

# TRANSMISSION OF A GROWTH-RETARDING FACTOR IN EUREKA LEMON TREES<sup>1</sup>

E. C. Calavan and L. G. Weathers<sup>2</sup>

University of California Citrus Experiment Station,  
Riverside, California

## INTRODUCTION

Poor growth and premature deterioration of Eureka lemon trees in several countries have been discussed by various workers (1, 5, 9, 10, 14, 16, 19, 23) during the past forty years. Lemon tree decline has been critical in California, where Eureka has been the principal variety grown. Some trees of other varieties, including Lisbon and Villafranca, have been similarly affected. Decline problems are varied and often complex. It is generally recognized that nutritional and climatic factors, as well as replant problems including nematodes and root diseases, are factors contributing to poor tree growth in many locations. These will not be discussed here.

This report is related principally to virus transmission experiments on lemon trees and to resultant effects on tree growth. A brief statement of preliminary results from some of these experiments was made in 1954 (7).

The purposes of the experiments described here were 1) to determine whether or not any transmissible factor is generally present in lemon trees declining from an unknown cause, 2) to evaluate specific and general effects of any transmitted factor, 3) to identify any transmissible agent found, and 4) to provide a basis for the development of disease control.

## MATERIALS AND METHODS

**Experimental Trees.** All trees used in these experiments were grown in commercial nurseries on seedling rootstocks. Trunk bases of most scions were between  $\frac{1}{2}$  and  $\frac{1}{3}$  inch in diameter at the time of planting. Lemon scions of the trees used in the first planting (spring, 1948) were approximately one year old; the orange scions were nearly two years old. The second planting (October, 1948) consisted of trees with 7-month-old scions.

In April and May, 1948, the following trees were planted near Oxnard, California, in one third of a 10-acre block on which citrus had not previously grown: 114 old-line Allen Eureka lemon, *Citrus limon* (Linn.) Burm., on sweet orange, *C. sinensis* (Linn.) Osbeck, rootstock; 500 old-line Eureka on grapefruit, *C. paradisi* Macf.; 16 UCLA No. 4 nucellar-line Eureka on grapefruit; 50 Cascade Washington Navel sweet orange on sour orange, *C. aurantium* Linn.; and 50 trees of Cascade Valencia sweet orange on sour orange.

In October 1948, the rest of the block, except 1 acre reserved for related projects, was planted to the following trees: 108 old-line Ledig Lisbon lemon (USDA, Shamel No. 12,006) on sweet orange, 108 nucellar-line Frost Eureka lemon on sour orange, and 108 nucellar-line Frost Eureka from each of two parents on sweet orange rootstock.

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<sup>2</sup> Associate Plant Pathologist and Assistant Plant Pathologist, respectively, Citrus Experiment Station, Riverside.

**Sources of Inoculum.** Diseased old-line lemon trees growing in Riverside, Ventura, or Santa Barbara counties were used as sources of buds and "spur buds" for tissue-graft inoculations of experimental trees. For convenience these sources are designated by number.

Sources of inoculum used in the spring planting were as follows: Nos. 1-6, collapsed (wilted) old-line Eureka trees; Nos. 7, 8, and 17, declined old-line Eureka trees; and No. 9, declined old-line open-type Lisbon trees. Four of the collapsed trees (Nos. 1, 3, 5, and 6) died soon after budwood was collected from them.

For the October planting, sources of inoculum were the following: collapsed trees, Nos. 2 and 4, used in the spring planting; Nos. 10-16, collapsed old-line Eureka trees; and Nos. 21-26, old-line Eureka trees with very severe shell bark (dry bark).

Two of the total of 13 collapsed source trees had shell bark on their trunks. The other 11 trees were from parent lines known to be susceptible to shell bark. Starch reserves in the young wood of the rootstock trunks of all collapsed inoculum source trees were found to be severely depleted, but those of the declined and of the non-collapsed shell-bark inoculum sources were nearly normal.

