Reaction of Exocortis-Infected and Healthy Trees to Experimental *Phytophthora* Inoculations

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In the last 20 years, the relationship between exocortis and stunting of citrus trees has been studied and the economic value of such virus-dwarfed trees has been suggested (Fraser *et al.*, 1961; Mendel, 1968; Cohen, 1968; Long *et al.*, 1972; Pompeu *et al.*, 1976). Rodriguez *et al.* (1974) compared yield and growth of nine groups of Hamlin nucellar orange trees on Rangpur lime rootstock inoculated with different strains of exocortis viroid. Studies carried out since 1947 (Rossetti and Bitancourt, 1947; Rossetti *et al.*, 1968; and Rossetti, 1969) have shown that the development of *Phytophthora* species is reduced on the tissue of citrus trees stunted or submitted to stress due to different causes, as compared to healthy nucellar trees.

In this paper, the dwarfing effect of exocortis on trees is analyzed. Resistance of virus-dwarfed trees to foot rot is studied by comparing growth of *Phytophthora* species on healthy nucellar Hamlin trees on Rangpur lime rootstock with growth on trees previously inoculated with different strains of exocortis.

MATERIALS AND METHODS

The trees chosen for this experiment belong to the same experimental plot described by Rodriguez *et al.* (1974), in which trees of nucellar Hamlin on Rangpur lime rootstock were planted in 1964 at the Limeira Experiment Station. Data for 1979 are based on five trees, while those for 1971, when used for comparison, were based on six trees.

Virus Content. The trees, from a single nucellar clone, had been inoculated before planting with eight isolates of exocortis classified as mild and severe. Control trees, here referred to as healthy, were free from exocortis, psorosis and xyloporosis, but carried tristeza virus, as did all the others. The trees used in this experiment were classified in nine groups according to their exocortis content: Group 1 was control-healthy trees, groups 2 to 5 were infected with four mild strains, groups 6 to 9 were infected with four severe strains. The trees of groups 2, 3, and 6 were also infected with psorosis. Trees in groups 8 and 9 were on the border of the experimental plot. Five trees of each group were randomly chosen for growth measurements, production measurements, and *Phytophthora* inoculations.

Growth. Measurements of the trees made in 1979 were compared with the 1971 measurements. The canopy volume was estimated by the formula $2/3\ \pi\ r^2h$, r being the average radius of the canopy and h the height of the canopy. The average trunk circumference was estimated by measurements taken 10 cm above the bud union. The degree of stunting due to exocortis was estimated in relation to the average canopy volume of healthy trees.

Production. Cumulative production of 1977 and 1978 in kg of fruit per tree was recorded for each treatment.

Phytophthora Inoculations: Isolates No. 3/74 of *Phytophthora citrophthora* (Sm. and Sm.) Leon. and No. 1/76 of *P. parasitica* Dast. were obtained originally from infected trees and were maintained in potato dextrose agar (PDA) in the collection of the Instituto Biologico. These were selected because of their pathogenicity in previous tests

on lemon trees. Trees were inoculated with discs of cycelial mats from 5-day-old PDA cultures of each fungus, twice in the rootstock trunk and twice on the scion trunk, approximately 10 to 20 cm below and above the bud union, by the method of Rossetti and Bitancourt (1947). The lesions produced by the fungi were measured 45 days after inoculation. Growth of the fungi in the cambium tissue was estimated by measuring the length and mean width of the lesions, which gave an approximate area and by weighing the outline of the lesions drawn on wax paper. The latter method was considered more accurate. The 16 treatments were in completely randomized blocks with five replications. Data were transformed to log X. Treatment effects were analyzed by F values and Tukey's test.

RESULTS

Symptoms. Healthy trees and trees infected with mild strains of exocortis did not show bark scaling typical of exocortis on the trunk of the rootstock. All groups of trees infected with severe strains of exocortis had one or more trees, of five, showing exocortis bark scaling. Scaling was rated in three degrees as follows: in group 6, two trees showed mild, two severe, and one very severe symptoms; in group 7, three trees showed mild and one severe symptoms; in group 9, two trees showed mild and one very severe symptoms (table 1).

