Failure to Transmit Citrus Blight by Limb Grafts*

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ABSTRACT. Four healthy 4-yr-old Valencia sweet orange trees on rough lemon rootstock were planted 1 to 1.5 m away from each of 12 blight-affected Valencia sweet orange trees on Carrizo citrange rootstock (15-yr-old). For the 12 replications, a limb-grafted and a non-grafted control tree were protected from natural root-graft transmission by 1.2 m deep soil barriers. A non-barrier control tree and a root-grafted receptor tree were also placed equidistant from each donor tree. These treatments were established in 1986 on a deep sandy soil site with no history of citrus blight. Limb and root grafts were maintained for 6 yr between the receptor and donor trees. Two of the twelve donor trees eventually proved to be blight-free. All receptor trees root-grafted to blight donor trees were positive for the 12-kd blight-associated protein by year 5, and these trees showed some visible symptoms of blight at least by year 6. Two of the non-barrier control trees and one of the barrier control trees were positive for the blight protein by year 6, but showed no visible blight symptoms. Extensive mingling of root systems had occurred beyond the barrier extension and under the non-barrier trees. None of the limb-grafted receptor trees showed symptoms of citrus blight or were positive for the blight protein after 6 yr suggesting that no graft transmissible agent for this disorder is present in the aboveground portion of the tree.

Index words. root grafts, citrus blight proteins.

The cause of citrus blight still remains unknown, but repeated root-graft transmission from blighted donor trees to healthy receptor trees indicates that a graft transmissible agent is involved (5, 6). Earlier attempts to transmit citrus blight by budding from blight-affected trees or reconstituting trees by grafting small stems and roots from blight trees were unsuccessful (7). The purpose of this study was to determine if blight could be transmitted from affected (donor) trees to healthy (receptors) through long-term grafts of aboveground parts, which would presumably provide a long-term source of the infectious agent even if one existed for only short periods above ground.

MATERIALS AND METHODS

Donor trees were selected from a block of 15-yr-old Valencia sweet orange on Carrizo citrange rootstock. These blight-affected trees were selected on the basis of the high concentration of bark zinc (1) and moderately reduced water uptake by the syringe method (4) compared to healthy looking trees but with only a slight wilted appearance. Representative early decline trees had zinc levels in bark tissue from 87 to 168 ppm compared to 63 to 72 ppm for apparently healthy trees. Water uptake into the trunk was 2.6 to 5.8 ml/min compared to 11 to 13 ml/min for these same sets of trees. Therefore 12 trees with bark zinc levels above 90 ppm were selected (Table 1). These 12

TABLE 1
ZINC CONCENTRATION IN BARK OF VALENCIA SWEET ORANGE TREES ON CARRIZO CITRANGE ROOTSTOCK USED AS DONOR TREES

Replication no.	Bark zinc concentration (ppm)	
	Before transplanting	After transplanting[z]
1	92	123
2	102	60
3	99	57
4	194	129
5	256	225
6	170	109
7	219	190
8	257	177
9	248	356
10	191	217
11	146	165
12	100	152

[z]See dates in Table 2.

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trees were transplanted June 1986 with a 1.2-m diameter tree spade into a typical deep sandy soil site with no previous history of blight.

Four receptor trees of 4-yr-old Valencia sweet orange on rough lemon rootstock were planted equidistantly around the perimeter of each donor tree (Fig. 1). Two receptor trees were separated from each donor tree and from each other by an in-ground barrier of corrugated fiberglass roofing sheets covered on both sides with 0.15 mm black plastic film. The barriers were installed to a depth of 1.2 m with lateral extensions of 1.2 m.

One of the two trees separated by the barrier was limb grafted to the donor tree and the other was left as a control with no root or canopy grafts to the donor tree (Fig. 1). The other two trees had no soil barrier and one was root-grafted to the donor tree. The root grafts and limb grafts (ca. 2 to 3/plant) were made 2 months after transplanting. Limb grafts and barrier controls of two adjacent replications were inclosed on the same side of the H designed soil barrier in case limb grafts became affected. Every two-replication set was randomized for side of barrier for these two treatments. The root graft and soil contact only treatments were randomized for the remaining two transplanting locations in every replication. This allowed for statistical analysis by factorial if only part of the trees in a treatment developed citrus blight. Additional grafts were made at later dates as the roots and canopies developed (Table 2). One limb grafted and one barrier control were lost to Phytophthora foot rot.

Receptor trees were monitored for development of blight. In the fifth and sixth years after grafting, all trees were analyzed for the presence of a blight-specific 12-kd protein in mature leaves by the immuno-dot method (2).