**Plot Designs and Inoculation Procedures.** Tree spacing in the spring planting was 11 feet in the rows, with 12 feet between the rows, except for a 30-foot box drive near the middle of the plot. One hundred seventy (23.3 per cent) of the trees, representing all varieties planted, were kept as noninoculated controls in 33 groups of 2 to 10 trees each. All other trees were inoculated in groups of 2 to 5 trees each. To prevent crowding, almost three fourths of the lemon trees in the spring planting were removed in 1952, with very little change in the ratio of inoculated trees to controls.

Tree spacing in the fall planting was 24 feet in the rows, with 24 feet between the rows, except for two 30-foot box drives. Each of the four stionic groups comprising this 16-row planting filled 4 rows, which were divided into two strips, of 2 rows each, located about 200 feet apart. One hundred twelve control trees were regularly placed throughout this planting in such a way that each inoculated tree was within 34 feet of a paired noninoculated tree of the same stionic combination. In the northern portion of the October planting, 1 multiple-inoculum-source plot, and 1 plot for each of inoculum sources 2, 4, 10, 11, 12, 13, and 14 were established. Each plot was 1 or 2 trees wide and extended across all 16 rows. Twenty-four trees in the multiple-source plot received one bud from each of sources 2, 4, and 14. Inoculations from source 14 also were made in locations scattered throughout this planting. In the southern portion of the planting, trees inoculated from sources 21 to 26 were planted between the controls. Other trees inoculated from sources 14, 15, or 16 were also included in this area.

These plot designs permitted adaptation of cultural practices to the needs of each stionic group and, by providing many pairs of inoculated trees and controls throughout the planting, greatly reduced the effect of variable soil conditions on evaluation of results.

Inoculations were made by inserting two shield buds and one "spur bud," with a short woody branch, into T-cuts through the bark of the scion trunk or primary branches. Buds were wrapped with budding cloth for about 3 weeks and were not allowed to grow into shoots. Survival was carefully checked and rebudding was done on any tree where more than one bud died. With the exception noted elsewhere, each individual inoculated tree was budded from only one inoculum source. All inoculations were completed prior to the development of the first flush of growth after planting.

**Measurement Data.** Measurements of tree growth were initiated in 1950 after it became apparent that neither the lemon nor the orange trees were showing any visible reaction to the inoculations. Trunk-circumference measurements, to the nearest millimeter, 6 inches above the bud union on each tree were made annually with flexible steel tapes during the winter period when trees were semidormant. The first measure-

ments were made in January 1950, after completion of inoculations and prior to any measurable effect of inoculation on growth. The trunks of most of these trees have remained nearly round, without apparent differences in shape between inoculated trees and controls. Webber (27, 28) found cross sections of citrus trees to be suitable indices of their total growth. All measurements have therefore been converted into cross-section areas to permit more critical analyses of growth differences among trees of various diameters.

## RESULTS

Visible effects on tree growth were first observed in 1953 in several nucellar-line Frost Eureka trees. Prior to that time the only symptoms noted were crinkly-leaf psorosis transmitted from source 10. Affected trees inoculated from various sources showed subnormal shoot growth, were abnormally open because of poor growth and premature defoliation, and were generally smaller and more chlorotic than controls. The chlorosis appeared to be a reaction to soil salts aggravated by inoculation, especially in Frost Eureka on sour orange rootstock. Exposed fruits were conspicuous on many inoculated nucellar-line Eureka trees. Since 1953, size differences between controls and inoculated trees (fig. 1) have increased continuously, and the apparent overall condition of some inoculated trees has fluctuated between good and poor.

No symptoms of shell bark or collapse have as yet been caused by the inoculations, and thus far there has been no evidence to indicate transmittal of any factors causing lemon sieve-tube necrosis or sour orange rootstock necrosis, described by Schneider (22, 23).

