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Author

Rossetti, Victoria

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Testing for Exocortis

SYMPTOMS OF EXOCORTIS are usually visible 4 to 8 years after graft inoculation (1, 2, 3). This fact has led several authors to undertake investigations for a quicker detection of the virus infection.

Childs, Norman, and Eichhorn in 1958 (4) showed color reactions of the ray cells in cross sections of the bark of exocortis-infected *Poncirus trifoliata* treated with certain reagents such as phloroglucinol-HCl.

In 1957 Moreira (5) observed scaling of the bark on branches of Rangpur lime which had developed from healthy budwood grafted to exocortis-affected citrus trees. More recently he has observed that symptoms, starting with yellow patches and followed by typical cracking and scaling, will develop within a few months (6). This method should provide for a valuable field test for the quick detection of exocortis virus on citrus trees to be selected as a source of healthy budwood.

To verify the efficiency of this method as a field test, an experiment was carried out at the Limeira Citrus Experiment Station of the Instituto Agrônômico, the results of which are reported here.

Methods and Material

The experiment was carried out in a number of 3-year-old trees originating from the following budwood sources:

1. A 14-year-old healthy Bahianinha orange tree derived from a nucellar seedling, grafted on Rangpur lime—Limeira Citrus Experiment Station.
2. A 20-year-old diseased Bahianinha orange tree grafted on Rangpur Lime—Limeira Citrus Experiment Station.

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3. A 20-year-old diseased Bahianinha orange tree grafted on trifoliolate orange—Limeira Citrus Experiment Station.
4. A 7-year-old diseased Bahianinha orange tree grafted on Rangpur lime—Limeira Citrus Experiment Station.
5. A 20-year-old apparently healthy Pera orange tree grafted on Rangpur lime—Limeira Citrus Experiment Station.
6. A 20-year-old apparently healthy Pera orange tree grafted on trifoliolate orange—Limeira Citrus Experiment Station.
7. A 4-year-old diseased Pera orange tree grafted on Rangpur lime—Limeira, Fazenda Citra.
8. A 16-year-old healthy Hamlin orange nucellar seedling—Limeira Citrus Experiment Station.
9. A 6-year-old diseased Hamlin orange tree grafted on Rangpur lime—Araras, Fazenda Campo Alto.
10. A 16-year-old healthy Marsh-seedless grapefruit seedling—Limeira Citrus Experiment Station.
11. A 6-year-old healthy nucellar Marsh-seedless grapefruit tree grafted on trifoliolate orange—Limeira Citrus Experiment Station.
12. A 20-year-old diseased Marsh-seedless grapefruit tree grafted on Rangpur lime—Limeira Citrus Experiment Station.
13. A 20-year-old diseased Marsh-seedless grapefruit tree grafted on trifoliolate orange—Limeira Citrus Experiment Station.

In all cases of diseased trees, exocortis was identified from the bark symptoms.

In February and March, 1956, buds from each source were grafted on 4 rootstocks—Cleopatra tangerine, Caipira sweet orange, Rangpur lime, and trifoliolate orange—which were then kept in the nursery until November, 1957, when 7 plants of each stock-scion combination were planted in an orchard at a distance of 4 x 4 meters. In May, 1959, the trees of different stock-scion combinations were top-worked with healthy buds from seedlings of Rangpur lime and trifoliolate orange. A total of 12 budding, 6 each of those 2 varieties, were made on the branches of a number of trees of each stock-scion combination, except on the trees on Trifoliata rootstock because their poor development permitted only a small number of grafts.

