Experimental Transmission of Citrus Blight

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ABSTRACT

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Eight mature, blight-affected Pineapple sweet orange (*Citrus sinensis*) trees on rough lemon (*C. jambhiri*) rootstock were transplanted to an area of low blight incidence. Healthy trees of bearing age and of the same cultivar were transplanted next to each blight-affected donor tree and several roots each of the healthy, receptor trees and the blighted donors were splice-grafted together. An equal number of control trees was transplanted to an adjacent row. After 3 yr, receptor trees had significantly higher concentrations of zinc in trunk phloem than the controls. After 4 yr, seven of eight receptor trees had typical visual symptoms of blight, significantly higher concentrations of zinc in trunk wood, lower water uptake, and amorphous plugs in the vessels of the trunk. Only one of eight control trees developed these typical blight symptoms. This is the first report of experimental transmission of citrus blight.

Citrus blight is a vascular wilt disease of unknown cause. Losses from the disease have been severe, reaching 500,000 trees per year in Florida, with loss rates in individual groves as high as 10–20% per year (11). Progress in blight research has been slow because blight does not develop in trees less than 5–8 yr old and the disease has not been induced experimentally (10). In early work, Rhoads (7) concluded that blight was a nonparasitic disorder after failure to graft-transmit or propagate the disease. Even after blight was more thoroughly characterized and diagnostic tests became available, bud propagations and reconstitutions of blighted trees from sprouted roots and buds of affected trees failed to reproduce the disease (9,13). Feldman and Hanks (3) reported reduction of root growth in rough lemon seedlings graft-inoculated with tissue of trees affected by blight (synonymous with young tree decline and sandhill decline), but reduced root growth has never been documented as a symptom of blight and typical characteristics of blight were not reproduced. Salibe et al (8) reported poor growth and symptoms of zinc deficiency in seedlings inoculated from trees affected with *marchitamiento repentino*, but water conductivity and zinc levels in wood were not determined and such symptoms do not occur on nonbearing trees under field conditions. Lima and Borducchi (6) also graft-transmitted a "zinc accumulation factor" to young trees from blight-affected sources, but these trees did not show decline symptoms. Transmission of a zinc accumulation factor was not confirmed in other studies (13). In all previous work, no one has artificially induced a tree to decline from blight or reproduced all of the known symptoms of the disease in a mature tree. It has been generally agreed that the malady was nontransmissible (10).

Most studies have concentrated on transmission of blight symptoms to young seedlings or propagation of the disease by reconstituting new trees from roots and buds of blight-affected trees. In this paper, we present the first evidence that blight can be transmitted experimentally and that all the characteristics of the disease can be reproduced in mature trees.

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MATERIALS AND METHODS

Trees with mild blight symptoms and healthy trees were transplanted adjacent to each other and their roots joined by grafting. Fifteen-year-old Pineapple sweet orange (*Citrus sinensis*) trees on rough lemon (*C. jambhiri*) rootstock with mild symptoms of blight and high concentrations of zinc in trunk wood were selected in a severely blighted grove near Lake Hamilton, FL. In April 1979, eight of these trees were selected as donors, pruned severely to remove all wood smaller than 5 cm in diameter, and transplanted with a root ball about 1.1 m in diameter at the surface with a truck-mounted Vermeer tree spade (Vermeer Manufacturing Co., Pella, IA). These trees were transplanted to a grove of low blight incidence near Lake Alfred, FL, and allowed to become established during the summer. In October 1979, eight healthy 6-yr-old Pineapple sweet orange trees on rough lemon rootstock from a grove in Lake Alfred unaffected by blight were pruned and transplanted as described. These receptor trees were planted adjacent to the donor trees with the trunks about 1.2 m from those of the donors. Five to 10 roots about 1–2 cm in diameter from each receptor tree were splice-grafted to similar roots on the corresponding donor tree. Eight additional healthy 6-yr-old Pineapple sweet orange trees on rough lemon were transplanted similarly from the same grove as the receptor trees to an adjacent row 4.5 m from the blight-affected donors as ungrafted controls for the root-grafted receptors.

At various times during the experiment, several diagnostic techniques were used to evaluate the status of the donor, receptor, and control trees. Canopy symptoms were rated on a scale of 0 = healthy, 1 = mild (leaves small with symptoms of zinc deficiency, internodes short, slight wilt but little or no thinning of foliage), 2 = moderate (leaves small, often flaccid, with symptoms of zinc deficiency, canopy sparse with some twig

Table 1. Diagnostic characteristics of the blight-affected donor trees during the experiment.

Characteristic	Date	Value[a]
Wood zinc	April 1979	13.3 μg/g
Phloem zinc	April 1981	120.8 μg/g
Phloem zinc	July 1982	159.4 μg/g
Wood zinc	October 1983	16.2 μg/g
Water uptake	October 1983	0.0 ml/sec
Canopy rating	October 1983	2.1[b]

[a] Values are the averages for all eight donor trees.
[b] Rated on a scale of 0 = healthy to 3 = severe blight.

Table 2. Zinc concentration in receptor and control trees during the experiment

		Zinc (μg/g)[a]	
Characteristic	Date	Receptor	Control
Phloem zinc	July 1979	44.1 ns	41.1
Phloem zinc	April 1981	67.1 ns	62.1
Phloem zinc	July 1982	96.8*	57.7
Wood zinc	August 1982	1.14 ns	1.18

[a] Values are the averages of the eight receptor and eight control trees; ns = not significant and * = significant ($P < 0.05$) according to Student's *t*-test.