Data derived from trunk measurements of the past eight years have been subjected to analysis of covariance for pairs (24) according to types of trees, regardless of inoculum source, in order to adjust for any significant effect of January 1950 size upon later growth. Data for each year, beginning in January 1950 and continuing for the seven-year period 1951-1957, inclusive, are presented in table 1, which shows the actual variations among paired trees. Analyses according to tree type were confined to pairs of sister trees. Inoculated and control trees of each pair were planted on the same day in adjacent positions in the same row, and since that time, or until their removal, they have received comparable treatment. Pairs which failed to meet this stand-

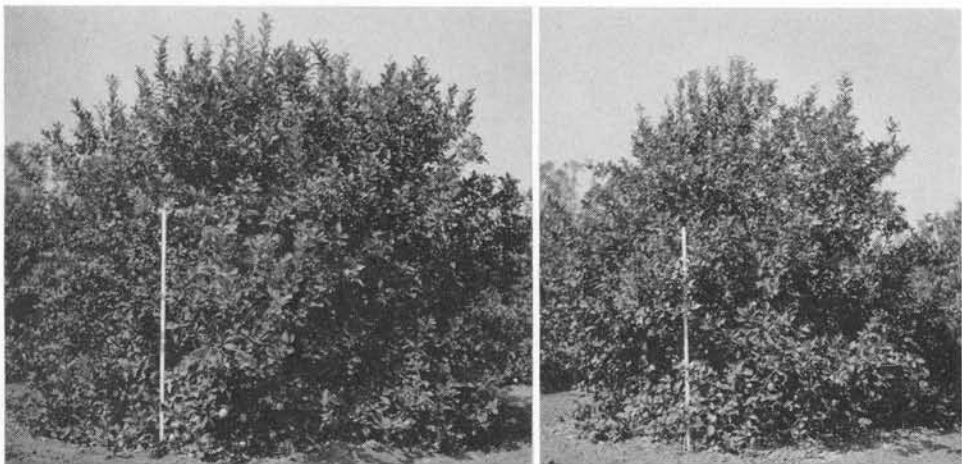


Fig. 1. Nucellar-line Frost Eureka lemon trees on sour orange rootstock (listed as pair 12 in table 1). Left, noninoculated control. Right, tree inoculated in 1949 from source No. 14. Stakes 6 feet high. (Photographed separately 25 feet from trunks, October 1957, by R. G. Platt.)

ard were omitted. In making up pairs the right or left position for the inoculated tree was chosen at random whenever there was a choice. A separate analysis of left versus right compared to the controls used in group F, table 2, showed no significant difference, at the 5 per cent level, between right and left positions.

Results of 55 analyses of covariance of data arranged like those in table 1, plus seven analyses of variance for 1950 size, are condensed in table 2. Growth retardation of inoculated nucellar-line Frost Eureka trees on sweet orange and on sour orange rootstocks has been significant each year since 1951 for the F group and since 1953 for the E group, and is highly significant in both groups for the entire 1950-1957 period.

The inoculated nucellar-line UCLA Eureka trees on grapefruit rootstock have shown the greatest stunting of any group. Inoculum source Nos. 1 and 3, used singly in these trees, were not available for use on the Frost Eureka. Even with only four pairs available for analysis, the effect was significant in certain years and for the total period.

There was a significant effect of inoculation on growth in the sweet orange trees on sour orange rootstock for two years (1953-1955). With only four valid pairs available for analysis, growth differences for the 1950-1956 period are not significant. The mean differences plotted in figures 2 and 3 show similar trends between oranges and nucellar-line Frost Eureka lemons. Three of the four inoculated sweet orange trees received grafts from source Nos. 3 or 4, which were collapsed old-line Allen Eureka; the other tree was inoculated from source No. 9, a declining old-line Lisbon lemon. Further com-

Table 1. SIZE OF TRUNK CROSS SECTIONS IN 1950 AND COMPARATIVE INCREMENTS OF GROWTH 1950-1957 FOR 15 PAIRS OF INOCULATED AND NONINOCULATED NUCELLAR-LINE FROST EUREKA LEMON TREES ON SOUR ORANGE ROOTSTOCK<sup>1</sup>

