Transmission of Citrus Blight by Root Graft Inoculation[z]

L. W. Timmer, R. H. Brlansky, K. S. Derrick, and R. F. Lee

ABSTRACT. In June, 1987, 12 mature, blight-affected Valencia sweet orange trees on rough lemon rootstock were transplanted next to an equal number of healthy trees of the same cultivar. Eight to ten roots were approach-grafted to the adjacent healthy tree. By April 1989, 6 of the 12 previously healthy trees had visible blight symptoms and by October 1989, 7 of 12 showed symptoms. All symptomatic trees had reduced water uptake, amorphous plugging in xylem vessels and zinc accumulation in trunk wood. None of the nongrafted control trees was affected by the disease. In a second experiment, 14 Valencia sweet orange trees on rough lemon rootstock were graft-inoculated with 8 root pieces each with slow water flow from blight-affected trees, a second group of 14 was inoculated with root pieces with fast water flow from blighted trees, and a third group served as non-inoculated controls. After 2 yr, 11 of 14 trees inoculated with roots with slow water flow began to decline and showed all of the diagnostic characteristics of blight. Eight of 14 trees inoculated with roots from blighted trees but with fast water flow, developed symptoms of the disease. All nongrafted control trees remained healthy. By 2-1/2 yr after inoculation, 13 of 14 trees inoculated with slow-flow roots, 9 of 14 inoculated with fast-flow roots and only 1 of 14 of the nongrafted controls was affected by blight. Blight appears to be caused by an infectious agent that can be transmitted by root grafts.

Citrus blight is a disease that causes decline of the tree canopy and renders trees unproductive. Tree loss to blight in Florida is estimated to be over one-half million trees per year (14). Blight or very similar diseases affect citrus production in Brazil, Argentina and South Africa (1, 16). The disease is characterized by low water uptake in trunk wood (5), the presence of amorphous plugs in xylem vessels (1), and elevated zinc concentrations in trunk wood (15). The cause of the disease is not known. In the early years, blight was considered a non-parasitic disorder because of failures to transmit the disease by budwood (9). More recent attempts to transmit the disease in vegetatively propagated material or to reproduce the disease by reconstituting trees from roots and scion material of blight-affected trees also have failed (2, 12, 17). Inoculation of citrus seedlings in the greenhouse produced some reduction in root growth, but did not reproduce any of the known field symptoms of blight (3). Reproduction of some characteristics of "declinio" and "marchitamiento repentino," two blightlike declines by graft inoculation have been reported (6, 11).

More recently, we demonstrated that blight could be transmitted to healthy trees by approach-grafting (4, 13). We also transmitted blight by graft inoculation of trees with root pieces (4). The current experiments confirm and extend the results on graft transmission of citrus blight.

MATERIALS AND METHODS

Mature Valencia sweet orange trees on rough lemon rootstock which were affected by citrus blight were selected in a commercial orchard near Lake Alfred, Florida. The 12 donor trees selected were in mild to moderate decline, showed no water uptake by the syringe injection test (5) and averaged 6.03 μg/g of zinc in the trunk wood (14). Five healthy trees in the same orchard took up an average of 0.25 ml/sec and averaged 0.35

μg/g Zn of trunk wood. Donor trees were severely pruned and transplanted to the experimental orchard using a Vermeer tree spade (Vermeer Manufacturing Co., Pella, IA) in June 1987.

Receptor trees were 25-yr-old Valencia sweet orange on rough lemon rootstock planted on a 7.5 x 7.5 m spacing. Donor trees were placed within the row about 1-2 m from the trunk of the receptor. Eight to 10 roots about 4-10 mm in diameter from each donor and receptor tree were connected by approach-grafting in June 1987. The tree within the row adjacent to the inoculated tree and closest to each donor served as a nongrafted control. When examined 6 months after inoculation, nearly all of the grafts had taken except for one case where the donor tree had died. Receptor trees remained attached to the donor trees for one year at which time the donor trees were removed mistakenly with a front end loader.

