

# Reaction of Pera Sweet Orange Trees Preimmunized Against Tristeza to Inoculations with *Phytophthora citrophthora*

E. Feichtenberger, V. Rossetti, A. A. Salibe and D. A. Oliveira

**ABSTRACT.** Previously studies have shown that the development of *Phytophthora spp.* is reduced in stunted citrus trees, or in trees stressed from different causes. In this paper, the relationship between citrus tristeza virus (CTV), in preimmunized and non-preimmunized nucellar Pera sweet orange trees on Rangpur lime rootstock, and experimental *P. citrophthora* inoculations is reported. Trees were evaluated for CTV effects using stem-pitting, canopy volume, and trunk circumference as parameters. The degree of stem-pitting of non-preimmunized trees was much higher than on preimmunized plants. No significant differences were detected in the trunk circumference. The average canopy diameter of preimmunized trees was smaller than that of non-preimmunized ones. Plants were inoculated with mycelial disks of *P. citrophthora* above and below the budunion. Differences in lesion size between treatments were significant only on the scion. The lesions were smaller in preimmunized trees, thus indicating an increased resistance to *P. citrophthora* in them.

Studies are in progress in Brazil on resistance of citrus trees to *Phytophthora spp.* which cause foot-rot. For this reason the interaction between *Phytophthora* root-rot and some virus or virus-like diseases is being studied. The development of *Phytophthora spp.* is reduced in citrus trees stunted by the citrus exocortis viroid (5), or in trees stressed due to other causes (4) as compared to healthy, vigorous trees.

In this paper we report the reaction of Pera sweet orange trees preimmunized with citrus tristeza virus (CTV) and non-preimmunized trees to experimental inoculations with *P. citrophthora* (Sm & Sm) Leon.

## MATERIALS AND METHODS

**Test plants.** Preimmunized budwood was collected in the best commercial orchard of preimmunized nucellar Pera sweet orange trees in Bebedouro County in the State of São Paulo. Non-preimmunized budwood, of the same nucellar variety, was selected at the Limeira Experimental Station. It was the same material used in the Brazilian preimmunization program (1, 2).

Twenty plants of each group were grown on Rangpur lime rootstock and planted in 1978, on a 1.20 x 0.40 m spacing, at the Lageado Experimental Station of UNESP, in Botucatu County, State of São Paulo, Brazil.

**Growth of trees.** Measurements of preimmunized and non-preimmunized trees were made in April, 1982. The canopy volume was estimated by the formula  $\frac{2}{3}\pi r^2 h$ ,  $r$  being the average radius of the canopy and  $h$  the height of the canopy. The average trunk circumference was measured 10 cm above the budunion.

**Stem-pitting rate.** In April 1982, 10 twenty-cm long branches from each plant were collected, peeled and graded for stem-pitting intensity from 1 (no symptoms) to 5 (very severe symptoms).

**Phytophthora inoculations.** Isolate IB 2/83 of *P. citrophthora* 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 to lemon trees in previous tests. A previously described method (4) was used to inoculate the trees, twice in the

TABLE 1  
DEVELOPMENT OF *PHYTOPHTHORA CITROPHTHORA* IN INOCULATED  
TRUNKS OF NUCELLAR PERA SWEET ORANGE TREES ON RANGPUR LIME,  
PREIMMUNIZED AND NON PREIMMUNIZED AGAINST CITRUS TRISTEZA  
VIRUS

Treatment	Tree height (m)	Canopy* volume (m <sup>3</sup> )	Trunk circumference (cm)	Stem† pitting	<i>Phytophthora citrophthora</i> lesion‡	
					Rootstock (Rangpur lime) cm <sup>2</sup>	Scion (Pera sweet orange) cm <sup>2</sup>
Non-preimmunized	2.8	0.9 a§	22	2.2 a	8.1 a§	31.1 a§
Preimmunized	2.9	0.6 b	24	1.2 b	5.4 a	20.3 b

\*  $2/3\pi r^2 h$ ; r = average radius of canopy; h = height of canopy. Values are mean of 20 replicates.

† Stem-pitting intensity graded from 1 (no symptoms) to 5 (very severe symptoms). Values are means of 10 branches per plant, collected from 20 replicates.

‡ Lesion size (length x mean breadth) 66 days after inoculation. Values are means of 40 replicates.

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

rootstock and twice in the scion, about 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 cambium and wood were measured (length x mean breadth) to estimate their area. Data were transformed to log x. Treatment effects were analyzed by F values and Tukey's test.

## RESULTS AND DISCUSSION

**Growth of trees.** The average height of preimmunized and non-preimmunized trees was almost the same (table 1). However, the average canopy volume of preimmunized trees was significantly less than that of non-preimmunized trees (table 1).

The average trunk circumference, measured 10 cm above the budunion, was the same in pre-immunized and non-preimmunized trees (table 1).

**Stem-pitting rate.** The degree of stem-pitting in the branches was 45.5% lower on trees preimmunized against tristeza than on those of the non-preimmunized group.