Tree pair	Trunk size January 1950†		Growth increment 1950-1957†	
	Control trees	Inoculated trees	Control trees	Inoculated trees
	cm <sup>2</sup>	cm <sup>2</sup>	cm <sup>2</sup>	cm <sup>2</sup>
1.....	2.0	1.3	139.7	124.7
2.....	2.0	2.6	133.1	106.4
3.....	1.1	1.3	157.9	128.6
4.....	2.2	3.8	141.5	135.9
5.....	1.9	3.7	124.1	142.1
6.....	1.7	2.4	152.4	120.5
7.....	1.1	1.2	154.4	138.5
8.....	2.7	3.0	126.5	96.7
9.....	1.1	1.0	175.4	142.7
10.....	1.1	1.1	162.9	149.5
11.....	0.7	1.5	130.5	113.4
12.....	0.9	0.9	154.6	123.3
13.....	3.2	3.1	126.0	108.8
14.....	1.1	1.1	140.6	112.6
15.....	0.9	1.2	153.9	123.0
Total.....	23.7	29.2	2173.5	1866.7
Mean.....	1.58	1.95	124.45	144.90
Mean difference‡.....	-37*			
Adjusted mean§.....			122.01	147.15
Adjusted mean difference‡.....			24.95***	

<sup>1</sup> Example of kind of data used in preparation of table 2.

† Calculated from circumference measurements 6 inches above the bud union.

‡ Stated as mean of controls less mean of inoculated trees. Single and triple asterisks indicate significance at the 0.05 and 0.001 levels, respectively.

§ Adjusted for significant influence from 1950 size, according to analysis of covariance for blocks.

Table 2. SUMMARY OF TRUNK CROSS-SECTION DATA FROM SEVEN GROUPS OF CITRUS TREES SHOWING MEAN SIZES OF CONTROLS IN JANUARY 1950, MEAN ANNUAL AND MEAN TOTAL GROWTH INCREMENTS OF CONTROLS FOR A SEVEN-YEAR PERIOD, AND MEAN DIFFERENCES IN SIZE AND GROWTH INCREMENTS BETWEEN CONTROLS AND INOCULATED TREES

Variety <sup>a</sup>	Tree pairs used	Class <sup>b</sup>	Size <sup>c</sup> Jan. 1950	Trunk cross section in square millimeters							
				Growth increment <sup>e</sup>							
				1950-51	1951-52	1952-53	1953-54	1954-55	1955-56	1956-57	1950-57 <sup>d</sup>
A.....	4	C $\bar{x}$	743	920	1338	1467	1680	1978	1495	.. <sup>e</sup>	8,878 <sup>e</sup>
		$\bar{d}$	43	50	270	362*	348*	263	.. <sup>e</sup>	1,573 <sup>e</sup>	
		L.S.D.	NS	NS	NS	NS	258	296	NS	.. <sup>e</sup>	NS
B.....	21	C $\bar{x}$	1110	1567 <sup>f</sup>	1325	1667	1630	1862	1414	1705	11,209
		$\bar{d}$	83	43 <sup>f</sup>	31	71	-10	35	75	37	360
		L.S.D.	NS	NS	NS	NS	NS	NS	NS	NS	NS
C.....	22	C $\bar{x}$	566	1145	1727	1941	2078 <sup>f</sup>	2400	1968	2025	13,272
		$\bar{d}$	-12	12	-24	65	-15 <sup>f</sup>	158	119	193	484
		L.S.D.	NS	NS	NS	NS	NS	NS	NS	NS	NS
D.....	4	C $\bar{x}$	1365	2240	1990	2545	2177	3233	2508	2958	17,650
		$\bar{d}$	67	392	440*	710*	492**	988	440	873	4,335*
		L.S.D.	NS	NS	295	471	460	NS	NS	NS	NS
E.....	23	C $\bar{x}$	323	1129 <sup>f</sup>	1856 <sup>f</sup>	2287	2462 <sup>f</sup>	3186	2266	2340	15,613
		$\bar{d}$	28	18 <sup>f</sup>	106 <sup>f</sup>	347***	363***	653***	415***	369***	2,443***
		L.S.D.	NS	NS	NS	171	242	280	262	320	913
F.....	24	C $\bar{x}$	363	1316 <sup>f</sup>	1989	2274 <sup>f</sup>	2658	3090 <sup>f</sup>	2360	2404	16,078
		$\bar{d}$	-6	83 <sup>f*</sup>	116*	255 <sup>f***</sup>	310***	475 <sup>f***</sup>	431***	307**	1,951***
		L.S.D.	NS	80	100	206	263	291	271	234	1,103
G.....	15	C $\bar{x}$	158	887 <sup>f</sup>	1823 <sup>f</sup>	2165 <sup>f</sup>	2347 <sup>f</sup>	3040	2273	2102	14,715 <sup>f</sup>
		$\bar{d}$	-37*	34 <sup>f</sup>	180 <sup>f*</sup>	415 <sup>f***</sup>	488 <sup>f***</sup>	503 <sup>f***</sup>	452**	195*	2,495 <sup>f***</sup>
		L.S.D.	36	NS	149	325	132	387	345	152	1,435

