLE 1. AVERAGE HEIGHT OF PLANTS AND TRUNK CIRCUMFERENCE (10 CM ABOVE BUD UNION) OF FIVE SCION VARIETIES ON TOLERANT ROOTSTOCKS FOLLOWING INOCULATION WITH SEVERE AND MILD STRAINS OF EXOCORTIS VIRUS. MEASUREMENTS WERE MADE 1½ YEARS AFTER BUDDING AND INOCULATION

Scion varieties	Virus strain	Height of plants	Trunk circumference
Harvey lemon	Severe	— ^a	— ^a
	Mild	99.0	6.8
	Control	161.0	7.8
Etrog citron	Severe	40.0	2.1
	Mild	125.8	4.7
	Control	153.3	5.7
Rangpur lime	Severe	84.6	5.5
	Mild	89.3	6.8
	Control	144.0	9.5
Ling Ming	Severe	51.9	2.9
	Mild	108.0	5.4
	Control	139.1	6.7
Trifoliolate orange	Severe	55.5	2.9
	Mild	90.6	5.0
	Control	149.0	6.3

^aAll plants died about one year after budding and inoculation. At that time the average height was about 50.0 cm and the trunk circumference 2.0 cm.

Discussion and Conclusions

Rangpur lime has been recommended by Moreira (2) as a fast and reliable indicator plant for exocortis virus. Salibe (3, 4) reported that the indexing when using Rangpur lime as an indicator is apparently not affected by the presence of other viruses.

The Rangpur lime trees in Brazil are severely affected by rust mite, *Phyllocoptruta oleivora* (Ashm.), and frequent control sprays are required to avoid russetting of the test plants.

The result of the experiments here reported has shown that several citrus varieties may be used as indicator plants when testing for exocortis virus. Harvey lemon and Etrog citron were found to be fast indicators and to react to both the mild and the severe strain of the virus, developing conspicuous symptoms. The Harvey lemon was the best indicator plant for the mild strain. It was found that all mandarin-lime varieties react to exocortis virus in a manner similar to that of Rangpur lime and may also be used as reliable indicator plants.

Trifoliolate orange showed conspicuous symptoms of exocortis only when inoculated with a severe strain of the virus. In the presence of mild

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exocortis virus, however, the trifoliolate orange plants showed only some stunting, being considered a poor indicator of this virus.

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