Roots for the root-piece inoculation test were collected from three blight-affected Pineapple sweet orange trees on rough lemon rootstock in a commercial orchard near Lake Alfred, Florida. The donor trees showed no water uptake using the syringe test (5) and averaged 14.1 μg/Zn/g of trunk wood. Root pieces 4-10 mm in diameter and 10-15 cm long were collected, washed, and surface disinfested with 0.5% NaOC1 for 3 min. Water from a burette attached to one end of the root piece was drawn through by a vacuum of about 90 kPa applied to the opposite end of the root. Flow was measured for 1 min. Roots were divided into slow-flow—those allowing passage of less than 10 ml/min and fast-flow—those allowing passage of greater than 50 ml/min. Roots with intermediate flow were not used.

Receptor trees were 25-yr-old Valencia sweet orange trees on rough lemon rootstock. Roots of receptor trees were exposed using a high-pressure stream of water. Trees were inoculated by grafting with 8-10 donor root pieces using side grafts, approach grafts or cleft grafts in April 1987. The treatments, consisting of inoculation with slow-flow roots, fast-flow roots, and the nongrafted controls, were replicated on 14 single trees and arranged in a randomized complete block design.

Canopy condition of the trees was rated as previously (13) on a scale of 0 = healthy to 3 = severe decline. Water uptake into the trunk was determined using the syringe injection technique (5). The zinc concentration was determined by previously described methods (15). The number of amorphous plugs in vessels was counted in xylem collected 2-3 cm in from the cambium by methods described previously (1).

RESULTS

In the test where entire blight-affected trees were used as donors, the receptor trees showed moderate uptake of water and all had low concentrations of zinc in the trunk wood prior to grafting (Table 1). One year after inoculation, all receptor trees still appeared healthy and the zinc content of trunk wood was no higher than the nongrafted control trees. The average water uptake of inocu-

TABLE 1
WATER UPTAKE BY THE SYRINGE INJECTION TECHNIQUE AND THE ZINC CONTENT OF TRUNK WOOD OF RECEPTOR VALENCIA SWEET ORANGE TREES ON ROUGH LEMON ROOTSTOCK INOCULATED BY GRAFTING ROOTS IN JUNE 1987 TO THOSE OF ADJACENT, TRANSPLANTED, BLIGHT-AFFECTED TREES AND OF NONGRAFTED CONTROLTREES

Date of analysis Treatment	Water uptake (ml/sec)[z]	Zn content (μg/g)[z]
May 1987		
Inoculated	0.26 ± 0.13	1.47 ± 0.27
Control	0.23 ± 0.09	1.35 ± 0.21
May 1988		
Inoculated	0.33 ± 0.07	3.79 ± 0.78
Control	0.59 ± 0.90	3.13 ± 0.26

[z]Values are means of 12 inoculated trees and 14 control trees ± the standard error.

TABLE 2
TREE CONDITION, WATER UPTAKE BY THE SYRINGE INJECTION TECHNIQUE, ZINC CONTENT OF TRUNK WOOD AND THE PERCENTAGE OF VESSELS WITH AMORPHOUS PLUGS OF VALENCIA SWEET ORANGE TREES ON ROUGH LEMON ROOTSTOCK INOCULATED BY GRAFTING OF ROOTS TO THOSE OF BLIGHT-AFFECTED TREES IN JUNE 1987 OR OF NONGRAFTED CONTROL TREES

Date of analysis Treatment Tree condition	No. of trees	Canopy rating[z,y]	Water uptake (ml/sec)[z]	Zn content (μg/g)[z]	Vessels with amorphous plugs (%)[z]
April 1989					
Inoculated					
Symptomatic	6	1.1 ± 0.1	0.02 ± 0.02	7.30 ± 0.70	26.3 ± 9.0
Asymptomatic	6	0.0	0.59 ± 0.25	1.48 ± 0.30	12.7 ± 7.8
Control					
Asymptomatic	14	0.0	1.07 ± 0.13	1.15 ± 0.38	4.4 ± 1.8
October 1989					
Inoculated					
Symptomatic	7	1.9 ± 0.2	0.01 ± 0.01	4.79 ± 0.65	28.8 ± 7.0
Asymptomatic	5	0.0	0.89 ± 0.20	1.65 ± 0.32	6.6 ± 3.4
Control					
Asymptomatic	14	0.0	1.00 ± 0.12	1.64 ± 0.11	2.5 ± 1.9

