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Experimental Transmission of Declinio by Approach-Root-Grafting in São Paulo State, Brazil

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ABSTRACT. Two types of citrus declinio (CD) transmission trials using approach root grafts were carried out: 1) *Adult trees*. In 1987, twenty pairs of 12-yr-old trees, one of each pair being diseased (donor) and the other of each pair being healthy (receptor) were approach-root-grafted to each other. A year later most of receptor trees and many controls were showing symptoms of CD. Results showed that adult trees from affected orchards in S. Paulo State should not be used as receptors for CD. 2) *Three-yr-old plants*. In 1984 three plants (receptors) of six, planted in 1981 around diseased (donors) and healthy adult trees with five replications were approach-root-grafted. The three ungrafted plants remained as controls. In 1987 all young plants were dug out and each was replanted and used as root-graft donors between two adult healthy trees (receptors) to test for retransmission of CD. In early 1988 the previously inoculated plants showed poor development; ten of eleven were dead in November when they were 7 yr old. Control plants were normal. These results lead to the conclusion that declinio has been transmitted by approach root grafting. This is the first report of graft transmission of CD in Brazil.

After several unfruitful attempts to experimentally transmit declinio of citrus trees in the São Paulo State, Brazil, using classical methods reported by different research groups, the first positive results were finally obtained by the staff of the São Paulo Biological Institute. The method applied was by approach root grafting of 14-yr-old diseased trees to young nursery plants.

In 1943 Bitancourt (3) showed psorosis transmission in the field from a diseased to an adjacent healthy tree by natural root graft. In 1988, Broadbent *et al.*, (4) reported natural transmission of exocortis occurred in Australia when the root systems of adjacent trees overlapped and individual woody roots were closely appressed. In a few cases, some degree of union was evident. Other examples of root graft transmission of plant pathogens are reviewed by Epstein (6).

In 1984, in Florida the causal agent of citrus blight was successfully transmitted by experimental approach root graft using mature affected trees as donors and apparently healthy trees from the same orchard as receptors (12). Positive results also were obtained in a second experiment in Florida within 2 yr after approach

root grafting mature trees on Carrizo citrange rootstock to blighted trees on rough lemon rootstock (7). The results suggest that the cause of blight is a graft transmissible infectious agent.

The purpose of this work was to study the transmissibility of declinio in Brazil. Several types of experiments were carried out in different locations of the São Paulo State (9, 10). In this paper the results of two different experiments by approach root grafting are described and discussed.

MATERIAL AND METHODS

Trees were diagnosed as healthy and declinio affected by using syringe water injection, zinc analysis of the trunk wood and the number of amorphous plugs.

The syringe injection method was used to measure water uptake into the trunk (5, 8). When water uptake was higher than 0.30 ml/sec the trees were considered to be free of declinio. When the water uptake was about 0.1 to 0.3 ml/sec or less, trees were considered declinio-affected; the canopy of those trees was visibly declined. For zinc analysis, trunk wood samples were collected, dried and analyzed by

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atomic absorption spectroscopy as described by Wutscher *et al.* (13).

For determination of the number of amorphous plugs, horizontal core samples each about 5 cm long were taken from the scion trunk wood about 20 to 30 cm above the bud union with a 5 mm Haglof increment borer (Forestry Suppliers, Jackson, MS, 39204). Cores were fixed overnight in 3% glutaraldehyde in 0.06 M Na-K phosphate buffer, pH 6.8 with 0.02% sodium azide. The glutaraldehyde solution was removed and the cores were stored in the phosphate buffer at 4° C. Cross sections 20 μ m thick were cut from the core at a depth of 2 to 3 cm from the cambium using a cryostat. Ten sections from each core were mounted on microscope slides, and the number of amorphous plugs in 200 xylem vessels was counted under the microscope at 100X magnification (1, 11).

The method of root-grafting used in these experiments was essentially the same as that used previously in Florida (7). The method consisted of grafting three to five secondary roots, approximately 0.5 cm in diameter, from declinio-affected trees to young receptor plants. Donor and receptor roots were lightly scraped to expose the cambium along 6 to 10 cm of one side, placed together, and then firmly fastened together with plastic strips.

For canopy rating, the following visual symptoms were evaluated: zinc deficiency symptoms in leaves; wilting of part or all of the canopy; leaf drop; twig dieback; abnormal flowering; general canopy decline; internal growth of new shoots; reduced size of fruit.