Growth. The average degree of tree stunting due to exocortis in relation to the average volume of the canopy of control trees, was 40 and 76 per cent, respectively, for mild and severe strains (table 1). When compared to the 1971 measurements, the difference between stunting degree in 1979 was approximately 8 and 30 per cent, respectively. Trees infected with mild exocortis in 1979 had more than double the volume estimated in 1971, while trees with the severe strains averaged only 46 per cent more than in 1971.

The average trunk circumference, measured 10 cm above the bud union, was approximately 59.0 cm for healthy trees (table 1). Trunk circumference averaged 16 and 38 per cent less, respectively, for trees infected with mild and severe strains.

Production. The average cumulative yield of 2 years (1977 and 1978) in kg of fruit per tree is shown in table 1. As compared with the average cumulative yield of the control trees, yield for mild and severe exocortis-affected trees was reduced by 12 and 58 per cent, respectively. As compared with the average 4-year cumulative yield for 1968-1971, control trees produced 53 per cent more fruit per tree, while the production increased by 28 per cent for trees with mild strains and was reduced by 25 per cent for trees with severe strains.

Phytophthora Inoculations: Results of the development of *Phytophthora parasitica* and *P. citrophthora* when inoculated on the trunk of the scion are shown in table 2. The weights of wax paper where lesion outlines were drawn correlate with the approximate areas of the lesions. Thus, the lesion maps of *P. parasitica* and *P. citrophthora* on the trees of the healthy control group weighed 109.2 and 48.9 g, respectively, for the scion and 17.9 and 11.4 g for the rootstock (table 2). Compared with the healthy control trees, the average lesion size of both fungi on the scions of trees infected with the mild strains was, respectively, 43.6 and 43.8 per cent smaller; for the trees infected with the severe strains, the lesions were 54.3 and 40 per cent smaller, respectively. On the Rangpur lime rootstock, lesions of *P. parasitica* and *P. citrophthora* were 43.4 and 2.6 per cent smaller, respectively, on trees infected with mild strains and 50.7 and 18.4 per cent, respectively, for trees infected with severe strains (table 2).

In the analysis of variance, F values showed that the effect of stunting due to exocortis on the lesion size was significant at the 5 per cent level. Also the lesions were significantly larger (5 per cent level) on the scion than on the rootstock. Because the interaction of fungus X position was significant, an analysis within positions was carried out and regression lines were calculated.

The first degree linear regression was significant at the 5 per cent level both on the scion and on the rootstock. Fig. 1 shows that the reduction of lesion size is proportional to the reduction of tree size due to exocortis inoculation. Lesions of *P. parasitica* were larger than lesions of *P. citrophthora* on the scion. Lesions caused by both fungi on the rootstock were similar and a single regression line was calculated for both species (fig. 1).

DISCUSSION AND CONCLUSIONS

The four mild strains of exocortis viroid, when used to inoculate trees before planting, did not induce typical exocortis scaly bark symptoms on the trunks of Rangpur lime rootstocks. The effect on tree size was conspicuous mainly after the first decade, and reduced it by 32 to 41 per cent compared with healthy trees. This reduction in the volume of the canopy was maintained in the following years, the variations estimated in 1979 being from 36 to 47 per cent (table 1). Production was not altered from 1968 to 1971 by the mild exocortis strains. It was reduced by 12 per cent compared with control trees, considering the 1977 and 1978 yields, but the reduction averaged 28 per cent almost 20 years after inoculation. The average trunk circumference of infected trees was about 16 per cent smaller than the trunk circumference of healthy trees in 1979. These mild strains, or related factors, induced greater resistance to *Phytophthora parasitica* and *P. citrophthora* infection on sweet orange (Hamlin orange trunk). Lesion size on exocortis-infected trees was reduced 43.6 and 34.8 per cent, respectively, compared to healthy trees, a highly

