Transmission Trials of Citrus Declinio using Various Inocula

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ABSTRACT. Trials were begun in 1987 in São Paulo State, Brazil to determine whether the disease can be experimentally transmitted to healthy trees by any methods other than root grafting. Various tissues and tissue extracts were used as inocula. By 1992 no positive results had been obtained.

Citrus declinio, which appears to be identical to blight (1,8,12), was first observed in Brazil in 1970 (7). It has since caused estimated losses of 10 million trees. In the southern part of São Paulo State the affected trees seldom die, but in the north, death can occur 2-3 yr after symptom appearance, possibly of the different soil and climatic conditions there.

The cause of declinio/blight remains a mystery, but the positive results of approach root grafting in Brazil (4,5,6) and elsewhere (2,3,9,10), and the failure of transmission by bud grafts (11), indicate that it may be caused by a root restricted organism.

Trials were begun in 1987 to determine whether declinio/blight could be experimentally transmitted using a variety of inocula. An orchard of some 470 5-7-yr old Natal sweet orange on Rangpur lime trees on a farm in southern São Paulo State was used. The inocula from declinio-affected trees and the methods of inocula used were as follows:

1. Side grafts of five root pieces per plant on roots.
2. Side-grafts of 10 root pieces per plant on branches.
3. Six to eight trunk bark discs (2 cm diameter) per plant on trunks.
4. Five root bark discs (2 cm diameter) per plant on roots.
5. Four to five cylinders (4.5 cm x 0.4 cm) from the trunk forced into the trunk of each tree.
6. 10-15 bud grafts per plant in branches.
7. 10-12 chip inoculations per plant, using young twig bark on twigs.
8. 10 leaf midrib pieces per plant on twigs.
9. Soil suspension: 100g soil in 200ml buffer phosphate-PBS pH 7.4 (0.01 M KH_2PO_4, 0.15 M NaCl) filtered, injected into the trunk.
10. Root and rootlet extracts: tissue of 100 g of roots and rootlets ground in 500ml buffer PBS (0.01 M KH_2 PO_4, M NaCl, pH 7.4), left for 24 hr then centrifuged at 10,000*** g for 10 min, supernatant concentrated by lyophilization, then injected into the trunk.

Ten plants in each row were inoculated with one of the above inocula. For controls, one uninoculated plant was left between each inoculated one, and in addition 10 control plants were prepared by inoculating trees with tissues from apparently healthy trees.

In a separate trial begun in 1989, 50 young Valencia sweet orange on Rangpur lime trees were each inoculated with 10-12 buds from diseased trees. For controls, a further 50 plants were inoculated with healthy buds. All the plants were planted out at the Bebedouro Experiment Station, in rows with inoculated and control plants alternately.

While some of the inoculated and control trees in the first trial began to show some declinio-like symptoms 3-5

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yr after inoculation, none gave a positive reaction with water uptake tests and the observed symptoms were mostly caused by *Phytophthora*. Regular observations were being conducted to determine whether any methods other than root grafting can be used to transmit declinio.

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