Transmission of the Stubborn Pathogen in Citrus by Leaf-Piece Grafts

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THE STUBBORN pathogen is not transmitted consistently by tissue grafts from affected plants (2, 5, 6). However, a high percentage of transmission can often be obtained by large side grafts even when transmission by buds from the same source is low (5). Routine indexing for the stubborn pathogen is done by side grafting citrus stems into 10 or more indicator seedlings, a laborious procedure requiring large amounts of tissue (1). The discovery, in 1966 (5), that the stubborn pathogen is readily transmitted by small grafts from some young expanding leaves of field-grown stubborn trees suggested that young leaves may be excellent sources of infection and that similar leaves from suspect shoots might be useful in indexing. This paper reports further studies on transmission of the stubborn pathogen by grafting small pieces of leaves of various ages and young stem tips from donor plants into sensitive indicator seedlings.

Experiments and Results

EXPERIMENT 1: TRANSMISSION FROM SWEET ORANGE LEAVES OF VARIOUS AGES AND YOUNG STEM TIPS TO SWEET ORANGE.—Donor plants were 10 glasshouse-grown Madam Vinous sweet orange seedlings infected with the C-189 isolate of the stubborn pathogen (4), which is free of known citrus viruses. In June 1968 we inoculated from these donors 110 young Madam Vinous seedlings by rectangular leaf pieces, 3–5 x 10–15 mm in size, collected at different stages of maturity and inserted under rectangular bark flaps cut at the top and both sides. Midveins, present in all graft pieces except those from lateral margins, were shaved lightly on the lower side, which was placed next to the cambial side of the bark flap. All indicator plants were kept in Riverside in a glasshouse at 25–33°C, cut back 1 month after inoculation, and trained to single shoots, which were examined periodically for 6 months. All positive reactions were detected as typical young-leaf symptoms of stubborn (3) within 3 weeks after cutting back. The categories of leaf inocula used and results are in Table 1. The stubborn pathogen was transmitted readily by a single tissue graft from a very young stubborn leaf, but was rarely transmitted by grafts from fully expanded leaves of Madam Vinous sweet orange.

Young, growing shoot tips, 8–10 mm long, from the same donor plants were defoliated, cut to a V-shape at the base, inserted (1 graft

TABLE 1. EFFECT OF AGE AND PORTION OF LEAF ON TRANSMISSION OF STUBBORN VIRUS BY LEAF-PIECE GRAFTS FROM MADAM VINOUS SWEET ORANGE DONORS TO MADAM VINOUS SEEDLINGS

Class,[a] approx. age of leaves and portions used	Approx. length (cm)	Grafts per plant	Indicator plants infected of 10 inoculated (%)
Newly unfolded, 2 days	1		
Blade, petiole		1	80
Young expanding, 1 week	3		
Blade base		1	80
Petiole		1	50
Fully expanded, mottled, 4 weeks	6		
Blade tip		1	0
Lateral margins, blade		2	10[b]
Blade base		1	10[b]
Petiole		1	0
Mature, mottled, 7 months	6		
Blade base		1	0
Petiole		1	0
Mature, symptomless, 7 months	8		
Blade base		1	10[c]
Petiole		1	0

a. Ten leaves of each class were used.
b. Transmission from lateral margin and basal portions of same leaf blade.
c. Transmission from a leaf formed before the donor plant was inoculated.

per indicator) under bark flaps of 10 glasshouse-grown Hinckley sweet orange seedlings, and maintained in the same way as the leaf-grafted seedlings. Four of the 10 plants showed typical young-leaf symptoms of stubborn (3) within 3 weeks after being cut back, thus indicating that the stubborn pathogen occurs near the tips of growing stems.

EXPERIMENT 2: TRANSMISSION FROM LEAVES OF 5 CITRUS VARIETIES TO GRAPEFRUIT.—Donor plants were 3 glasshouse-grown seedlings each of Cuban shaddock, Duncan grapefruit, Madam Vinous sweet orange, Mexican lime, and Rangpur lime. All were infected with the Minneola tangelo isolate of the stubborn pathogen (7), which is free of known citrus viruses. In November 1968, leaf pieces (about 3 x 15 mm, and including the midvein) from the blades of young expanding and young fully expanded leaves were grafted into T-cuts on 150 young glasshouse-grown Duncan grapefruit seedlings at Indio. The plants were cared for as in Experiment 1 but at slightly cooler temperatures. The results (Table 2) confirm those from earlier experiments and show that the stubborn pathogen was readily transmitted from plants of all 5 varieties by single leaf-piece grafts from young expanding leaves and produced typical mottled-leaf symptoms (7) in grapefruit seedlings. Grafts from recently expanded leaves of Cuban shaddock, Madam Vinous sweet orange, and Mexican lime only occasionally transmitted the pathogen. Under winter conditions the incuba-

tion period of the stubborn pathogen was longer than in experiment 1. No transmission was detected from expanded leaves of Duncan grapefruit or Rangpur lime.