RESULTS AND DISCUSSION

The first receptor tree with visible symptoms, permanent leaf wilt and zinc deficiency, appeared in 1990 on a root-grafted tree, 4 yr after the grafts were established. After yr 6, seven of 12 root-grafted receptor trees showed stunting with foliar zinc deficiency symptoms; three additional root-grafted receptors showed dieback and two trees had no symptoms. Although the receptor trees

soil barrier, total physical exclusion
soil barrier and limb grafted
no soil barrier, root grafted
no soil barrier, no root grafts

Fig. 1. Diagram of layout of two replications of experiment. The H figure represents the soil barrier which was 1.2 m deep and the lateral extensions were 1.2 m long. The donor and receptor tree positions are given in the legend above.

TABLE 2
TIME SCHEDULE FOR EXPERIMENT ACTIVITIES IN TRANSMISSION EXPERIMENT

Year	Activity	Date
1	Donor trees tested for zinc	Apr 86
1	All trees transplanted	Jun 86
1	Initial grafting	Aug 86
1	Grafts checked	Dec 86
1	Donor trees zinc retested	Dec 86
1	Additional limb & root grafts	Mar 87
2	Additional limb grafts	Apr 88
2	Limb grafts rechecked	Aug 88
3	Rechecked limb grafts	Apr 89
4	Additional limb grafts	Mar 90
5	First blight protein assay	Mar 91
5	Additional limb grafts	Jul 91
6	Second blight protein assay	May 92

were 4 yr old at the time of the grafting, they were small as a result of being grown and held in 5.4 liter pots. They required some time to come into production, a condition that appears to be necessary for symptom expression. Using similar age material but field grown 4-yr-old receptor trees, Marais and Lee (5) saw visible symptoms of blight in the receptor trees within 2 yr of root grafting. The donor trees for the two root-grafted trees without symptoms were found to have low zinc levels on reexamination in December 1986, after planting, (Table 1) and were therefore probably not blight-affected.

Further assaying with the blight protein immuno-dot in 1991, confirmed that these donor trees did not have blight and were essentially donor control trees (Table 3). All other donor trees and their corresponding root-grafted receptor trees were positive for the blight protein. No other receptor trees were positive in year 5.

In 1992 the trees were analyzed again for the presence of the blight protein by the immuno-dot test and again no limb-grafted receptor trees were positive for the protein (Table 3). One barrier control tree and two soil contact trees were positive in addition to the ten donor and ten root-grafted receptor trees that were positive the previous year. Since all of the root grafts with positive donor trees became affected with citrus blight as in previous studies (5, 6) and none of the limb grafted receptors did, no statistical analysis was made.

Root development for at least 0.5 m beyond the lateral barrier extension between the donor and the barrier-protected-control trees was examined in September 1992 by digging out below the root system, undercutting, to expose the root system. A dense root mass was found immediately beyond the barrier with intermingling of roots from the two trees. It is presumed that some

TABLE 3
IMMUNO-DOT ASSAY FOR 12-KD BLIGHT PROTEIN IN DONOR AND EXPERIMENT TREES YR 5 AND 6 AFTER GRAFTING

	Donor		Root grafted		Limb grafted		Control		Soil contact	
	Year		Year		Year		Year		Year	
Replication no.	*5*	*6*	*5*	*6*	*5*	*6*	*5*	*6*	*5*	*6*
1	+[z]	+	+	+	−	−	−	−	−	−
2	−	−	−	−	−	−	−	−	−	+
3	−	−	−	−	−	−	−	−	−	−
4	+	+	+	+	−	−	−	+	−	+
5	+	+	+	+	NT[y]		−	−	−	−
6	+	+	+	+	−	−	−	−	−	−
7	+	+	+	+	−	−	−	−	−	−
8	+	+	+	+	−	−	−	−	−	−
9	+	+	+	+	−	−	−	−	−	−
10	+	+	+	+	−	−	NT		−	−
11	+	+	+	+	−	−	−	−	−	−
12	+	+	+	+	−	−	−	−	−	−

[z]Positive (+) or negative (−) for 12-kd blight protein.
[y]NT = no tree.

natural root-grafting had occurred. However, one of the blight-protein-positive trees exposed by soil contact was in a replication with one of the negative donor trees (Table 3). This tree apparently became affected by some other mode of transmission, unknown, but development of visible symptoms should be observed before concluding positively that this tree has citrus blight.

Although no evidence of transmission by limb-grafts occurred in this experiment over a 6-yr-period, two possible factors other than an inability to transmit this disease through aboveground tissues must be considered. The donor trees already had restricted water flow and aboveground movement of a causal agent may have also been restricted. Secondly, because of the weakness of blight-affected donor trees and the occurrence of a severe freeze in December, 1989, it was difficult to maintain the same limb-grafts for the entire 6-yr period. Limb-grafts had to be reestablished about every 2 yr or at least three times on some trees. However, the putative pathogen has been transmitted by root grafts in as little as 3 to 7 months (3), suggesting that having some limb grafts for the entire 6 yr as in this study should have been more than long enough if the agent were available in the canopy of the plant for transmission. Further, the declining condition of donor trees does not impede root-graft transmission. The results of this study and previous work (5, 6) suggest that the root transmissible agent of citrus blight does not reside in the aboveground portions of the citrus tree.

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