Results and Discussion

In September, 1959, some of the grafts of Rangpur lime were already showing yellow patches on the bark; and in October, 5 months after

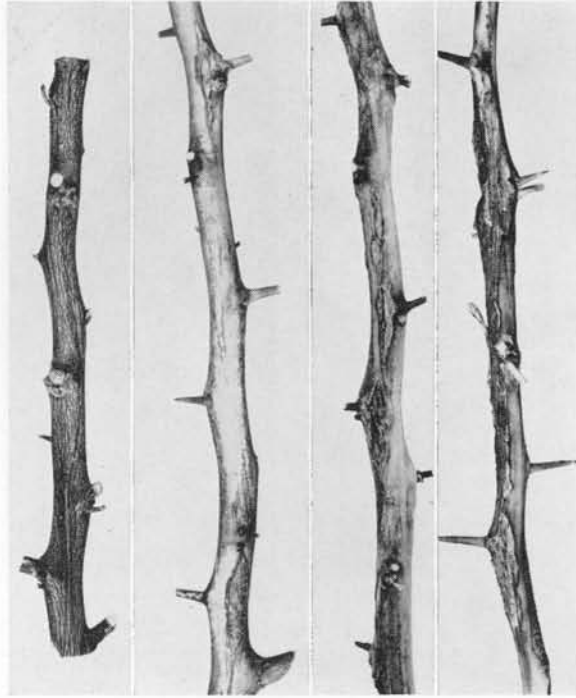


FIGURE 1. Young branches of Rangpur lime showing different intensity of symptoms of exocortis. The first branch at left is healthy.

budding, most of those budded on diseased trees were showing cracks in the bark (Figs. 1 and 2).

Table 1 gives the results read 9 months after the trees were top-worked. Only the number of trees on which the top-worked shoots had a satisfactory growth are recorded; most of the Trifoliata buddings developed poorly or not at all. Because of its fast development, Rangpur lime gives a better test than trifoliolate orange. Tests in which symptoms were observed, such as yellow patches, cracks, and scaling of the bark along the shoots of the test variety, are recorded as positive, and those in which no such abnormalities were found as negative. In most cases, the shoots of Rangpur lime showed symptoms when the top-worked tree had originated from diseased budwood, and developed normally healthy when the tree was from healthy budwood, thus proving that the test is reliable for early detection of symptoms of exocortis virus in citrus trees.

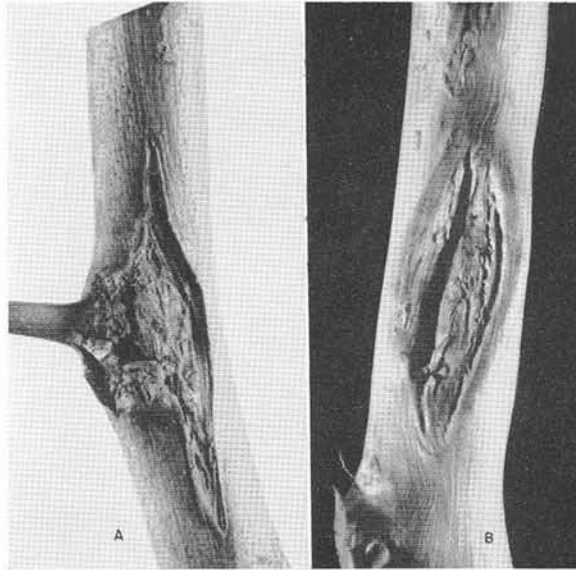


FIGURE 2. *A. A lesion showing cracking and scaling of the bark in a branch of Rangpur lime. B. A lesion when the bark is removed; gum is formed in the wood, inside the crack.*

Nevertheless, some exceptions were found. With 4 of 12 trees originating from healthy nucellar Bahianinha, the test was unexpectedly positive on the Rangpur lime shoots. To be sure that those trees were diseased, buds from 3 of them and buds from healthy Rangpur lime were grafted on Caipira sweet orange seedlings in 5 replications each. In a few months, the Rangpur lime shoots showed marked symptoms of the disease in all replications in 2 of the cases, thus proving that those 2 trees had been infected with exocortis virus, whereas for the third tree, only 1 of the 5 replications is now—10 months after budding—showing some discoloration of the bark. More recently, still another test was made: buds from the same 4 nucellar Bahianinha trees, as well as from the mother tree from which they had originated, were budded on Rangpur lime seedlings, but none of them are showing symptoms yet, 3 months after budding.