[z]All values are the means ± the standard error.
[y]Canopy decline rated on a scale of 0 = healthy to 3 = severe decline.

lated trees was about half that of control trees. By April 1989, six of the inoculated trees showed mild decline symptoms with wilt, thin canopies, twig dieback and occasional zinc deficiency patterns in the leaves. Water uptake by these trees was low, the zinc content of wood was high, and amorphous plugs were common in xylem vessels (Table 2). Asymptomatic, inoculated trees had lower water uptake and more amorphous plugs in vessels, but the zinc content was about the same as nongrafted control trees. By October 1989, one additional tree had developed symptoms and most of the symptomatic trees had reached the moderate stage of decline. All symptomatic trees had reduced water uptake, elevated zinc levels, and a high percentage of vessels with amorphous plugs. The remaining five asymptomatic, grafted trees had water uptake and zinc content comparable to the nongrafted controls and only slightly higher numbers of amorphous plugs. One of these five trees was the one where the donor tree died.

In the root piece grafting experiment, all treatments had the same water uptake and zinc levels in trunk wood (Table 3) at the time of grafting. By 14 months after grafting, no trees had developed symptoms of blight. Water uptake by grafted trees was slightly lower than in the controls and the zinc content was about the same. However, one tree grafted with fast flow roots had a water uptake of 0.03 ml/sec and a zinc content of 9.75 μg/g.

TABLE 3
WATER UPTAKE BY THE SYRINGE INJECTION TECHNIQUE AND ZINC CONTENT OF TRUNK WOOD OF VALENCIA ORANGE TREES ON ROUGH LEMON ROOTSTOCK INOCULATED WITH SLOW-FLOW OR FAST-FLOW ROOTS FROM BLIGHT-AFFECTED TREES IN APRIL 1987 AND OF NONGRAFTED CONTROL TREES

Date of analysis Treatment	Water uptake (ml/sec)[z]	Zn content (μg/g)[z]
March 1987		
Slow-flow roots	0.15 ± 0.01	2.16 ± 0.25
Fast-flow roots	0.15 ± 0.03	2.34 ± 0.28
Control	0.17 ± 0.03	2.26 ± 0.19
June 1988		
Slow-flow roots	0.60 ± 0.28	3.61 ± 0.61
Fast-flow roots	0.50 ± 0.22	2.68 ± 0.55
Control	0.85 ± 0.30	2.64 ± 0.36

[z]Values are means of 14 trees ± the standard error.

TABLE 4
TREE CONDITION, WATER UPTAKE BY THE SYRINGE INJECTION TECHNIQUE, ZINC CONTENT OF TRUNK WOOD AND THE PERCENTAGE OF VESSELS WITH AMORPHOUS PLUGS OF VALENCIA SWEET ORANGE TREES ON ROUGH LEMON ROOTSTOCK INOCULATED WITH SLOW-FLOW AND FAST-FLOW ROOTS FROM BLIGHT-AFFECTED TREES IN APRIL 1987 OR OF NONGRAFTED CONTROL TREES