**Phytophthora inoculations.** The development of *P. citrophthora* on the inoculated trunk of the rootstock and of the scion in pre-immunized and non-preimmunized 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 sweet orange scion. On the Rangpur lime rootstock the size of lesions that developed in preimmunized trees was statistically similar to the size of lesions in non preimmunized trees (Table 1). Statistically significant differences were found only in lesion size on the scion of the trees.

Once again, the development of experimental lesions resulting from *Phytophthora* inoculations was correlated to the vigor of the trees, measured in this experiment by the canopy volume. The area of the lesions was 34.7% lower on the trees preimmunized against tristeza, in which the canopy volume was 33.3% lower than on the non-preimmunized trees. It is possible that the spacing used (1.20 m x 0.40 m) and the orientation of the trees in the field played some role in the differences of canopy volume observed between the trees of both groups in this trial. The trunk circumference values were practically the same on the trees of both groups or a little higher on the pre-immunized trees and did not interfere in the fungus development. In previous experiments, the lesion size was correlated with the amount of water translocated in the cambial region, measured by the electric conductivity in that region (3).

## LITERATURE CITED

1. COSTA, A. S. and G. W. MÜLLER  
1980. Tristeza control by cross protection: A U.S.-Brazil cooperative success. *Plant Disease* 64: 538-541.
2. MÜLLER, G. W. and A. S. COSTA  
1977. Tristeza control in Brazil by preimmunization with mild strains. 1977. *Proc. Int. Soc. Citriculture* 3: 868-872.
3. ROSSETTI, V.  
1969. Studies on *Phytophthora* species on citrus in Brazil. *Proc. 1st Int. Citrus Symp.* 3: 1211-1216.
4. ROSSETTI, V. and A. A. BITANCOURT  
1951. Estudos sobre a (gomose de *Phytophthora*) dos citros. II. Influência do estado de vegetação do hospedeiro nas lesões experimentais. *Arg. Inst. Biol. São Paulo.* 20: 73-94.

- 5. ROSSETTI, V., J. POMPEU, JR., O. RODRIGUEZ, M. H. VECHIATO, M. L. DA VEIGA, D. A. OLIVEIRA, and J. TEÓFILO SOBRINHO  
 1980. Reaction of exocortis-infected and healthy trees to experimental *Phytophthora* inoculations, p. 209-214 *In Proc. 8th Conf. IOCV. IOCV, Riverside.*

Development in exocortis-infected trees was significantly lower in the number of lesions in the periderm and in the thickness of the periderm (Table 1). Significant differences were found only in trees with the most of the trees.

Thereafter, the development of experimental lesions resulting from *Phytophthora* inoculations was restricted to the sites of the trees treated in this experiment by the canopy volume. The mean of the lesions was 14.7% lower in the trees protected against the disease, in which the canopy volume was 11.1% lower than in the non-protected trees. It is possible that the greater need for light in the trees in the protected zone is the difference of canopy volume observed between the trees of both groups is due to the lack of transpiration. The trees transpired more water than the trees of the control group, as the light in the protected zone and did not increase in the canopy development. In previous experiments, the trees also were associated with the amount of water transpired in the control trees measured in the same conditions in the region.

Development of lesions in the periderm of exocortis-infected and non-protected trees was almost the same as in the trees of the control group. However, the mean of the lesions was 14.7% lower in the trees protected against the disease, in which the canopy volume was 11.1% lower than in the non-protected trees.

The greater need for light in the trees in the protected zone is the difference of canopy volume observed between the trees of both groups is due to the lack of transpiration. The trees transpired more water than the trees of the control group, as the light in the protected zone and did not increase in the canopy development. In previous experiments, the trees also were associated with the amount of water transpired in the control trees measured in the same conditions in the region.

DISCUSSION

The results of this experiment show that exocortis-infected trees have a lower number of lesions in the periderm and a lower thickness of the periderm when compared with healthy trees. This is due to the fact that exocortis-infected trees have a lower canopy volume and, therefore, a lower need for light. The greater need for light in the trees in the protected zone is the difference of canopy volume observed between the trees of both groups is due to the lack of transpiration. The trees transpired more water than the trees of the control group, as the light in the protected zone and did not increase in the canopy development. In previous experiments, the trees also were associated with the amount of water transpired in the control trees measured in the same conditions in the region.

The results of this experiment show that exocortis-infected trees have a lower number of lesions in the periderm and a lower thickness of the periderm when compared with healthy trees. This is due to the fact that exocortis-infected trees have a lower canopy volume and, therefore, a lower need for light. The greater need for light in the trees in the protected zone is the difference of canopy volume observed between the trees of both groups is due to the lack of transpiration. The trees transpired more water than the trees of the control group, as the light in the protected zone and did not increase in the canopy development. In previous experiments, the trees also were associated with the amount of water transpired in the control trees measured in the same conditions in the region.