Declinio does not affect trees until they reach bearing age, about 5-yr-old or more. Canopy rating based on severity of visual symptoms was from 0 to 3: 0.0 = healthy; 1.0 = initial declinio stage; 2.0 = intermediate stage; 3.0 = more advanced stage.

Root-grafting of mature adjacent trees in an affected orchard. Twenty pairs of adjacent adult trees (healthy and declinio affected) of 12-yr-old

Pera sweet orange on Rangpur lime rootstock, were selected in an orchard in February 1987. The condition of the trees was evaluated as described above.

Fourteen of the 20 pairs of trees consisted of one declinio-affected tree (donor) and one adjacent healthy tree (receptor). The remaining six pairs of trees consisted of two healthy adjacent trees in the same orchard, also root-grafted, as controls.

The trees were evaluated by canopy rating in January, May and October 1988. The trees were rated for water uptake, zinc and plugging before starting the experiment.

Approach root-grafting mature diseased trees to 3-yr-old trees. In 1981 six, 1-yr-old nursery plants of Valencia sweet orange on Rangpur lime rootstock free of declinio symptoms were planted around each of five 14-yr-old declinio-affected trees of the same combination.

In 1984 when the plants were 3 yr old, three young trees of each group of receptors were root-grafted to the mature donor trees. The other three young trees were maintained as non-root-grafted controls and to monitor soil effect, or natural spread of declinio.

In 1987, all the young 6-yr-old trees were up-rooted, pruned and each one, now serving as donor, was replanted between two healthy mature trees (receptors). Subsequently, they were root-grafted to the mature, healthy trees, to test retransmission. The transplanted trees were irrigated and their trunks were protected against sunburn by painting with lime.

RESULTS

Tree to tree root-grafting of mature adjacent trees. In February 1987, the diagnostic tests were positive for declinio in the diseased donor trees (D) and negative for the healthy (H) receptors and controls as shown in Table 1. Microscopic observations showed high numbers of amorphous plugs only in the donor, declinio-af-

TABLE 1
MEANS AND RANGES OF WATER ABSORPTION BY TRUNK SYRINGE INJECTION AND DECLINIO VISIBLE SYMPTOM RATINGS IN
PAIRS OF ADULT PERA SWEET ORANGE TREES ON RANGPUR LIME ROOTSTOCK, INOCULATED BY APPROACH ROOT GRAFT,
AND IN PAIRS OF CONTROL TREES

Pairs of trees submitted to appr.-root-graft ^z	Water uptake (ml/30 sec)		Visual symptoms ^y on:								
	Feb. 12, 1987		Feb. 12, 1987		Jan. 28, 1988		May 3, 1988		Oct. 26, 1988		
	D	H	D	H	D	H	D	H	D	H	
14 pairs D x H											
Range	0.0-2.0	15.0-25.0	1.0-3.0	0.0	1.0-3.0	0.0-3.0	1.0-3.0	0.5-3.0	1.5-3.0	0.5-3.0	
Mean	0.3	21.8	2.42	0.0	2.64	0.50	2.64	0.90	2.90	1.40	
6 pairs H ₁ x H ₂											
Range	H ₁ 17.6-30.0	H ₂ 9.0-37.5	H ₁ 0.0	H ₂ 0.0	H ₁ 0.0-1.0	H ₂ 0.0-1.0	H ₁ 0.5-1.0	H ₂ 0.0-1.5	H ₁ 0.5-3.0	H ₂ 0.5-3.0	
Mean	22.5	17.6	0.0	0.0	0.33	0.42	0.75	0.75	1.33	1.83	

^zD = donor, declinio-affected trees; H = receptor, apparently healthy trees, approach root grafted in pairs D x H. H₁ and H₂ = control pairs: apparently healthy, also approach-root-grafted in pairs.

^yrated on a scale of 0 = healthy to 3 = severe decline.

ected trees. Visual symptoms of decline, ranged from 0 to 3 in four observations from February 1987 to October 1988 (Table 1). For the pairs of adjacent trees D x H, the mean water uptake measurement when the experiment was started for decline trees (D) was 0.03 ml/sec and for healthy trees (H) was 0.73 ml/sec. The mean visual symptoms rating increased over 20 months for D trees, from 2.42 to 2.90. For the root-grafted H trees, these ratings increased from 0.0 to 1.4. For the pairs of adjacent control healthy trees H₁ x H₂, the initial mean water uptake was 0.75 and 0.59 ml/sec, respectively. The mean ratings of visual symptoms increased over 20 months from 0.0 to 1.33 for H₁ and from 0.0 to 1.83 for H₂, showing that decline was beginning to occur in these trees in this high incidence area.

