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Strains of Exocortis Virus

It has become increasingly evident that the virus of exocortis must occur in the form of numerous strains (2, 3, 4, 6, 7). This hypothesis is borne out by observations in commercial groves of trees of Hamlin and Baianinha Navel orange [*Citrus sinensis* (L.) Osbeck] on Rangpur lime (*C. limonia* Osbeck) rootstock. All trees of these two varieties in Brazil originated from single parent trees that are known to have been infected with exocortis virus. Both varieties are free from xyloporosis virus; the trees of Hamlin orange are also free from psorosis virus. Variation in time after budding for symptoms to develop on the Rangpur lime rootstock and the severity of these symptoms suggest that the strains of virus vary from mild to severe.

Observations on Commercial Groves and Rootstock Plantings

In a 13-year-old commercial grove of Hamlin orange on Rangpur lime rootstock in Araras county, 50 trees were chosen at random and rated for the degree of severity of exocortis symptoms in the rootstock. The trunk circumference 10 cm above the bud-union was measured as an expression of tree size and the numerals 0, for no symptoms, 1, for slight scaling, 2, for moderate scaling, and 3, for intense scaling and gumming were assigned to the trees. A highly significant negative correlation, $r = -0.821$, between trunk diameter and severity of scaling was detected.

Comparable results were obtained in a similar study of a 9-year-old Baianinha Navel orange grove on Rangpur lime rootstock in Limeira county. However, no apparently healthy tree was found in this grove.

Observations on numerous other groves led to the conclusion that exocortis symptoms (scaling and gumming of the rootstock) were less conspicuous in groves in which little or no fertilizer had been applied and in which the trees had made slow growth. Trees showing scaling of the rootstock without stunting were not found.

Examinations were made in a Marsh seedless rootstock experiment laid out at the Limeira Station in 1936. All buds had been taken from a single parent tree. Two of 36 trees on Rangpur lime and one of 16 trees on trifoliate orange rootstock were apparently healthy. The others were showing exocortis symptoms with different intensities. The two apparently healthy trees on Rangpur lime were very vigorous and productive. The healthy tree on trifoliate orange was twice as large as the diseased ones, but was still stunted as compared to the trees on other rootstocks. All 52 trees were tested for exocortis, and all proved to be infected. The indicator plants developed exocortis symptoms within 4-6 months, except those inoculated with buds taken from the apparently healthy trees; the latter required three additional months and showed only mild symptoms.

In another rootstock experiment, with Baianinha Navel top planted in 1949 at the Tietê Experiment Station, three trees on Rangpur lime and two trees on trifoliate orange, of 12 trees budded on each rootstock, failed to develop exocortis symptoms. The other trees on these rootstocks were stunted and showing symptoms in various degrees. All buds here were also from a single parent tree. All 24 trees were tested for exocortis virus by the Rangpur lime method (8). The time required for the indicator plants to start showing symptoms and the severity of the yellowing, cracking, and scaling of the bark of branches that they displayed were directly related to the severity of symptoms observed in the field trees. The indicator plants that were inoculated with buds from trees with severe exocortis developed symptoms within 4-5 months whereas those inoculated with buds from the apparently healthy trees showed symptoms only after 9 months.

Experimental Tests

In a commercial grove of Hamlin orange on Rangpur lime rootstock, two trees were selected: one was stunted, with the bark of the rootstock severely scaled, and the other was more vigorous and had but little scaling. Buds from these two trees were grafted onto healthy Rangpur lime seedlings in November, 1954. One year later, five trees of each selec-

tion were transplanted to the field. Bark scaling on the rootstock started to develop four years later on the test trees grafted with buds from the severely diseased parent tree. Some bark cracks, with eventual shelling, started to occur only after two additional years on the test trees propagated from the mildly affected parent tree. The average trunk circumference 10 cm above bud-union of the trees with severe and mild exocortis, respectively, was 27.1 and 38.7 cm in October, 1962. The average production per tree per year, 1959-1963, was, respectively, 438 and 734 fruit. In October, 1962, the ten trees were tested for exocortis virus by the Rangpur lime method (8). The indicator plants inoculated with buds from the tree with severe exocortis developed severe symptoms within 110 days whereas those inoculated with buds from the trees with mild exocortis required four additional months to start showing symptoms.

