

# Experimental Transmission of Citrus Blight in South Africa

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**ABSTRACT.** Twelve healthy 4-yr-old Valencia sweet orange trees on Troyer citrange rootstock were transplanted to a 15-yr-old orchard of the same variety in which a high incidence of citrus blight occurred. The trees were planted in pairs on the north and south sides of a blight affected tree. Several roots of one of the pair of healthy receptor trees were approach-grafted to roots of the adjacent blighted donor trees. After 23 months, four of six of the receptor trees exhibited typical visual symptoms of blight. Three years after grafting, symptomatic receptor trees had reduced water uptake, significantly higher concentrations of zinc in trunk phloem and amorphous plugs in vessels of the trunk xylem. None of the six control trees developed these typical blight symptoms.

*Index words.* root grafts.

The first description of a decline resembling citrus blight in the Republic of South Africa was reported in 1979 by Da Graca and Van Vuuren (4). In 1982 reports of severe cases of this type of decline were received from several Eastern and Northern Transvaal citrus producing areas, consequently a survey was conducted in the major citrus producing areas of South Africa and Swaziland to verify the presence of citrus blight and to determine its distribution (7, 10, 11). It was during this survey that the syringe injection technique as a rapid diagnostic test for citrus blight was developed (8). It was also in 1982 that cooperative blight research between researchers at CREC, Lake Alfred, Florida and researchers in South Africa commenced. To further verify that citrus blight in South Africa was in fact identical to that in Florida and to gain more etiological data on the blight organism it was decided to conduct transmission experiments in areas where blight was severe. These experiments were based on those conducted by Tucker, *et al.* (15) and Lee, *et al.* (9) who demonstrated the presence of an infectious agent in the roots of blight affected trees. All previous research, including most recent work by Hopkins (5), has been unable to artificially reproduce all of the known symptoms of the disease in bearing trees.

This paper reports on the results obtained in one of the transmission

experiments in which roots of apparently healthy 4-yr-old trees were approach grafted to 15-yr-old blighted trees.

## MATERIALS AND METHODS

In August 1985 twelve 4-yr-old apparently healthy Valencia orange trees on Troyer citrange were removed from a non-blighted orchard and transplanted to a 15-yr-old blighted orchard of Valencia orange on rough lemon rootstock. The receptor trees were selected by means of the syringe injection technique (8) and then pruned severely to remove all wood smaller than 3 cm in diameter and planted, open-rooted by hand in holes 1 m x 1 m. After transplanting, the trees were painted with white water-based paint to prevent injury by sunburn. All trees were treated with granular formulations of metal-axyl and aldicarb at recommended dosages, at intervals of 12 weeks. The donor trees were selected on the basis of high zinc (16) and restricted water uptake (8) in trunk xylem.

The receptor trees were planted in pairs equidistant (1.5 m) from a blighted tree used as donor. One of the pairs of trees had five to six of its roots, 1-1.5 cm in diameter, approach grafted to the roots of the donor tree. Ungrafted trees constituted the controls. The status of the receptor and control trees was monitored for visual signs of decline every two months.

In February 1989 all control and receptor trees were evaluated using several diagnostic techniques. Canopy symptoms were rated on a scale of 0-3; 0 = healthy; 1 = mild (leaves small with blotchy mottle patterns and zinc deficiency symptoms, no thinning of the foliage); 2 = moderate (leaves small, often flaccid with blotchy mottle patterns and zinc deficiency symptoms, small fruit, delayed blossom and sparse canopy); 3 = severe (canopy sparse, twig dieback substantial, small fruit, delayed blossom, wilt and trunk sprouts common). Zinc concentration in trunk phloem tissue was determined by the method of Albrigo and Young (1). Bark samples were taken above the bud union in order to obtain a large enough sample without removing too much bark and causing a girdling effect in the scion. Water uptake was measured by the syringe injection method of Lee *et al.* (8). Core samples 5 mm in diameter by 4 cm long were removed from the trunk with a Haglof increment borer and fixed in 3% glutaraldehyde. Cross sections 30- $\mu$ m thick were made from the cores at 2-3 cm depth from the cambium. Six sections from each core were mounted on microscope slides and the number of amorphous and filamentous plugs in 200 vessels was counted for each tree (2).

## RESULTS AND DISCUSSION

At the initiation of the experiment the donor trees had a mean trunk zinc content of 25.8  $\mu$ g/g compared with 5.4  $\mu$ g/g in healthy trees in the same grove. The water uptake of the donor trees was 0.01 ml/sec compared with 0.58 ml/sec in the healthy trees.

By July 1987 the receptors at tree no. 1, 3, 5 and 6 started exhibiting zinc deficiency symptoms. The receptor trees at tree no. 2 and 4 had subsequently died. By March 1988 the zinc deficiency and blotchy mottle symptoms had spread to the top of the canopies in all remaining receptor trees.

