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Distribution and Movement of Exocortis Virus in Citrus Trees

VERY FEW STUDIES have been conducted on the distribution and movement of viruses in citrus trees. Costa *et al.* (1) reported studies on the movement and distribution of tristeza virus while Price and Knorr (4) reported similar studies with tristeza and psorosis viruses. Recently, Moreira (3) developed a quick field method for detecting exocortis virus. Using this method, some studies on the distribution and movement of exocortis virus in citrus trees were conducted at the Limeira Citrus Experiment Station.

Distribution of Exocortis Virus

Buds from a nucellar Pera sweet orange seedling [*Citrus sinensis* (L.) Osbeck] that had been inoculated in the nursery with exocortis virus were propagated on Rangpur lime rootstock. Five years later all trees were exhibiting symptoms. These trees were also carrying tristeza virus, since it is present in practically all citrus trees in Brazil. Material taken from one of these Pera orange trees included leaf pieces, very young twigs, buds, pieces of bark from branches, pieces of bark from the rootstock, and pieces of root. In September, 1960, ten Rangpur lime (*C. limonia* Osbeck) seedlings were inoculated with each type of material. These seedlings were cut back. Inspections of new sprouts revealed that all indicator plants inoculated with young twigs, buds, bark pieces from scion and from rootstock, and root pieces had developed exocortis symptoms within 4-6 months after budding. Seedlings inoculated with leaf pieces did not start to show symptoms until 15 months after budding and then developed only mild symptoms, which were indicative of a mild strain of the virus.

Thirty months after budding there were no visible differences among test plants inoculated with buds, twigs, or bark pieces. All plants inoculated with leaf pieces were, however, more vigorous exhibiting only small yellow areas in the bark of branches. All plants inoculated with root pieces were stunted and had more severe symptoms than the others. Pieces of wood and peel of fruit were also used in this test, but none of them took and the test plants were eliminated.

This experiment was repeated with inoculating material taken from infected trees on different rootstocks. Leaf pieces always transmitted mild strains or failed to transmit. Root pieces always transmitted. Root pieces from trees on certain rootstocks, such as Rangpur lime, appear to have transmitted a more severe strain of exocortis virus than that transmitted by buds from the same tree.

In another test, a Baianinha orange tree and a Hamlin orange tree on Caipira sweet orange rootstock were selected as sources of budwood. Both trees were carrying exocortis and tristeza viruses; the Baianinha orange tree also carried psorosis virus. In August, 1960, 100 buds from different branches around each tree were used to inoculate Rangpur lime seedlings, only one bud for each seedling. At the end of 15 months, 8 of the test plants inoculated with buds from the Baianinha orange tree had developed very severe symptoms of exocortis; 67 had severe symptoms; and 13 had mild symptoms. Two test plants failed to show symptoms. Nine of the test plants inoculated with buds from the Hamlin orange tree developed very severe symptoms, 71 developed severe symptoms, and 16 developed mild symptoms; 4 seedlings failed to develop symptoms.

A test was conducted to determine whether or not virus is translocated in the phloem. Six 3-year-old Rangpur lime seedlings were pruned, leaving a single main branch. In September, 1960, they were inoculated with exocortis virus by budding with many buds. At the same time, a ring of bark 5 cm wide was removed from below the inoculating buds of each seedling. Later, the seedlings were cut back above the point of inoculation. Yellowing and bark splitting, indicative of exocortis virus developed in the sprouts above the ring but not in the sprouts produced below the ring. Exocortis virus apparently was not translocated across the ring, suggesting that it moves in the phloem, not in the xylem.

Movement of Exocortis Virus

In March, 1960, a homogeneous lot of 102 Rangpur lime seedlings

was budded with buds from an exocortis-infected Hamlin orange tree at a point 15 cm above soil level. Only one infected bud was budded on each seedling. Some non-budded seedlings were left to serve as controls. The following day and every second day thereafter, 6 seedlings chosen at random were cut back and the buds used for inoculation of the 6 seedlings were removed. Symptoms appeared on one of the 6 seedlings from which the inoculating buds were removed on the 5th day, on 4 seedlings of the lots from which the buds were removed on the 7th and 9th days, on 5 from which the buds were removed on the 11th day, and on all other inoculated seedlings; none of the controls developed symptoms. Five test plants developed only mild symptoms, indicative of mild strains of exocortis virus. Apparently, 5-13 days is necessary for exocortis virus to move from the inoculating buds into the rootstocks.

In October 1960, 56 vigorous 2-year-old nucellar Valencia orange trees on Rangpur lime rootstock were inoculated with exocortis virus by budding with a single bud at a point 30 cm above the union. The tops of 4 trees chosen at random were removed by cutting at the bud union on the 1st, 5th, 10th, 14th, 16th, 18th, 20th, 22d, 24th, 26th, 28th, 33d, 38th, and 44th day after inoculation. The sprouts that developed from the rootstock were observed for symptoms of exocortis. Symptoms appeared on the test plants whose tops were removed 20 days or more after inoculation, indicating that at least 20 days were necessary for the exocortis virus to pass from the infected bud into the Valencia orange tree and move 30 cm down.

In another test, six 5-year-old Rangpur lime seedlings were inoculated by budding with 5 exocortis virus-infected buds into roots with a diameter of 2 cm. After budding, the roots were recovered with earth. Inspections 2 months later showed that budding had been successful. At this time, the seedlings were cut back 50 cm above soil level and new sprouts were allowed to grow. One year later, some of these sprouts were as tall as 2 meters but healthy; they were still symptomless another year later. The experiment suggests that movement of exocortis virus from the roots to the top must be very slow.

Discussion and Conclusions

It seems that only mild strains of exocortis virus exist in the leaves of infected trees. This fact may explain why such trees show no leaf symptoms in contrast to trees infected with psorosis virus, for instance. Indications were obtained that a more severe strain of exocortis virus occurs in

the roots of infected trees on certain rootstocks, such as Rangpur lime. Moreover, buds from an infected tree vary in the severity of the strain of exocortis virus they carry; some apparently are completely free of virus. This fact suggests the possibility of obtaining buds free from exocortis virus by a great number of propagations from infected trees. Salibe and Moreira (5) have reported that severe and mild strains of exocortis virus occur in the same tree.

From 5 to 13 days are necessary for exocortis virus to move from the inoculating bud into the rootstock. Price and Knorr (5) have observed that 8-14 days are necessary for tristeza and psorosis viruses to pass from the bud into the stock. Mendel (2) established that the first cell bridge and water and food exchange between tissues of the bud and the rootstock occur normally 5 days or more after budding. This would be the limiting period for the virus to move from the bud and infect the rootstock.

Exocortis virus required a period of 20 days to pass from the infected bud into nursery trees and to move 30 cm down the stem. Movement from the roots to the top must be very slow if it occurs at all. Price and Knorr (4) reported that tristeza and psorosis viruses, after entering the tree, move rapidly in both directions.

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