RANGPUR LIME DISEASE AND ITS RELATIONSHIP TO EXOCORTIS

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INTRODUCTION

The Rangpur lime has been used in Brazil more or less extensively as a rootstock for several orange varieties since the early citrus plantings were established. When the tristeza disease invaded and destroyed those Brazilian citrus groves which were budded on sour orange and sweet lime (lima da Persia), it was demonstrated (*5*) that the Rangpur lime was tolerant to tristeza virus. Since then, nurserymen and growers have favored its use as rootstock for many kinds of citrus plants. However, the young plantings soon showed symptoms of a "new disease" that seemed to be related to the Rangpur lime rootstock. The behavior of *Poncirus trifoliata* and Rangpur lime as rootstocks for some sweet orange, grapefruit, and mandarin clones in the tests at the Limeira, Ribeirão Preto, and Tieté Stations gave us evidence that the "new disease" was caused by the exocortis virus (*6*). Olson and Shull (*9*), in Texas, and Reitz and Knorr (*10*), in Florida, were able to confirm that thesis. Olson and Shull demonstrated also that Rangpur lime shows xyloporosis symptoms when as a rootstock it has been budded with infected scion varieties. Grant *et al.* (*3*) reported later that they found xyloporosis symptoms on Rangpur lime in Brazil.

This paper reports on additional studies made in Brazil on transmission of exocortis virus, symptomatology of affected trees, and the effects of the disease on fruit production.

MATERIALS AND RESULTS

Incubation Period in Young Trees. One hundred Rangpur lime seedlings were budded in the nursery in May, 1950, with clonal buds from the following varieties (20 each): Bahianinha, Barão, and Pera oranges, Marsh seedless grapefruit, and Eureka lemon. The Bahianinha orange and Marsh seedless grapefruit clones were exocortis and psorosis virus carriers. In March, 1953, the trees were transplanted at 4 × 4 meters. Several Marsh grapefruit trees and one Bahianinha orange tree showed the first symptoms of exocortis at the trunk base in November, 1954. Up to that time, all trees of these two varieties were in good condition, even better than the trees of the same varieties on sweet orange and tangerine rootstocks. One year later almost all the trees on Rangpur lime began to show gumming and bark shelling on the rootstock trunks, and the tops showed stunting. In October, 1957, not a single tree had a healthy appearance. No symptoms were detected in the trees of the other three varieties (Barão and Pera oranges and Eureka lemon).

In another test, seedlings of trifoliate orange, Troyer citrange, and Rangpur lime (10 each) were budded in September, 1954, with buds from a Hamlin orange clone that was an exocortis carrier. Later, in December, 1955, five trees of each combination were

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transplanted at 7 × 7 meters. No symptoms of exocortis had appeared in the trees of these three lots up to October, 1957.

Incubation Period in Adult Trees. Buds from 30 new nucellar clones of Bahianinha orange were budded in 1940 on sour orange seedlings. In 1943 these trees were inarched with two Rangpur lime seedlings each. In spite of the fact that the Bahianinha orange old clone was an exocortis virus carrier, no symptoms developed on the Rangpur lime inarches of the nucellar trees. In November, 1954, three of the inarched trees were inoculated by inserting several Bahianinha exocortis-virus-carrier buds in their scaffold branches. Twenty-five months later, in December, 1956, some gumming and bark shelling could be seen at the bases of the Rangpur lime inarches in the three trees.

In another test, eight Pera orange trees, 18 years old and free from exocortis virus (four on Rangpur lime and four on trifoliate orange rootstocks) were inoculated in November, 1954, by buds of Pera and Hamlin oranges from exocortis-affected trees. Up to October, 1957, no exocortis symptoms had appeared in the two rootstocks.