In February, 1960, 100 trees of Hamlin orange, one year old in the nursery, were topworked with healthy nucellar Rangpur lime and trifoliate orange buds to serve as indicators of exocortis virus. All young Hamlin trees were daughters from a single parent tree that was known to be carrying a severe strain of virus. The sprouts of Rangpur lime and trifoliate orange were periodically inspected. Symptoms started to appear within 120 days in the sprouts of Rangpur lime on certain plants and after five months in sprouts of trifoliate orange. A last inspection was made one year after budding. The Rangpur lime sprouts were found in 9 trees to be showing yellowing, cracking, scaling, and gumming in the bark of branches; in 63 trees to be showing yellowing and some cracking, and in 16 trees to be showing only yellow areas in the branches. The Rangpur lime sprouts in 4 trees were vigorous and apparently healthy. The plants showing mild symptoms developed those symptoms later than plants with severe symptoms. This difference was attributed to the presence of different strains of virus in the various plants. The trifoliate orange sprouts developed symptoms only in the trees in which the Rangpur lime sprouts exhibited severe symptoms. In those trees in which the Rangpur lime sprouts showed mild symptoms, the trifoliate orange sprouts were stunted but had no other symptoms indicative of exocortis virus except that they eventually developed small yellow blotches.

A series of tests was carried out to determine whether or not certain mild strains of exocortis virus would interfere with establishment of a severe strain of the virus in a plant. Thirty Rangpur lime seedlings were infected with mild strains of exocortis virus obtained from Hamlin

orange, a Baianinha Navel orange, and a Marsh seedless grapefruit tree, respectively, on Rangpur lime rootstock showing mild symptoms of exocortis. Ten seedlings were inoculated in August, 1960, with buds from each tree. Twenty days later all seedlings were cut back and new sprouts were allowed to grow. These sprouts were inspected subsequently and found to be showing mild symptoms of exocortis. One year later, each seedling was inoculated by budding with a severe strain of exocortis virus. The seedlings were once again pruned back. Five months later, the new sprouts on all seedlings exhibited severe symptoms of exocortis.

In October, 1959, ten Rangpur lime seedlings were infected with a mild strain of exocortis virus from a Baianinha Navel orange tree on Rangpur lime rootstock with very mild symptoms. The seedlings were cut back 20 days later and new sprouts were allowed to grow. In February, 1960, these seedlings were grafted with buds from a nucellar Baianinha Navel orange tree and, at the same time, inoculated with a severe strain of exocortis virus. Three and one-half years later, all trees were stunted and showed severe scaling of the rootstock. Nucellar Baianinha Navel orange trees without exocortis virus were used as controls and found to be apparently healthy at that time.

Discussion and Conclusions

The observations reported here, which were made of trees on Rangpur lime rootstock, revealed differences in the severity and type of scaling, period of incubation, and degree of stunting in commercial groves and rootstock plantings, which suggest the existence of a number of strains of exocortis virus. Similar variations were reported to occur in groves on trifoliate orange rootstock (1, 3, 4). Fraser and Levitt (4, 5) reported the possible existence of a strain of exocortis virus that causes stunting, but not scaling, of the trifoliate orange rootstock. Trees on Rangpur lime rootstock affected by exocortis are not so stunted as those on trifoliate orange (2, 9). Stunted trees on Rangpur lime were observed in commercial groves but were generally poorly fertilized groves. Trees with scaling on the rootstock without stunting were not found.

Various experimental tests have substantiated the existence of several strains of exocortis virus, as indicated by different incubation periods and the severity of symptoms induced on Rangpur lime test plants. Calavan and Weathers (2, 10) obtained similar results when inoculating with exocortis virus from various sources into trees on different rootstocks.

Rangpur lime plants were observed to develop exocortis symptoms

when infected with mild or severe strains of exocortis virus, whereas trifoliate orange showed symptoms only when infected with the severe strains. The mild strains of virus induced only stunting in trifoliate orange scions.

From trees infected with a severe strain of exocortis virus, buds carrying mild strains were obtained, suggesting that mild and severe strains may exist in the same tree. It was found also that apparently healthy buds may be obtained occasionally from trees infected with exocortis virus.

The mild strains used in interference tests did not protect trees against infection with a more severe strain of the virus.

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