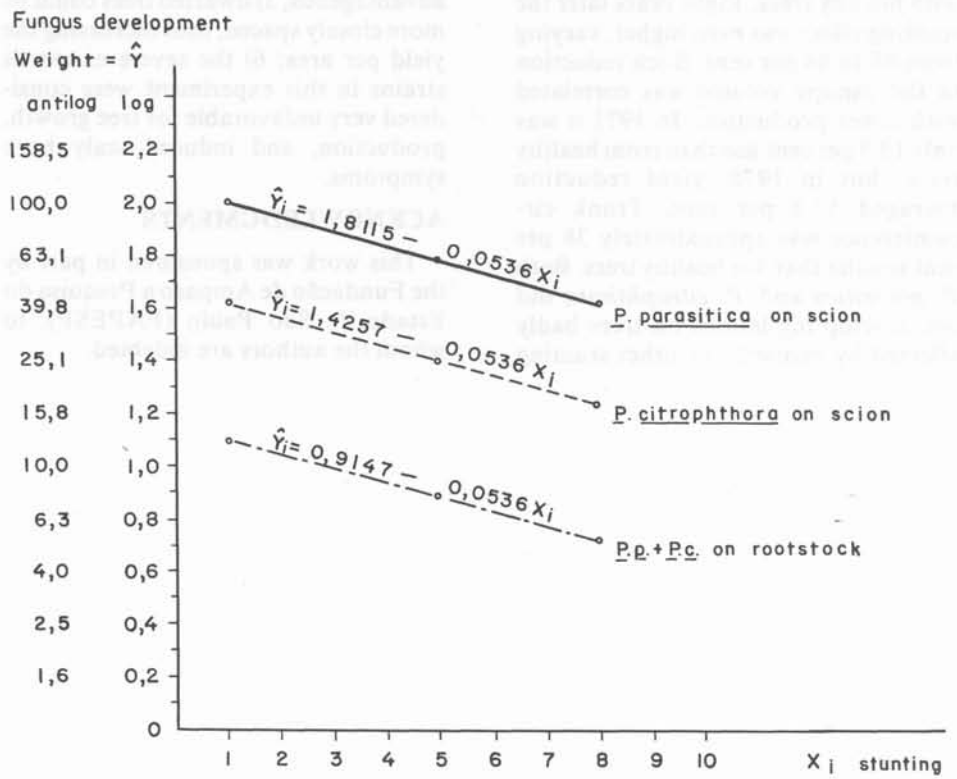

Fig. 1. Regression lines showing that as exocortis stunting effect increases, lesions due to Phytophthora inoculations decrease.

significant difference. Rangpur lime is resistant to foot-rot gummosis, thus the lesions on the rootstock were much smaller than those on the scion. Nevertheless, resistance increased, mainly to *P. parasitica*, when compared with healthy trees (table 2). Thus, the dwarfing effect of the mild exocortis strains used in this experiment seems advantageous for nucellar Hamlin orange trees on Rangpur lime rootstock, because it may reduce harvesting costs, permit more efficient spraying for pest and disease control, lower spraying costs, and make trees more resistant to phytophthora foot rot.

The four severe strains of exocortis viroid had a disastrous effect on the trees. All of them caused more or less severe scaly bark symptoms on the Rangpur lime rootstocks, with strain 6 inducing the most severe symptoms. They reduced tree size from 54 to 63 per cent in the first decade, as compared with healthy trees. Eight years later the stunting effect was even higher, varying from 68 to 84 per cent. Such reduction of the canopy volume was correlated with lower production. In 1971 it was only 13.5 per cent less than from healthy trees, but in 1978, yield reduction averaged 57.8 per cent. Trunk circumference was approximately 38 per cent smaller than for healthy trees. Both *P. parasitica* and *P. citrophthora* did not develop big lesions on trees badly affected by exocortis or other stunting factors, as has already been demonstrated in previous work by Rossetti and Bitancourt (1947). In our experiment, the lesions on trees infected with a severe strain did not differ consistently from those on trees infected with a mild strain, although they were somewhat smaller for both fungi on the scions and on the rootstocks.