Exocortis Symptoms Following Top-working. Two 5-year-old Hamlin orange trees on Caipira sweet orange rootstock, symptomless carriers of exocortis virus, were top-worked in several branches with buds from seedlings of trifoliate orange and Rangpur lime. Thirty months after top-working, the Rangpur lime and trifoliate orange limbs on both Hamlin and Bahianinha exhibited several yellowing spots on the bark. Later, the bark split lengthwise and typical (*6*) exocortis bark shelling appeared. These results show that the symptomless trees of Hamlin on Caipira sweet orange were carriers of exocortis virus and that virus transmission took place.

Symptom Expression in Affected Trees. Three clonal varieties of sweet orange (Hamlin, Bahianinha, and Maracaña) planted in the rootstock test plots at Tieté Station in 1948 were exocortis virus carriers, as indicated by the presence of symptoms on trifoliate orange and Rangpur lime rootstocks. In these tests each root and scion combination was represented by three trees in each of four blocks. Some variation in symptom expression was noted in the six combinations, as is shown in table 1. In a total of 72 trees, 13 still showed no symptoms ten years after planting. This could have resulted either from delayed development of symptoms or from the chance-use of some virus-free buds from exocortis-carrier trees. Many Bahianinha orange plantings on Rangpur lime rootstock show variations in degree of symptom expression and a small percentage of apparently healthy trees despite the fact that we now know that all the old

Table 1. NUMBER OF SWEET ORANGE TREES ON TRIFOLIATE ORANGE AND RANGPUR LIME ROOTSTOCKS AT TIETÉ STATION, SHOWING VARIOUS DEGREES OF EXOCORTIS SYMPTOMS*

(0 = no symptoms; 5 = extensive bark shelling)

Scion (sweet orange)†	Rootstock											
	Trifoliate orange						Rangpur lime					
	0	1	2	3	4	5	0	1	2	3	4	5
Hamlin	2	3	4	1	2	0	2	3	2	2	1	2
Bahianinha‡	1	1	2	0	2	6	5	4	0	0	0	3
Maracaña	0	1	2	2	2	5	3	4	1	0	1	3
Total	3	5	8	3	6	11	10	11	3	2	2	8

* Each rootstock and scion combination was represented by a total of 12 trees in 4 blocks planted December, 1948. Data shown are from records as of October, 1957. Each scion variety originated from buds from a single tree.

† No xyloporosis symptoms were found when the 3 sweet orange sources used were budded to sweet lime (lima da Persia) rootstock.

‡ This Bahianinha navel orange source known to be a carrier of the psorosis virus.

clones of Bahianinha and Hamlin oranges cultivated in São Paulo orchards have been symptomless carriers of the virus for a long time.

To determine if some buds from an exocortis-carrier tree may be virus-free, Rangpur lime seedlings were budded with buds from apparently healthy old-clone trees of Bahianinha and Hamlin oranges on Rangpur lime, ten propagations being made from each. Another 20 Rangpur lime seedlings were budded with buds from diseased trees of these same combinations. Five trees of each lot were transplanted one year later, and the other five remained in the nursery. In October, 1957, three years after budding, no symptoms could be detected in any of these trees, and their trunk measurements showed no significant differences. A study of these trees will be continued.

Growth and Yield Reductions Caused by Exocortis. No experimentally designed plantings have been available as yet for determining the effect of exocortis on growth and yield of citrus trees. However, some indication of the stunting effect of exocortis was obtained by measuring diseased and symptomless trees in two plantings.

In a 10-year-old planting of Hamlin orange trees budded on Rangpur lime, 12 per cent of the trees had no symptoms of exocortis. These apparently disease-free trees had an average trunk diameter 32 per cent larger than the diseased ones. In a 7-year-old planting of Pera orange budded on Rangpur lime, 50 per cent of the trees showed no symptoms. The average trunk circumference of these symptomless trees (40 cm) was twice that of the trees showing exocortis (20 cm).