<sup>a</sup> A = Washington Navel orange on Florida sour orange and Valencia orange on Florida sour orange; B = old-line Eureka lemon on grapefruit and old-line Eureka lemon on sweet orange; C = old-line Ledig Lisbon lemon on sweet orange; D = young-line UCLA No. 4 Eureka lemon on grapefruit; E = young-line Frost (del Tio) Eureka lemon on sweet orange; F = young-line Frost (CES) Eureka lemon on sweet orange; G = young-line Frost (del Tio) Eureka lemon on sour orange rootstock.

<sup>b</sup> C $\bar{x}$  = mean for controls;  $\bar{d}$  = mean for controls less mean for inoculated trees; L.S.D. is at level indicated by asterisks. *Note:* To obtain mean for inoculated trees use (C $\bar{x}$  -  $\bar{d}$ ).  
<sup>c</sup> Calculated from wintertime trunk circumference measurements 6 inches above the bud union. Single, double, and triple asterisks indicate significance at the 0.05, 0.01, and 0.001 levels, respectively. NS indicates no significance at the 0.05 level.

<sup>d</sup> Total of seven annual increments, except for minor discrepancies from adjustment for regression (<sup>f</sup>).

<sup>e</sup> "A" group removed in 1956.

<sup>f</sup> Figure adjusted for regression because 1950 size had a significant effect, at 0.05 level, on growth increment.

