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Reaction of Exocortis and Rumble Diseased Lemon Trees to *Phytophthora citrophthora* Inoculations

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ABSTRACT. Previous studies showed that mild strains of citrus exocortis viroid (CEV) induced stunting of citrus trees and higher resistance to *Phytophthora* infection. In this paper the reaction of exocortis and rumble-like diseased trees to *P. citrophthora* experimental inoculations was investigated. Ten-year-old lemon plants grafted on Rangpur lime were inoculated in May 1974 with buds carrying a severe strain of CEV. In March 1977 other plants in the same experimental plot were inoculated with bark from plants showing rumble-like fruit symptoms and severely affected by exocortis. In April 1982, the trees were evaluated and the average canopy volume and trunk circumference of exocortis infected and exocortis + rumble-like diseased trees were smaller than for exocortis-free control trees. Trees were trunk inoculated in April 1982 with mycelial disks of *P. citrophthora*, above (scion) and below (rootstock) the budunion. Lesions were measured 66 days later and significant differences in size of *P. citrophthora* lesions were found for those in the scion. Lesions were much smaller in exocortis infected and exocortis + rumble-like diseased trees than in exocortis-free trees. These data confirm previous studies that show that when trees affected by CEV or other stunting factors are inoculated with *Phytophthora*, the resulting lesions are smaller than those on vigorous healthy trees.

The potential use of exocortis-dwarfed citrus trees has been studied in Australia (1, 4, 6); in Brazil (7, 9), and in the USA (2, 3).

Previously reported studies carried out in Brazil have shown that the development of *Phytophthora* spp. is reduced in stunted citrus trees, or in trees submitted to stress due to different causes compared to vigorous healthy trees (8, 9).

In this paper we report the reaction of exocortis and rumble-like diseased trees to *Phytophthora citrophthora*. We compared the lesions induced by the fungus on healthy lemon trees with those on trees inoculated with a severe citrus exocortis viroid (CEV) strain, and on trees inoculated with material collected from plants showing both rumble and severe exocortis symptoms.

MATERIALS AND METHODS

Test plants. Sicilian lemon plants were selected for this experiment. In July 1973 they were grafted on Rangpur lime rootstock

and planted at Lageado Experimental Station of UNESP, in Botucatu County, State of São Paulo, Brazil.

Experimental design. The experimental design used was a randomized complete block with 3 treatments and 7 replicate trees per treatment.

Exocortis inoculation. When the plants were 2 years old they were inoculated with exocortis using buds carrying CEV. The source of inoculum was from a tree carrying a severe exocortis strain from a badly affected orchard located in Mogi-Guaçu County, State of São Paulo. This tree was free of xyloporosis and psorosis. This orchard has been observed for the last 7 years and, up to now, none of the trees have shown typical symptoms of rumble (5).

Rumble source. The rumble material used was collected originally from trees of several commercial lemon orchards in the State of Rio Grande do Sul and taken to the Taquari Experimental Station in Rio Grande do Sul. Selections from Taquari were propagated at the

Experimental Station of Limeira, in the State of São Paulo. Later, typical fruit symptoms of rumple (5) were observed on those plants. In March 1977, the Sicilian lemon plants of the experimental plot on the Lageado Farm, in Botucatu, were inoculated with bark from trees in Limeira that showed rumple symptoms and that were severely affected by CEV.

Growth of trees. Measurements of lemon trees were made in April 1982. The canopy volume was estimated by the formula $\frac{2}{3} r^2 h$, where r is 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 budunion. The degree of stunting due to exocortis was estimated in relation to the average canopy volume of healthy trees.

Phytophthora inoculations. Isolate no. IB 02/83 of *P. citrophthora* (Sm. & Sm.) Leon. was obtained from infected citrus fruits and maintained in the Culture Collection of the Instituto Biológico. It was selected on the basis of its pathogenicity shown in previous tests on lemon trees. The method of Rossetti and Bitancourt (8) was used to inoculate the trunk of the trees, twice in the rootstock and twice in the scion, approximately 10 to 20 cm below and above the budunion. After 66 days the bark was stripped from the trunk and lesions on the surface of the exposed wood were measured (length x mean breadth) to estimate their approximate area. Data were transformed to log x and analyzed statistically by F values and Tukey's test.

RESULTS AND DISCUSSION

Rumple symptoms. Fruit symptoms similar to those described for rumple (5) were found first only on trees inoculated in March 1977

with material from lemon trees showing typical rumple symptoms. These results suggest that we were able to transmit rumple. However, in one of the final readings in the experimental plot at Botucatu, made in the summer of 1983, similar symptoms were found also in plants inoculated in March 1974 with buds carrying only CEV. This shows that the possible transmissibility of rumple in this experiment is questionable.

Growth of trees. Data on growth of healthy and diseased lemon trees are presented in table 1. The average height of healthy and infected trees was almost the same. However, the average canopy volume of exocortis-infected trees was smaller than that of exocortis-free plants (table 1). The degree of tree stunting due to exocortis or to rumple-like disease + exocortis infection in relation to the average canopy volume of control trees, was 28.2 and 24.7% respectively (table 1). Stunting is probably due to the CEV acting directly or indirectly on the trees. The plants infected by exocortis alone were inoculated in 1974, whereas those carrying exocortis + rumple-like disease were inoculated approximately 3 years later. This might explain why the canopy volume in the first group differs from control trees more than that of the second one.

The trunk measurements of CEV-infected trees were significantly lower than those for control trees. The average trunk circumference was 43 cm for healthy trees. For trees inoculated with CEV and rumple-like material + CEV, the average trunk diameters were 16.3 and 18.6% lower, respectively, than that for exocortis-free control trees.