Approach root-grafting mature diseased trees to 3-yr-old plants. In 1987, three years after the plants had been root-grafted, and both the decline inoculated and healthy control plants, then 6-yr-old, had been transplanted, all of them suffered the effect of shade from the canopy of the adjacent mature healthy trees. They sprouted normally after pruning, but their growth was poor. The water uptake measurements for the mature, now receptor trees were somewhat irregular, varying from 0.27 to 0.35 ml/sec. Diagnostic tests were not applied to the transplanted trees (previously inoculated and controls).

One year after transplanting, the young trees which had been previously graft-inoculated to decline-affected trees started to decline, whereas the healthy non grafted control trees were not declining. In November 1988, when the trees which had survived the transplanting were 7.5-yr-old (4.5 yr after-inoculation), 9 of 11 plants previously graft inoculated died, and in April 1989, only one was still alive.

The 12 control non-inoculated plants which survived transplanting and had been graft-inoculated in 1984,

which were also root-grafted to mature healthy trees in 1987, were normal, but in November 1988, two of them died. All the others were in good condition on April 1989.

Three of the mature trees which were root-grafted in 1984 to the previously root-graft inoculated plants, showed obvious visible symptoms of decline, and their water uptake was 0.0 ml/sec. None of the 12 mature trees root-grafted to the non inoculated control plants showed visible symptoms of decline.

DISCUSSION

In the first experiment, tentative transmission of decline by approach root-grafting 12-yr-old adjacent trees within a heavily decline-affected orchard, was not positive. Results showed that the healthy appearing controls H₁ and H₂ (Table 1) developed high degrees of visible decline symptoms within 16 months. The progress of the disease was slower on control trees, but the final readings were close or reached even higher degrees than in the inoculated trees root-grafted to affected donor trees (Table 1).

In Florida, blight transmission was positive in mature trees when they were removed from an orchard and transplanted in another place, where healthy receptor trees were approach-root-grafted to blight-affected donors.

The results of this experiment suggest that development of decline on mature trees within very severely affected orchards in the State of São Paulo may occur quickly, probably faster than the development of blight in Florida. For this reason, mature apparently healthy trees from severely decline-affected orchards in the State of São Paulo may not be useful as receptors for transmission experiments, for they might very well already have the disease but at a level too low for detection by current diagnostic methods.

In the second experiment described, with young plant, 3-yr-old

trees were used for receptors and later for donors. While the evidence of declinio symptoms and characteristics develop more showly in young plants, the results, in the State of São Paulo, are more reliable than when using adult trees.

In this experiment, the orchard was not so severely affected by declinio as it was in the first experiment. Ten of the 11 (91%) experimentally inoculated plants showed declinio symptoms about 3 yr after inoculation and died 1 to 2 yr later. For the non-grafted controls which had been exposed to the same environmental conditions, only 2 of 12 plants (16.6%) died.

In the State of São Paulo, declinio-affected trees are killed very frequently by the disease. Dead trees may be commonly seen in rows, in clusters or in isolated cases, especially when stress factors such as poor soil and severe drought may hasten the process especially in the northern part of the state. The adverse conditions, that the young trees were subjected to such as the effect of excessive shade, after being transplanted between healthy trees may have had an effect on their growth and survival. Their sprouting was normal but growth was retarded. However, most of the plants which had been previously root-grafted to diseased donors started to show visible declinio

symptoms 6 months after transplanting and died when were 6 to 7 yr old. While they were not subjected to declinio diagnostic tests, the visual symptoms results suggested that the causal agent of declinio had been successfully transmitted by approach root grafting.

Retransmission to mature healthy trees was tried in this experiment. Three of the 12 mature trees that were root-grafted to inoculated plants showed declinio symptoms, and their water uptake was 0.02 ml/sec. Considering the results of the first experiment, it is possible that the mature trees could have already been affected by declinio, however at the time of this report none of those which were root grafted to the control plants had shown visible declinio symptoms.

The results of the second experiment suggest that declinio was transmitted to the 3-yr-old plants, causing death of almost all the inoculated plants by the time they reached 6-7 yr of age. This may indicate that declinio is caused by a transmissible pathogen.

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