By February 1989 all receptor trees yet alive were exhibiting visible symptoms of blight accompanied by restricted water uptake, zinc accumulation in the trunk bark and high numbers of amorphous plugs (table 1). These results are similar to those obtained by Tucker *et al.* (15) and Lee *et al.* (9). Similar results have recently been obtained in two experiments in Letsitele and Natal in which healthy six and 12-yr-old trees on rough lemon were root-graft inoculated using pieces of roots collected from blight affected trees (Marais and Lee, unpublished).

The numbers of amorphous plugs in the receptor trees (table 1) are not

TABLE 1  
DIAGNOSTIC CHARACTERISTICS OF RECEPTOR AND CONTROL TREES 45 MONTHS  
AFTER INITIATION OF EXPERIMENT

Tree no.	Canopy rating <sup>2</sup>		Water uptake (ml/sec)		Bark Zn ( $\mu$ g/g)		Amorphous plugs/200 vessels	
	Receptor	Control	Receptor	Control	Receptor	Control	Receptor	Control
1	2.0	0.0	0.0	0.83	47	31	16	2
2	— <sup>3</sup>	0.0	—	0.34	—	30	—	3
3	2.0	0.0	0.0	0.34	235	49	10	2
4	—	0.0	—	0.40	—	32	—	2
5	2.0	0.0	0.0	0.50	59	29	15	2
6	1.5	0.0	0.0	0.50	53	36	8	3
Mean	1.9	0.0	0.0	0.49	98.5	34.5	12.3	2.3

<sup>2</sup>Rated on a scale of 0 = healthy to 3 = severe

<sup>3</sup>Missing trees

exceptionally high, possibly as a result of a rootstock effect, but are high enough to cause reduced water uptake and induce decline (2, 3). The poor water uptake in the control trees (table 1) may be related to the high numbers of filamentous plugs in the trunk xylem (2, 13). The age and trunk diameter of the trees as well as external water stresses may also contribute to restricted water flow. Field observations have shown that healthy trees which are drought stressed have restricted water flow. Young, healthy trees which have just come into bearing also tend to exhibit reduced water uptake.

Trunk and height growth were reduced in the receptor trees as a result of blight (table 2). The bark zinc levels in the receptor trees varied from 1.5 to 5.0 times higher than in the controls (table 1). The levels were much lower than they would have been had the trees been propagated on rough lemon rootstock. This influence of rootstock on relative zinc levels in healthy and blighted trees has been reported by Albrigo and Young (1). Delayed bloom and smaller than normal sized fruit, characteristic of blighted trees, occurred in the receptor trees. The root grafts between donor and receptor trees will be microscopically examined upon completion of the experiment at the end of 1990.

The results obtained in this experiment as well as those in which root piece inoculations were conducted (Marais and Lee, unpublished) serve to support the findings of Tucker *et al.* (15) and Lee *et al.* (9) that citrus blight is caused by a graft transmissible infectious agent localized in the roots. The nature of the agent is still obscure. Hopkins (5) found that *Xylella fastidiosa* could on inocula-

TABLE 2  
TRUNK AND HEIGHT GROWTH OF  
RECEPTOR AND CONTROL TREES  
45 MONTHS AFTER INITIATION  
OF EXPERIMENT

Tree no.	Trunk circumference <sup>z</sup> (cm)		Tree height (m)	
	Receptor	Control	Receptor	Control
1	30.3	34.4	1.9	2.8
2	— <sup>y</sup>	31.9	—	2.0
3	28.2	29.0	1.8	2.5
4	—	32.5	—	2.1
5	30.3	34.4	2.3	3.0
6	31.7	33.3	2.2	2.8
Mean	30.1	32.6	2.1	2.5

<sup>z</sup>10 cm above bud union

<sup>y</sup>Missing trees

tion produce blight-like symptoms in citrus seedlings. He therefore implicated a xylem-limited gram-negative bacterium in the blight syndrome. This however appears to be negated by preliminary results in the root piece inoculation experiments conducted in Natal (Marais and Lee, unpublished) in which citrus blight was transmitted to mature trees by root pieces which had been treated with rolitetracycline.

Suppression of visible blight symptoms and zinc accumulation by tetracycline antibiotics (6, 12, 14) may just be an ameliorating effect of the antibiotic. Healthy trees which have been treated annually for over 6 yr in Letsitele with rolitetracycline have since developed blight and have been removed (Marais, unpublished). Whatever the nature of the agent, successful graft transmission has been a major breakthrough and has paved the way to studying the etiology of blight and evaluating rootstocks for blight tolerance.

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