Before being transplanted in the orchard, those trees had stayed in the

TABLE 1. NUMBER OF POSITIVE AND NEGATIVE TESTS OBSERVED 9 MONTHS AFTER TOP-WORKING TREES OF DIFFERENT STOCK-SCION COMBINATIONS WITH HEALTHY BUDS OF THE TEST VARIETIES RANGPUR LIME (R.I.) AND TRIFOLIATE ORANGE (T.) FOR EARLY DETECTION OF EXOCORTIS VIRUS

Origin of budwood		STOCK VARIETIES															Total no. of tests	
		Cleopatra			Caipira sweet orange			Rangpur lime			Trifoliolate orange							
		no. of trees	R.I. test	T. test	no. of trees	R.I. test	T. test	no. of trees	R.I. test	T. test	no. of trees	R.I. test	T. test					
47	Healthy Bahianinha	3	3	0	3	1	3	3	0	3	2	0	2	4	8			
	Infected "	4	5	0	4	4	0	3	2	0	1	0	1	1	0			
	Infected "	1	1	0	4	4	0	2	2	0				8	0			
	Infected "	2	2	0	2	3	0	2	3	0	1	0	2	2	0			
	Healthy Pera	3	0	3	2	0	2	4	0	5	0	1	2	0	2			
	Healthy "	5	0	5	2	0	2	3	0	4				0	11			
	Infected "	2	2	0	1	1	0	5	5	1				8	1			
	Healthy Hamlin	3	0	4	4	0	4	3	0	5	0	3		0	16			
	Infected "	2	2	0	4	5	0	2	4	0				12	0			
	Healthy grapefruit	3	0	3	4	0	3	3	0	3	3	0	2	0	15			
	Healthy "	4	0	4	2	0	1	2	0	1	0	1		0	8			
	Infected "	3	3	0	2	2	0	2	1	1	1	0	1	1	0			
	Infected "	3	3	0	1	1	0	3	3	0	1	1	0	8	0			

nursery for over a year, close to diseased grapefruit trees, and root grafting may have taken place, thus transmitting the virus to healthy nucellar Bahianinha plants. However, it seems improbable that natural root grafts should have occurred so frequently, and other means of transmission of the exocortis virus should be investigated. The occurrence of root grafts could not be verified because almost all the trees had been taken out of the nursery.

On 4 trees originating from diseased Pera orange buds (budwood source n.7), the test was negative when the 9 months' readings were made; but 12 months after budding, the symptoms started to show very definitely on 3 of them; on the fourth one they have not yet appeared. Buds taken from those trees were grafted on Rangpur lime seedlings; and 3 months after budding, the Rangpur lime shoots were already showing some discoloration and cracks of the bark in some of the replications. The same was observed on 1 tree of infected grapefruit.

The fact that symptoms of variable intensity and variable periods of incubation were observed in the test applied to trees originating from different budwood sources seems to point to the possible occurrence of different strains of the exocortis virus.

Objections may be presented that other bud-transmitted virus diseases might cause the same bark symptoms on the Rangpur lime and trifoliolate orange bud-developed branches. It is well known, and was confirmed by a recent survey carried out in the State of São Paulo by Rossetti and Salibe (7), that the Bahianinha variety in S. Paulo orchards is free from xyloporosis and Hamlin is free from psorosis. Since the test has shown positive symptoms on exocortis-infected trees of those two varieties and none on the noninfected ones, it seems that at least xyloporosis and psorosis can be ruled out.

In the State of São Paulo, a Budwood Certification Program is now being developed to provide for virus-free budwood for citrus nurseries. The results of the experiments reported in this paper seem to promise the availability of a reliable field test to be employed in that program for the selection of citrus trees free from exocortis.

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