Further evidence of the effect of exocortis on growth and yield was obtained from study of exocortis-infected Bahianinha and Hamlin trees on Rangpur lime and trifoliate rootstocks in comparison with healthy Pera on the same stocks. These data are presented in table 2. The growth of the three varieties on exocortis-tolerant sweet orange rootstock was nearly equal nine years after planting. However, when budded on Rangpur lime and trifoliate orange, the average trunk diameter of the healthy Pera orange trees was considerably larger than the average diameters of the two exocortis-infected varieties. If it is assumed that the growth of healthy Bahianinha and Hamlin trees would have been the same as healthy Pera, then the latter trees in this planting can be used as controls for determining the reduction in growth caused by exocortis infection. Following this assumption, as shown in table 2, exocortis-infected Bahianinha trees on Rangpur lime and trifoliate orange were 15.0 per cent and 33.8 per cent smaller, respectively, than healthy Pera trees on the same rootstocks. Similarly, the infected Hamlin trees were 22.1 per cent and 43.7 per cent smaller than the healthy Pera on the two exocortis-susceptible rootstocks.

Table 2 also includes some data concerning the effect of exocortis on yields. Here, too, it has been necessary to compare yields of two infected varieties with yields from healthy trees of a different variety. Another objection to these data is that yields are based on numbers of fruits during the first six years of production. This may explain why the infected Hamlin trees on Rangpur lime rootstock actually produced slightly more fruit than the healthy trees of Pera on that rootstock. However, the reductions in yields of the infected Hamlin trees on trifoliate rootstock and of infected Bahianinha trees on both rootstocks appear to be significant.

Exocortis Virus Transmission. As has been reported (*1, 6, 10*), it appears improbable that the exocortis virus is transmitted by insects. In the State of São Paulo, Brazil, no insect transmission has been verified. At Limeira Station many trees of susceptible combinations (Pera orange on trifoliate orange and on Rangpur lime) have grown for 20 years beside affected trees and are still free of exocortis. Also, up to the present, no evidence of seed transmission has been reported. At Limeira Station, 30 trees from new nucellar clones of Bahianinha orange originated from seeds from exocortis-affected trees were inarched with Rangpur lime in 1943, and none of these developed exocortis symptoms. Exocortis virus is transmitted by a high percentage of

Table 2. EFFECT OF EXOCORTIS ON AVERAGE TRUNK DIAMETER (MEASURED IN SEPTEMBER, 1957) AND TOTAL NUMBER OF FRUITS PRODUCED PER TREE IN SIX YEARS (1951–1956) IN ROOTSTOCK TEST PLOTS AT TIETÉ EXPERIMENT STATION (TREES PLANTED IN 1948)

Scion	Sweet orange (Caipira)*	Rootstock					
		Rangpur lime			Trifoliate orange		
	Trunk diameter	Trunk diameter	Difference in relation to sweet orange	Reduction caused by exocortis	Trunk diameter	Difference in relation to sweet orange	Reduction caused by exocortis
	cm	*cm*	*per cent*	*per cent*	*cm*	*per cent*	*per cent*
Bahianinha orange†	12.9	9.6	−25.5	15.0	4.7	−63.5	33.8
Hamlin orange†	12.4	8.8	−29.0	22.1	4.0	−67.7	43.7
Pera orange‡	13.4	11.3	−15.6	....	7.1	−47.0	....
	Total fruit per tree 1951–1956	Total fruit per tree 1951–1956			Total fruit per tree 1951–1956		
	no.	*no.*			*no.*		
Bahianinha orange†	1188	1171	− 1.4	11.0	384	−67.6	47.7
Hamlin orange†	1816	1361	−25.0	−3.4	433	−76.2	41.0
Pera orange‡	1278	1316	+ 2.8	....	734	−42.5	....