Table 3. Diagnostic characteristics of individual receptor and control trees at the end of the experiment

Tree no.	Canopy rating[a]		Water uptake (ml/sec)		Wood Zn (μg/g)		Amorphous plugs/ 200 vessels	
	Receptor	Control	Receptor	Control	Receptor	Control	Receptor	Control
1	1.0	0.0	0.20	0.95	10.0	3.5	7	0
2	1.0	0.0	0.07	0.49	6.4	2.4	19	0
3	1.0	0.0	0.00	1.25	8.8	2.1	57	3
4	1.0	0.0	0.07	1.06	9.2	2.1	8	0
5	1.0	0.0	0.00	1.03	14.6	2.1	15	0
6	1.5	0.0	0.03	1.22	4.9	1.8	10	0
7	1.0	0.0	0.00	1.14	8.0	4.8	21	0
8	0.5	1.0	0.46	0.00	3.6	8.2	4	101
Avg.	0.9*[b]	0.1	0.10*	0.76	8.2*	3.3	17.6	13.0

[a] Rated on a scale of 0 = healthy to 3 = severe.
[b] Significantly different from the control ($P<0.05$) according to Student's t test.

dieback), and 3 = severe (canopy thin, twig dieback substantial, trunk sprouts common). Zinc concentration in trunk wood and trunk phloem tissue was determined by the methods of Wutscher et al (12) and Albrigo and Young (1), respectively. Water uptake was measured by the syringe injection method of Lee et al (5). Core samples 5 mm in diameter by 6 cm long were removed from the trunk about 20 cm above the bud union with a Haglof increment borer (Forestry Suppliers, Inc., Jackson, MS). Cores were fixed immediately in 3% glutaraldehyde. The portion of the core between 2 and 3 cm from the cambium was sectioned transversely with a sliding microtome. Sections were observed microscopically and amorphous plugs typical of those found in blighted trees (2) were counted in a sample of 200 vessels for each tree.

RESULTS

At transplanting, blight-affected donor trees had 13.3 μg/g of zinc in the trunk wood compared with 2.2 μg/g in healthy trees in the same grove. After transplanting, the donor trees flushed vigorously but subsequently developed typical blight symptoms again and were in moderate decline by 1983. Throughout the experiment, wood and phloem levels of zinc remained high and the trees did not take up water (Table 1).

Receptor trees had low levels of phloem zinc at the outset and levels were still low in 1981. By 1982, zinc concentration in the phloem was significantly higher in receptor trees than in control trees but no difference was detected in trunk wood (Table 2). None of the receptor trees showed symptoms in 1982.

By October 1983, all grafted trees, but only one control tree, were showing visible symptoms of blight. Seven of eight grafted trees showed reduced water uptake, accumulation of zinc in trunk wood, and high numbers of amorphous plugs—all characteristics of blight (2,4,10) (Table 3). The eighth tree showed slight decline but was not confirmed as blighted by the other criteria. Seven of the eight nongrafted controls remained healthy in October 1983 as judged by all diagnostic criteria. The eighth tree was definitely blight-affected according to all diagnostic features.

Several root grafts on each tree were dug up and examined about 1 yr after grafting. We estimated that more than 75% of the grafts had taken. All donor-receptor combinations had one or more successful unions. In December 1983, three of the donor-receptor combinations plus two healthy controls and the blight-affected control were uprooted and the grafts and general root condition observed. Seventeen of the 18 root grafts observed on the three pairs of trees had formed successful unions. None of the receptor trees or controls showed any major root necrosis or feeder root decay. All three uprooted donor trees had necrosis of major roots typical of blighted trees in the advanced stage of decline.

The grove at Lake Alfred where the receptor and control trees were obtained was destroyed in 1982; however, 32 Pineapple sweet orange trees on rough lemon rootstock from that grove were transplanted to an area beside the graft-transmisson experiment. None of the transplanted trees have displayed blight symptoms although blight is present in groves about 300 m away.

DISCUSSION

We believe we have conclusively demonstrated experimental transmission of citrus blight for the first time. Seven of eight trees have developed all of the characteristics of blight after 4 yr of being root-grafted to blighted donors, whereas only one control tree was affected.

Transmission presumably occurred via the graft union, which implicates a systemic pathogen. Such an agent would necessarily be localized or erratically distributed because previous attempts to reproduce the disease by reconstituting blighted trees have failed (13).

It is conceivable, however, that the purported pathogen was carried with the soil transported with the donor trees and infected the proximate receptor trees but has not yet reached the more distant controls. Epidemiological studies have indicated that trees adjacent to affected trees are more likely to become diseased than those at greater distances (14). Such patterns of spread are consistent with soil transmission of citrus blight. The absence of root decay on recently affected trees indicates the disease is probably not caused by a soilborne root rot organism and confirms our previous conclusion (4) that major root necrosis is not the cause of decline of the canopy.

Considering this information, it is difficult to retain hypotheses that consider blight a physiological, nutritional, or soil condition problem.

With the apparent ability to induce blight experimentally, the search for a causal agent should be facilitated. Certainly, transplanting mature citrus to induce the disease is a slow, arduous task; however, it should now be possible to follow disease ontogeny more closely and focus the search for a causal agent.

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