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## A Graft-Transmissible Stunting Factor in Citrus

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CONSIDERABLE STUNTING was found in Sexton tangelo and Madam Vinous sweet orange seedlings used for indexing 30 trees for stubborn virus in 1966. Some leaves on certain stunted indicator plants were mildly to moderately mottled—similar to those reported on mildly affected stubborn plants (2)—but lacked diagnostic symptoms of stubborn disease (1, 5). This paper reports the results of experiments done in 1967 and 1968 to determine the persistence of the stunting factor in certain trees and its occurrence in others.

### *Methods and Materials*

EXPERIMENT 1: JULY 1967.—Donors were 23 trees, mostly normally vigorous and healthy, suspected of infection by the stunting factor on the basis of the 1966 tests. Twenty 4–6-cm bud sticks from each tree were side-grafted (1, 3) into 10 uniform seedlings of Madam Vinous sweet orange (MV) or Sexton tangelo (ST) in modified UC potting mix (4) in 1-gallon containers. Controls were 10 self-inoculated ST and MV and 10 ST and MV inoculated with severe stubborn sweet orange budwood. Seedlings were cut back to about 25 cm of stem, and all except the top 4 leaves were removed. Grafts were

wrapped with polyvinyl chloride budding tape, and each plant was enclosed in a polyethylene bag firmly secured to the container. Inoculated plants were randomized on a greenhouse bench and shaded with dark brown paper. The paper and polyethylene bags were removed in 14 days. Each plant was trained to a single indicator shoot and kept in a greenhouse where maximum temperatures averaged 38° and minimum temperatures 22°C.

Height and weight of all new shoots were measured 5 months after inoculation, and the plants were rated for leaf mottle, leaf size, and internodal length.

EXPERIMENT 2: MAY 1968.—Forty donor trees were selected for indexing on the basis of earlier results or for comparison with other trees of the same varieties. Each of 22 trees was indexed in the same way as in experiment 1 into 8 seedlings each of MV, ST, and Duncan grapefruit. Seventeen trees were indexed only in ST, and 1 was indexed only in MV. Controls were 2 sets—8 seedlings each—of self-inoculated ST, the same number of self-inoculated MV, and 8 plants each of ST and MV inoculated with stubborn virus from severely affected sweet orange. Height and weight of new shoots on all plants

were measured 2½ months after inoculation and again 4 months later on groups of plants that were visibly different from the controls. Maximum temperatures for the 60 days following inoculation averaged 36°, and minimum temperatures 21°C. Temperatures for the 60 days following the first cutback in July averaged 39° and 21°C, high and low, respectively. Total hours above 35°C for the first and second 60-

day periods were 184 and 288 respectively.

### Results

EXPERIMENT 1: JULY 1967 TESTS.— Significant stunting occurred in 9 of the 13 groups of ST and in 6 of the 12 groups of MV seedlings (Table 1). The maximum weight reductions found in ST and MV seedlings inoculated from certain donor trees were 44 and 27 per cent, respectively;

TABLE 1. AVERAGE WEIGHT AND PERCENTAGE REDUCTION OF 10 SEXTON TANGELO OR MADAM VINOUS SEEDLINGS INOCULATED JULY 1967 FROM DONOR TREES THAT HAD ABNORMAL INDEX RESULTS IN 1966

Donor selection	Sexton tangelo		Madam Vinous orange	
	Weight (g)	Weight reduction <sup>a</sup> (%)	Weight (g)	Weight reduction <sup>a</sup> (%)
Wilking mandarin	42.0 <sup>**</sup>	22		
Clementine mandarin			45.6 <sup>*</sup>	19
Frost Valencia orange 1	52.0	4		
Frost Valencia orange 2	49.5	9		
Cutter Valencia orange	45.4 <sup>*</sup>	16		
Olinda Valencia orange	40.4 <sup>***</sup>	25		
Fisher navel orange 1 <sub>b</sub>			52.0	6
Fisher navel orange 2 <sub>b</sub>			42.4 <sup>*</sup>	24
Red Blush grapefruit			45.1 <sup>*</sup>	19
Pomero trifoliate orange			48.7	12
Orlando tangelo	47.8	12		
Prior Lisbon lemon 1	40.2 <sup>***</sup>	26	47.1	16
Limoneira Lisbon lemon	30.3 <sup>***</sup>	44		
Monroe Lisbon lemon			43.1 <sup>**</sup>	23
Walker Lisbon lemon 1			45.8 <sup>*</sup>	18
Walker Lisbon lemon 2			40.1 <sup>**</sup>	27
Ross Eureka lemon 1			52.6	6
Ross Eureka lemon 2			51.2	8
Meek Eureka lemon			46.9	16
Cook Eureka lemon 1	61.3	0		
Cook Eureka lemon 2	43.8 <sup>**</sup>	19		
Cook Eureka lemon 3	32.9 <sup>***</sup>	39		
Cook Eureka lemon 4	31.9 <sup>***</sup>	41		
Cook Eureka lemon 5	30.5 <sup>***</sup>	44		
Stubborn-infected control	21.5 <sup>***</sup>	60	15.5 <sup>***</sup>	72
Self-grafted control	54.1		55.6	

a. Percentage below self-grafted control seedlings.

b. Tree developed typical stubborn symptoms and inverse coloration of fruit in 1968.

<sup>\*</sup>, <sup>\*\*</sup>, <sup>\*\*\*</sup> Significantly less than normal controls at the 0.05, 0.01, and 0.001 levels, respectively.

positive stubborn controls were more severely affected. Reduction in height of inoculated plants was generally proportional to reduction in weight (Fig. 1), and all plants of significantly affected groups usually were shorter and weighed less than the self-inoculated controls.