* Exocortis-tolerant rootstock.
† Exocortis carrier.
‡ Exocortis-free.

buds from diseased trees (table 1). Of 36 trees budded on trifoliate orange with buds from three affected sources, only three plants failed to show bark scaling after nine years. When the rootstock was Rangpur lime, 10 of 36 plants seemed to be disease-free. As mentioned before, a planting of Hamlin orange (exocortis-infected clone) budded on Rangpur lime exhibited 12 per cent of trees without scaling symptoms when the trees were ten years old. We do not know yet whether or not these apparently disease-free trees are carrying exocortis virus.

DISCUSSION

The trifoliate orange, some citranges, and the Rangpur lime have been recommended (*1, 2, 7*) as substitutes for sour orange in those areas where tristeza prohibited the use of this last-named rootstock. However, the first-named varieties are sensitive to exocortis virus, which is present in many clones of the main varieties cultivated in the various citrus areas.

The search for a safe method by which to determine in a short time whether a bud source is or is not infected is of maximum interest for the citrus budwood certification programs now under way in several citrus areas. According to some authors (*1, 6, 10*) the incubation period of exocortis is around four years. Olson and Shull (*9*) reported that bark-shelling symptoms appeared in the nursery trees two years after budding, and that if they were transplanted it required an additional year for the first symptoms to develop. Our observations do not agree with this report. The Rangpur lime budded with Bahianinha orange and Marsh grapefruit showed the first symptoms of exocortis four years after budding in transplanted trees. Hamlin orange from an exocortis source budded on trifoliate orange, Troyer citrange, and Rangpur lime in the nursery have not shown any symptoms after three years. This discordance could be explained by different ecological conditions, possible existence of different strains of the virus, or scion variety influence.

According to results obtained in one of our tests, it seems possible, under the conditions of the State of São Paulo, to shorten the incubation period to two years by inoculating adult Bahianinha orange trees budded on Rangpur lime. However, the same rootstock with Pera orange top has developed no symptoms after three years from inoculation.

An attempt to reduce the incubation period by top-working adult trees showed the possibility of obtaining symptoms within 30 months in the Rangpur lime sprouts. These results do not agree with data reported from Australia (*1*), where five years elapsed before symptoms developed on trifoliate orange top-worked onto exocortis-infected trees.

Great variation in symptom expression occurred when different top varieties were used in rootstock tests and commercial plantings. In spite of the fact that the top bud source was an exocortis carrier and the rootstock susceptible, some trees failed to show symptoms. One explanation for this could be again the existence of different strains of the virus. Benton *et al.* (*1*) suggested the possibility that an uneven distribution of the virus in the scion might be the cause of this irregularity. The three possibilities proposed by Reitz and Knorr (*10*) to explain such variation seem to be less acceptable than that suggested by Benton *et al.* (*1*). In our rootstock tests two 20-year-old grapefruit trees budded on Rangpur lime and one budded on trifoliate orange are still free from bark-shelling symptoms. Besides, it is known that Rangpur lime has a high nucellar rate (*8*). Our tests with buds from the two types of source trees possibly will clarify these doubts.

The intensity of symptom expression and the reduction of top growth and yield were always greater in trifoliate orange than in Rangpur lime. The vigor of Rangpur lime could be an explanation of these differences. Observations made in many citrus orchards gave us evidence that exocortis symptoms are stronger and the effects of the disease are more severe under conditions of poor soil fertility than under conditions of good soil fertility.

The peculiarity of exocortis in being transmissible only by tissue union (budding or grafting) emphasizes the importance of the budwood certification programs and the use of nucellar progenies already under way in some countries. It also opens up the possibility of using exocortis-sensitive rootstocks in some areas where no other roots are adapted to their particular ecological conditions.

Exocortis and other citrus viruses, such as that causing xyloporosis, are now present in much of the old-clone, commercial citrus and are not revealed except when certain scion and rootstock varieties are combined. Because of this situation the Fourth International Congress of Mediterranean Citriculture in 1956 approved the proposal (*7*) that, "To obtain the real reactions of one variety on a specific root the rootstock tests should be established only with seedling plants budded or grafted with scions from new nucellar clones."

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