Discussion and Conclusions

Our results indicate that a significant change in the host-pathogen relationship in a stubborn-infected leaf coincides approximately with the cessation of rapid expansion of the leaf blade. This change presumably could be due to inactivation of the pathogen, or to an absence or blockage of channels by which the pathogen normally moves within or away from donor tissue. The fact that the pathogen can be transmitted from occasional mature leaves (including one that first became infected after it was well developed) suggests that the stubborn pathogen persists in islands of tissue in some mature leaves of glasshouse-grown plants. It also persists in some shoots of field-grown plants while becoming apparently inactive in others (5).

We conclude from these and earlier experiments that young stem tips and very young leaves, but not mature leaves, may be useful for indexing, although young leaf-piece grafts from field trees often give a lower percentage of transmission than those from glasshouse-grown plants. The stubborn pathogen appears to be relatively abundant in tissues of rapidly growing young leaves on severely affected shoots of stubborn plants. It is more readily transmitted by small pieces of tissue taken from such leaves than from any other grafts of comparable size. Considering the relatively rapid upward movement of the stubborn pathogen in many of our vigorous young indicator plants, the failure of the pathogen to move through the xylem of girdled shoots (unpublished), and the results reported here, we suggest that the stubborn pathogen is present in the functioning phloem of many young growing leaves and shoot tips of affected trees.

TABLE 2. EFFECT OF DONOR VARIETY AND LEAF AGE ON TRANSMISSION OF STUBBORN VIRUS LEAF-PIECE GRAFTS FROM 5 DONOR VARIETIES TO DUNCAN GRAPEFRUIT SEEDLINGS

Donor variety and leaf-age class	Indicator plants infected[a] of 15 inoculated (%)
Cuban shaddock	
Young expanding	93
Young expanded	20
Duncan grapefruit	
Young expanding	67
Young expanded	0
Madam Vinous sweet orange	
Young expanding	93
Young expanded	7
Mexican lime	
Young expanding	93
Young expanded	20
Rangpur lime	
Young expanding	60
Young expanded	0

a. Based on mottle-leaf symptom 6 months after inoculation.

Literature Cited

1. CALAVAN, E. C. 1968. Stubborn, p. 35–43. *In* J. F. L. Childs (Chmn.), Indexing procedures for 15 virus diseases of citrus trees. U.S. Dept. Agr., Agr. Res. Serv., Agr. Handbook 333. U.S. Govt. Printing Office, Washington, D.C.
2. CALAVAN, E. C. 1968. A review of stubborn and greening diseases of citrus, p. 105–17. *In* J. F. L. Childs (ed.), Proc. 4th Conf. Intern. Organization Citrus Virol. Univ. Florida Press, Gainesville.
3. CALAVAN, E. C. 1969. Investigations of stubborn disease in California: Indexing, effects on growth and production, and evidence for virus strains, p. 1403–12. *In* H. D. Chapman (ed.), Proc. 1st Intern. Citrus Symp. Vol. 3. Univ. Calif., Riverside.
4. CALAVAN, E. C., and CHRISTIANSEN, D. W. 1961. Stunting and chlorosis induced in young-line citrus plants by inoculations from navel orange trees having symptoms of stubborn disease, p. 69–76. *In* W. C. Price (ed.), Proc. 2nd Conf. Intern. Organization Citrus Virol. Univ. Florida Press, Gainesville.
5. CALAVAN, E. C., ROISTACHER, C. N., and CHRISTIANSEN, D. W. 1968. Distribution of stubborn virus in trees of Citrus sinensis and C. paradisi at different seasons, p. 145–53. *In* J. F. L. Childs (ed.), Proc. 4th Conf. Intern. Organization Citrus Virol. Univ. Florida Press, Gainesville.
6. CARPENTER, J. B., and CALAVAN, E. C. 1969. Effects of stubborn virus on young Valencia orange trees, p. 1505–15. *In* H. D. Chapman (ed.), Proc. 1st Intern. Citrus Symp. Vol. 3. Univ. Calif., Riverside.
7. OLSON, E. O. 1969. Mottled-leaf symptom on index plants graft-inoculated from citrus trees showing various symptoms of stubborn disease, p. 1413–20. *In* H. D. Chapman (ed.), Proc. 1st Intern. Citrus Symp. Vol. 3. Univ. Calif., Riverside.