EXPERIMENT 2: MAY 1968 TESTS.— Significant stunting occurred in ST or MV seedlings graft-inoculated from 16 of the 40 trees indexed. Reduction in weight was as high

as 66 per cent on ST. Data are given in Table 2 for all groups that showed significant stunting and for a few used for varietal comparisons. MV seedlings generally were less severely stunted than ST seedlings, and Duncan grapefruit was not significantly affected by inocula from any of the 22 trees indexed to them. Nine groups of ST were stunted 30 per cent or more. Stunting was severest on ST seedlings inoculated by grafts from certain lemon donors.



FIGURE 1. Sexton tangelo seedlings. Left. Self-grafted controls. Right. Stunted plants (30 per cent shorter, 44 per cent less weight) graft-inoculated from apparently healthy Limoneira Lisbon lemon.

The stunting factor transmitted from 11 of 14 lemon trees significantly retarded ST seedlings. A stunting factor was transmitted also from certain trees of Valencia and navel orange, mandarin, tangelo, and grapefruit.

Chlorosis and mild mottling were sometimes associated with stunting in ST and MV. Often 1-4 plants in a group of 7 or 8 showed mottled, chlorotic, or abnormally pale green immature leaves. These symptoms usually disappeared as the leaves matured, but leaf mottle persisted in some of the more severely affected

ST seedlings. Most of the MV and ST seedlings lacked the small, strongly mottled leaves and very short internodes consistently present in the stubborn-positive controls.

Periodic indexing of certain donor trees into ST or MV over a period of 3 years gave somewhat inconsistent results. Eleven trees that were suspect in 1966 were re-indexed in 1967 and 1968; 5 indexed positive for stunting both years, 5 positive in only 1 of the 2 years, and 1 indexed negative both years.

TABLE 2. AVERAGE WEIGHT AND PERCENTAGE REDUCTION OF 7 OR 8 SEXTON TANGELO OR MADAM VINOUS SEEDLINGS INOCULATED MAY 1968 FROM SELECTED FIELD TREES

Donor selections	History <sup>a</sup>	Sexton tangelo		Madam Vinous orange	
		Weight (g)	Weight reduction (%) <sup>b</sup>	Weight (g)	Weight reduction (%) <sup>b</sup>
Campbell Valencia orange 1	C	35.9	15		
Campbell Valencia orange 2	B	34.9*	18		
Campbell Valencia orange 3	D	33.1*	22		
Olinda Valencia orange	C	28.2*	44		
Gillette navel orange	A	30.4**	28	21.0*	30
Red Blush grapefruit	A	30.6**	28	29.2	3
Prior Lisbon lemon 1	A	35.8	15	27.7	9
Prior Lisbon lemon 2	A	24.3***	43	19.4	34
Prior Lisbon lemon 3	A	19.4***	54	16.0***	47
Prior Lisbon lemon 4	C	12.2***	66	25.5	15
Prior Lisbon lemon 5	B			27.4	9
Limoneira Lisbon lemon 1	B	32.8	22		
Limoneira Lisbon lemon 2	A	28.7*	32		
Limoneira Lisbon lemon 3	B	26.1**	38		
Walker Lisbon lemon	A	32.6*	23		
Ross Eureka lemon 2	C	31.8*	25		
Ross Eureka lemon 1	C	24.2***	43		
UCLA Eureka lemon	B	32.5*	23		
Cook Eureka lemon 1	E	33.1*	22	26.1	13
Cook Eureka lemon 4	A	31.6**	25		
Stubborn-infected control		13.8***	65	16.1***	52
Self-inoculated control 1		42.3		30.8	
Self-inoculated control 2		42.2		29.4	

a. A, significant stunting in the 1967 index; B, some reaction in 1966 index; C, not previously indexed to this indicator; D, stubborn suspect donor; E, stunting not significant in 1967 index.

b. Percentage below self-grafted control seedlings.

\*, \*\*, \*\*\* Significantly less than normal controls at the 0.05, 0.01, and 0.001 levels, respectively.

*Discussion*

Inasmuch as the stunting factor transmitted from frequently indexed, vigorous, mostly normal appearing trees caused reactions resembling those considered to be mild stubborn disease (2) in stubborn-sensitive indicators, it may belong to the stubborn complex. However, this can be determined only by more definitive experiments with stubborn virus and the stunting factor. One of the donors (Fisher navel 2) developed stubborn symptoms only a year after a stunting factor was transmitted from it in the 1967 index. Another donor (Red Blush grapefruit) is the scion parent of several stubborn trees in another orchard but has not itself developed symptoms. A third donor (Campbell Valencia 3) was indexed because it was suspected of having stubborn disease.

Stunting of sensitive seedlings might be caused by a latent or apparently latent virus in some field trees or possibly by growth regulators or other materials present in inoculum tissue. Possibly not all the

stunting we observed was due to a single cause.

Stunting does not appear to depend on the variety of the donor plant. Grafts from certain Lisbon lemon, Eureka lemon, navel orange, Valencia orange, and grapefruit donor trees caused no reaction, whereas those from other donors of the same varieties caused highly significant stunting.

Warm temperatures apparently favor the stunting of inoculated indicator seedlings. Little stunting was noted under relatively cool growing conditions (May–July 1968), but strong reactions occurred later at higher temperatures in the same plants. We have observed similar effects of temperature on symptom development in plants inoculated with stubborn virus, as has been reported by Olson and Rogers (6).

The importance of the stunting factor transmitted from many normal appearing donors and its effect on their bud progeny is unknown and probably can be determined only by long-term experiments with progeny trees on various rootstocks in different climates.

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