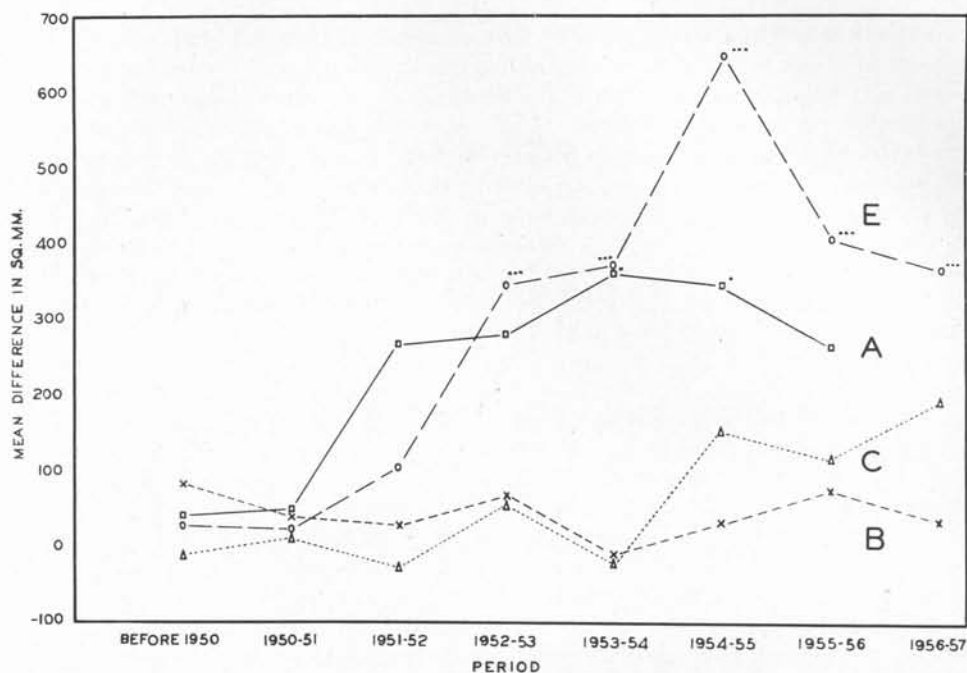


Fig. 2. Trends of mean differences expressed as annual growth increments for trunks of control trees less than for inoculated trees of four citrus varieties. (Derived from data in table 2. A = Washington Navel orange on Florida sour orange and Valencia orange on Florida sour orange; B = old-line Eureka lemon on grapefruit and old-line Eureka lemon on sweet orange; C = old-line Ledig Lisbon lemon on sweet orange; and E = young-line Frost (del Tio) Eureka lemon on sweet orange.)

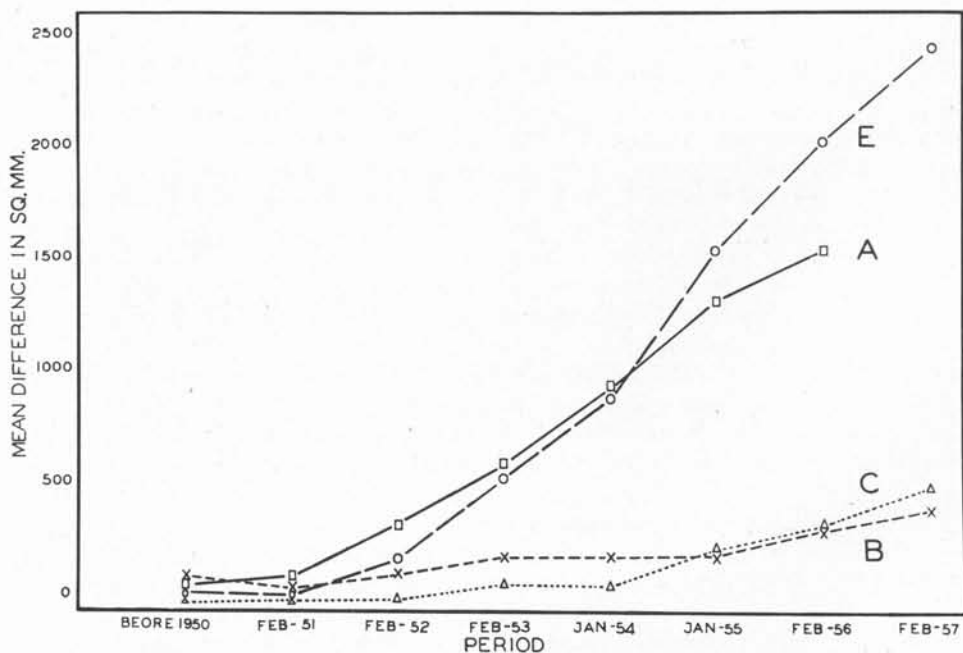


Fig. 3. Cumulative mean differences expressed as total growth increment for trunks of control trees less than for inoculated trees of the four citrus varieties described in figure 2.



parisons for 1950-1956, using all available sweet orange trees, show that average growth with 16 inoculated navel trees was 22 per cent less than with 5 control trees, and that average growth with 18 inoculated Valencia trees was 9 per cent less than with 6 control trees. The over-all average difference between inoculated trees and controls was 15 per cent.