From the above results, it may be concluded that: 1) some mild strains of exocortis have a dwarfing effect on trees of Hamlin orange on Rangpur lime rootstock, which seems advantageous for agricultural practices and control of diseases and pests; 2) such mild strains did not induce scaly bark symptoms; 3) trees inoculated with exocortis were more resistant to phytophthora foot rot; 4) under the conditions of this experiment, the effect of mild exocortis strains was greater on the tree growth than on fruit yield, even 20 years after inoculation, which seems to be economically advantageous; 5) dwarfed trees could be more closely spaced, thus increasing the yield per area; 6) the severe exocortis strains in this experiment were considered very unfavorable for tree growth, production, and induced scaly bark symptoms.

ACKNOWLEDGMENTS

This work was sponsored in part by the Fundacão de Amparo a Pesquisa do Estado de Sao Paulo (FAPESP), to whom the authors are indebted.

TABLE 1
DISEASE CONDITION, SIZE AND AVERAGE PRODUCTION OF TREES PLANTED IN 1964, AS MEASURED IN 1971 AND 1979

Strain of exocortis*	Bark scaling†	Canopy volume (m^3)‡		Circumference of trunk (cm)	Average cumulative yield (kg/tree)§§	
		1971	1979	1979§	1971	1978
Healthy 1	0	22.86	56.37	59.0	136.8	208.9
Mild 2	0	15.48	36.09	50.4	156.8	197.2
Mild 3	0	14.50	35.96	52.0	149.5	198.7
Mild 4	0	13.53	33.16	48.0	132.2	180.0
Mild 5	0	14.20	29.63	48.0	134.7	159.0
Average		14.43	33.71	49.6	143.3	183.7
Severe 6	5	8.46	16.15	37.2	128.5	95.5
Severe 7	4	10.59	11.97	37.2	108.0	79.7
Severe 8	1	—	17.93	40.0	—	104.7
Severe 9	3	—	9.31	32.0	—	73.1
Average		9.53	13.84	36.6	118.3	88.3

* 2, 3, 6 carry also psorosis; 8, 9 = border trees, not considered in 1971.
† No. of trees showing bark scaling due to exocortis, out of five trees.
‡ $2/3\ \pi\ r^2h$; r = average radius of canopy; h = height of canopy.
§ Measured 10 cm above bud union.
§§ 1971 = mean of 4 years' total production, based on 6 trees; 1978 = mean of 2 years' total production, based on five trees.

TABLE 2
DEVELOPMENT OF *PHYTOPHTHORA PARASITICA* (P.p.) AND *P. CITROPHTHORA* (P.c.) LESIONS ON INOCULATED TRUNK OF 15-YEAR-OLD NUCELLAR HAMLIN TREES ON RANGPUR LIME ROOTSTOCK INFECTED WITH DIFFERENT STRAINS OF EXOCORTIS VIROID

Strain of exocortis*	Degree of stunt (%)†	Size of P. lesions obtained by inoculation‡			
		on scion		on rootstock	
		P.p.	P.c.	P.p.	P.c.
Healthy 1	—	109.2	48.9	17.9	11.4
Mild 2	36	63.0	30.4	11.4	11.6
Mild 3	36	64.6	35.4	12.3	14.1
Mild 4	41	60.3	28.1	7.5	11.2
Mild 5	47	58.6	29.6	9.3	7.5
Average:	40	61.63	30.88	10.13	11.1
Severe 6	79	60.7	36.7	10.3	8.9
Severe 7	68	55.2	18.9	5.8	5.7
Severe* 8	71	37.6	27.2	10.0	11.9
Severe* 9	84	46.2	34.6	9.2	10.7
Average:	76	49.9	29.35	8.83	9.3

* Trees on border.
† Estimated in relation to healthy trees.
‡ Weight (g) of wax-paper outline of lesions.

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