Date of analysis / Treatment / Tree condition	No. of trees	Canopy rating[z,y]	Water uptake (ml/sec)[z]	Zn content (μg/g)[z]	Vessels with amorphous plugs (%)[z]
April 1989					
Slow-flow					
Symptomatic	11	0.9 ± 0.1	0.05 ± 0.02	10.0 ± 1.40	12.7 ± 4.4
Asymptomatic	3	0.0	0.33 ± 0.23	6.2 ± 2.89	4.5 ± 3.3
Fast-flow					
Symptomatic	8	1.0 ± 0.1	0.04 ± 0.03	6.4 ± 1.14	18.8 ± 6.7
Asymptomatic	6	0.0	0.90 ± 0.21	3.4 ± 1.25	2.8 ± 2.7
Control					
Asymptomatic	14	0.0	0.86 ± 0.12	2.5 ± 0.46	5.6 ± 2.1
October 1989					
Slow-flow					
Symptomatic	13	1.9 ± 0.2	0.04 ± 0.03	5.97 ± 0.67	22.7 ± 6.7
Asymptomatic	1	0.0	0.24	1.75	0.0
Fast-flow					
Symptomatic	9	1.4 ± 0.1	0.03 ± 0.03	4.38 ± 0.58	28.8 ± 4.8
Asymptomatic	5	0.0	0.39 ± 0.14	1.95 ± 0.57	18.4 ± 5.3
Control					
Symptomatic	1	0.5	0.00	4.25	17.5
Asymptomatic	13	0.0	0.86 ± 0.11	1.69 ± 0.20	3.8 ± 2.9

[z]Values are the means ± the standard error.
[y]Canopy decline rated on a scale of 0 = healthy to 3 = severe decline.

The first symptoms of blight were observed in February 1989, nearly 2 years after grafting. By April, 11 of the 14 trees grafted with slow-flow roots and 8 of 14 grafted with fast-flow roots showed distinct, but mild symptoms of blight, whereas all nongrafted control trees remained healthy. All symptomatic trees had low water uptake and high zinc content and amorphous plugs were common in the xylem vessels. The remaining three asymptomatic trees grafted with the slow-flow roots had reduced water uptake and elevated zinc compared to the nongrafted controls. The asymptomatic trees grafted with fast-flow roots were comparable to the nongrafted controls in all respects.

By October 1989, 13 of 14 trees grafted with slow-flow roots and 9 of 14 trees grafted with fast-flow roots had developed moderate decline and all showed reduced water uptake, elevated zinc and a high percentage of the vessels with amorphous plugs. One of the 14 nongrafted controls developed decline symptoms and the diagnostic criteria indicated the tree was affected by blight. Some of the asymptomatic grafted trees have reduced water flow and amorphous plugs in the xylem indicating that more trees may succumb to the disease.

DISCUSSION

Previous research (4, 13) and the current studies demonstrating transmission by root grafting leave little doubt that blight is a graft-transmissible disorder caused by an infectious agent.

The failure of earlier attempts to graft-transmit or propagate the disease (2, 9, 12, 17) are difficult to explain. In research conducted prior to the development of techniques for accurate diagnosis, it is conceivable that investigators confused blight with tree declines caused by excess water or fertilizers or by nematodes or soilborne fungi. Later research, however, certainly dealt with blight-affected trees. Many tests are in progress using different types of tissue or methods of inoculation. However, all tests where the disease has been transmitted successfully to date have included root xylem in the inoculum. Thus, the pathogen may be localized in xylem tissues reducing the possibility of transmission by buds or phloem tissue. The high rate of transmission obtained with roots from blighted trees with normal water flow in the current test suggests that the agent is well-distributed in root systems of affected trees. There is an indication that trees may be more likely to succumb or succumb more rapidly when larger amounts of inoculum are used. In previous studies, propagating material may have been selected which contained little inoculum or a low percentage of infected material. Since blight occurred in the control trees in most of these tests, transmission would have been difficult to detect if the percentage was low or symptom development was delayed. Recent evidence of the graft transmissibility of blight in South Africa (8) and of declinio in Brazil (10) substantiate the results obtained in our studies in Florida.

The causal agent of blight remains unknown, but current studies allow certain conclusions as to its nature. The graft transmissibility of blight in several experiments indicates that the disease is not attributable to cultural practices, physiological or other non-parasitic disorders as has been proposed (7). In other studies (L. W. Timmer and J. H. Graham, unpublished), healthy trees exposed to large amounts of soil from beneath blighted citrus trees have not developed symptoms of blight after 4 yr. We believe blight is caused by an infectious agent such as a virus, viroid or fastidious procaryote.

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