Old-line Eureka and Ledig Lisbon lemon trees were not significantly retarded by inoculations.

Additional analyses of covariance for pairs were made for groups of Frost Eureka lemon trees according to inoculum source. For these analyses each pair was composed of trees of the same stionic combination, planted adjacent to each other in the same row or diagonally across from each other in adjacent rows. Results of these analyses for the 1950-1957 period are shown in table 3. Inoculum from each source caused significant growth retardation. The group with trees inoculated from source No. 15 contains two inferior controls and misses significance for 1950-1957, but does show a significant effect for certain single years not listed in table 3.

Growth retardation in inoculated nucellar-line Eureka trees averages about 15 per cent for the 7-year period. For these trees this average dwarfing effect is approximately equivalent to the average growth per tree for controls (table 2) during 1956.

Indexing procedures to determine, so far as possible, the viruses present in numerous inoculated trees, controls, inoculum source trees used in these experiments, and numerous closely related selections were started in related experiments in 1948. From

Table 3. COMPARATIVE MEANS FOR SIZE IN JANUARY 1950 AND FOR GROWTH INCREMENTS OF TRUNK CROSS SECTIONS<sup>a</sup> OF PAIRED NONINOCULATED AND GRAFT-INOCULATED YOUNG-LINE FROST EUREKA LEMON TREES<sup>b</sup> FROM JANUARY 1950 TO FEBRUARY 1957, ACCORDING TO SOURCES OF INOCULUM

Inoculum source (no.) <sup>c</sup>	Tree pairs used (no.)	Trunk cross sections in square millimeters				Av. retardation in inoculated trees <sup>d</sup>		L.S.D. <sup>e</sup>
		Size, January 1950		Growth increment, 1950-57				
		Control trees	Inoculated trees	Control trees	Inoculated trees	per cent	sq. mm.	
2.....	12	295	298	14,805	12,141	18	2,664***	1564
4.....	12	304	263	16,662	13,971	15	2,745**	2441
10.....	11	266	228	14,791	11,651	20	3,140***	2599
11.....	12	297	335	16,492	14,635	10	1,851*	1351
12.....	6	330	325	16,088	13,098	18	2,990*	1922
13.....	11	327	295	16,212	14,249	11	1,963*	1560
14.....	20	228	204	14,413	12,727	11	1,686***	1498
15.....	10	317	332	15,124	13,200	10	1,924	.....
16.....	13	278	313	15,396 <sup>f</sup>	12,354 <sup>f</sup>	17	3,042****	2180
21.....	8	281	315	15,390	12,268	20	3,122**	2405
22.....	10	340	381	15,758	13,449	15	2,309***	1588
23.....	9	243	309	14,632	12,113	17	2,519**	2247
24.....	10	290	335	15,552	12,982	16	2,570**	2100
25.....	6	113	137	14,423 <sup>f</sup>	11,797 <sup>f</sup>	15	2,626****	2401
26.....	4	145	138	16,000	12,595	21	3,405*	1995
2 + 11 + 14.....	10	299	303	15,728	13,425	14	2,303**	1676

<sup>a</sup> Calculated from circumference measurements 6 inches above bud union.

<sup>b</sup> Groups E, F, and G in table 2, plus other trees of the same combinations.

<sup>c</sup> Source Nos. 2, 4, 10, 12, 13, and 15 were wilted old-line Eureka trees without shell-bark symptoms; Nos. 21 to 26 had very severe shell bark without wilting.

<sup>d</sup> Single, double, and triple asterisks indicate significance at the 0.05, 0.01, and 0.001 levels, respectively.

<sup>e</sup> L.S.D. at levels indicated by asterisks.

<sup>f</sup> Indicates figure has been adjusted for regression effect from tree size in 1950.