The results on growth of healthy and exocortis infected citrus trees confirm previous

TABLE 1
DEVELOPMENT OF *PHYTOPHTHORA CITROPHTHORA* LESIONS IN INOCULATED TRUNK OF 10 YEAR-OLD SICILIAN LEMON TREES ON RANGPUR LIME ROOTSTOCK

Treatment	Tree height (m)	Canopy volume* (m ³)	Degree of stunting (%)	Trunk circumference (cm)	<i>Phytophthora citrophthora</i> lesions†	
					Rootstock (Rangpur lime) cm ²	Scion (Sicilian lemon) cm ²
Control (exocortis-free)	4.4	36.5 a†††	—	43 a†††	31.6 a†††	615.8 a†††
Exocortis§	3.9	26.2 b	28.2	36 b	20.5 a	390.8 b
Exocortis†† + Rurple	3.6	27.1 ab	24.7	35 b	54.6 a	315.3 b

* $\frac{2}{3} r^2 h$; r = average radius of canopy; h = height of canopy values are means of 7 replicates.

† Estimated in relation to the average canopy volume of exocortis-free plants.

† Lesion size (length x mean breadth) 66 days after experimental inoculations. Values are mean of 14 replicates.

§ Plants inoculated in May 1974 with a bud carrying severe CEV.

†† Plants inoculated in March 1977 with material collected from lemon trees showing rurple symptoms and carrying severe CEV.

††† Figures followed by same letter do not differ significantly (Tukey test, $P = 0.05$).

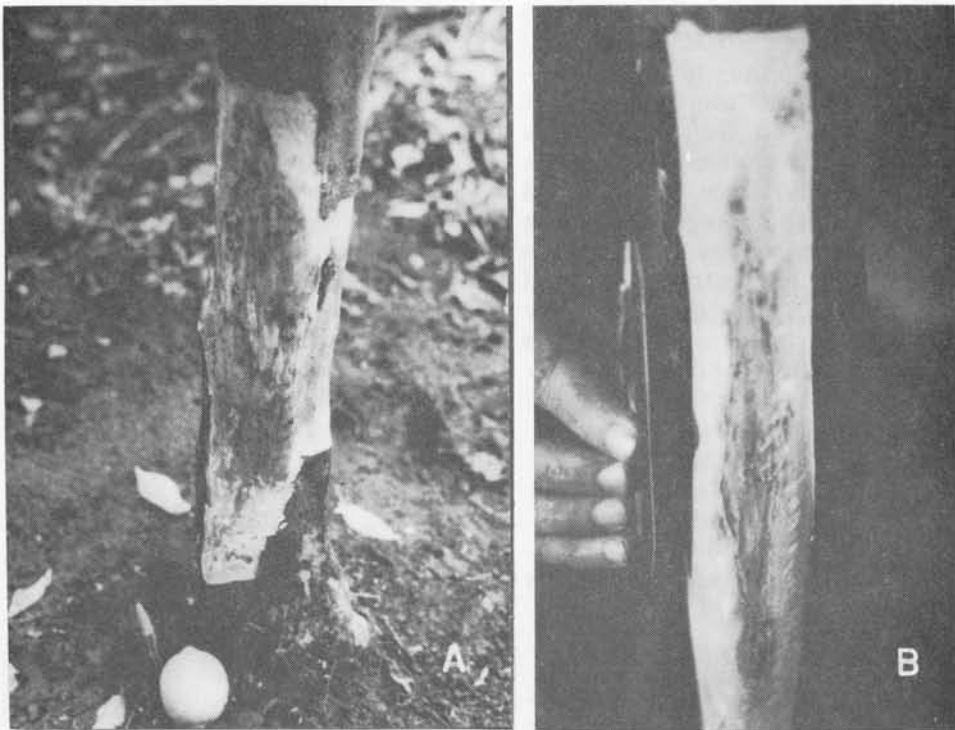


Fig. 1. Trunk of lemon trees on Rangpur lime rootstock inoculated with *Phytophthora citrophthora* showing lesions developed in 66 days in the scion trunk of: A—exocortis-free plant; B—exocortis infected plant.

studies that showed that CEV induced stunting of citrus trees (1, 2, 3, 4, 6, 7, 9).

Phytophthora inoculations. The development of *P. citrophthora* on the inoculated trees is shown in table 1. Rangpur lime is somewhat resistant to *Phytophthora*, thus, as expected, lesions on the rootstock were much smaller than those on the susceptible lemon scion. On the Rangpur lime, the size of lesions in exocortis-free control trees was similar to the size of lesions in exocortis and rumple-like + exocortis affected trees (table 1). Significant differences in lesion size were found only in lesions on the scion. On lemon, the average lesion size (length x mean breadth) caused by the fungus on exocortis-free control trees was 615.8 cm², whereas these values for exocortis infected trees, and for exocortis + rumple-like diseased trees, were

390.8 cm² and 315.3 cm², respectively. Compared with exocortis-free control trees, the average lesion size on the scion of trees severely affected by exocortis, and on trees showing rumple-like and severe exocortis symptoms, was 36.5 and 48.8% smaller respectively.

The results show that the development of *Phytophthora* on the trunk is directly correlated with the vigour of the trees measured by canopy volume (table 1). These data confirm previously reported results that when trees badly affected by CEV or other stunting factors are inoculated with *Phytophthora* the resulting lesions are smaller than the lesions produced on healthy vigorous trees (8, 9).

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