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Evidence for Strain Differences and Stunting with Exocortis Virus

RANGPUR LIME DISEASE, described by Olson (8) in 1952, is generally believed to be caused by the same virus, or virus complex, as that which causes exocortis (7, 10, 11). Therefore the name exocortis is used here irrespective of host variety.

Reports from various countries have indicated that exocortis virus causes stunting (1, 6, 7, 9, 12). Either exocortis virus from some sources evidently causes more stunting than that from other sources or some trifoliate orange (*Poncirus trifoliata* (L.) Raf.) and Rangpur lime (*Citrus limonia* Osbeck) rootstocks are less stunted by exocortis than others (1, 5).

This paper reports effects of exocortis virus upon the growth of 16 stionic combinations of citrus and presents evidence for the existence of strains of exocortis virus.

Materials and Methods

Experimental trees were propagated by grafting buds of parent trees free of exocortis virus onto seedling rootstocks; some were inoculated and others maintained as controls. Inoculations were made by grafting 2 or more buds from exocortis-infected trees (exocortis sources) into an experimental tree, care being taken that each tree received buds from only one source; exceptions were a few trees in Plot 1, which received buds from more than one source. Growth from buds used for inoculation was suppressed.

Plot 1, described previously (2), was set out in 1948 near Oxnard.

Plot 2, planted near Ventura in 1953, contains 3 6-tree blocks. Three trees of each block were inoculated in 1955, the others were left as controls.

Plot 3 contains 66 trees planted in two rows near Ventura in 1954. Alternate trees in almost 3/4 of the plot were inoculated in 1955. Alternate trees in the other portion were grafted with buds from trees presumed to be free of viruses. The other half of the trees in the plot were left as controls.

Plot 4 consists of greenhouse-grown plants set out in 1956 in Riverside, with 24′ x 10′ spacing. Half of the plants had been inoculated in 1955; some of the controls, following propagation, had been grafted with buds from trees presumably free of viruses. Growth from buds inserted after propagation was suppressed.

Plot 5 contains trees propagated in 1957 and planted in 1958 in Riverside, with 10′ x 12′ spacing. Each of the 2 varieties used as tops in this plot had been grown from a single parent tree. Half of the trees were inoculated in 1957; the remaining noninoculated trees were kept as controls.

Parent trees used as sources of buds for propagating the stionic combinations, those used as sources of buds for inoculations, and those used as sources for postpropagative budding of controls were indexed for viruses during the past 3 to 7 years. Experimental trees found to have been inoculated with buds from trees with mixed infections are omitted from Table 1; exceptions are trees inoculated with buds from one exocortis source used in Plot 1 (2) and some trees inoculated with buds from sources with both exocortis and vein enation viruses.

The parent trees used as budwood sources for propagating the stionic combinations have proved to be free of detectable viruses, excepting the young-line navel orange (*C. sinensis* (L.) Osbeck) which carried vein enation virus in many buds used for propagation.

The exocortis sources used—1 Clementine mandarin (*C. reticulata* Blanco), 9 Lisbon lemon (*C. limon* (L.) Burm.), 30 Eureka lemon—were indexed for viruses of exocortis, psorosis, tristeza, vein enation, and yellow vein. All 40 sources were found to contain exocortis virus; one source contained psorosis and cachexia viruses; 10 other sources had vein enation virus; 29 sources apparently carried only exocortis virus. The sources were also indexed for xyloporosis virus, using sweet lime (*C. limettioides* Tanaka) as an indicator, and for cachexia virus, using

TABLE 1. TRUNK GROWTH OF INOCULATED BUDLING TREES AND EXOCORTIS-FREE BUDLING CONTROLS

Plot No.	Top/Rootstock[a]	Period of study	Number of exocortis sources used	Number of trees[b]	Trunk growth extremes[c] cm²		Average trunk growth[c] cm²		Approx. stunting[d] %
					inoc.	control	inoc.	control	
1	Eur YL/Sw Or	1950-57	15	94	92-174	114-195	137***	159	14
1	Eur YL/Sr Or	1950-57	15	30	97-150	124-175	122***	147	17
1	Eur YL/Gpft	1950-57	2	8	118-153	166-185	134***	177	24
1	Sw Or OL/Sr Or	1950-56	3	8	58-83	77-113	73	89	18
2	Lis YL/TC	1956-59	2	18	88-130	119-154	112**	135	17
3	Val/TC	1956-59	3	48	23-57	54-79	46**	65	30
3	Val/TC	1956-59	0	18[e]	60-78[e]	55-75	69[e]	64	0[e]
4	Cala YL/MC	1955-60	4	22	48-67	58-85	55***	72	24
4	Val YL/MC	1955-60	2	12	37-52	51-74	44***	62	29
4	Val YL/T Or	1955-60	5	20	22-36	43-59	28***	52	46
5	Val YL/T Or	1957-60	4	8	5-18	22-33	13*	27	52
5	Nav YL/T Or	1957-60	2	6	7-12	17-20	9*	19	53
5	Nav YL/TC	1957-60	7	18	14-22	18-28	18**	23	22
5	Nav YL/Rang	1957-60	3	6	15-20	19-26	17*	22	23
5	Nav YL/OT	1957-60	5	16	15-28	17-26	23	22	0
5	Gpft YL/T Or	1957-60	5	10	10-16	21-27	13**	24	46
5	Gpft YL/TC	1957-60	3	6	21-25	24-32	23	27	15
5	Gpft YL/Rang	1957-60	11	22	13-29	21-38	21***	32	34

[a]Cala=calamondin; Eur=Eureka lemon; Gpft=grapefruit; Lis=Lisbon lemon; MC=Morton citrange; Nav=navel orange; OL=old-line; OT=Orlando tangelo; Rang=Rangpur lime; Sr Or=sour orange; Sw Or=sweet orange; TC=Troyer citrange; T Or= trifoliate orange; Val=Valencia orange; YL=young-line.

[b]Of which ½ were inoculated with buds containing exocortis virus, except as noted in footnote e.

[c]Cross-section areas calculated from circumference of trunks, 6 inches above bud union in Plots 1-4, and 2 inches above in Plot 5.

[d]Based on average trunk growth of inoculated and control groups.

[e]"Inoculum" source tree for this group contained no detectable viruses.

Note: Single, double, and triple asterisks indicate significant differences from the control at the 0.05, 0.01, and 0.001 levels, respectively.

Orlando tangelo (*C. reticulata* x *C. paradisi* Macf.) as an indicator. None of the sources has been found to carry xyloporosis virus, although insufficient time has elapsed to rule out its presence in more than 6 of the sources. Thirty-nine of the 40 sources are thus far negative for cachexia virus.

Vein enation virus has spread by vectors to some extent in all plots; tristeza virus has spread by vectors to some trees in Plots 4 and 5 only. Most of the spread occurred in 1959-60 and involved less than half of the experimental trees, as judged by random indexing.

Results

STUNTING.—Stunting was sometimes observed in inoculated indicator trees before external symptoms developed but sometimes could not be detected until bark symptoms were well developed. Most of the inoculated trees became stunted 1½ to 5 years after inoculation. The degree of stunting of trunks may be judged by consideration of the data in Table 1. Stunting of tops having exocortis-indicator rootstocks (Fig. 1) usually was more severe than indicated by cross-section areas of trunks.

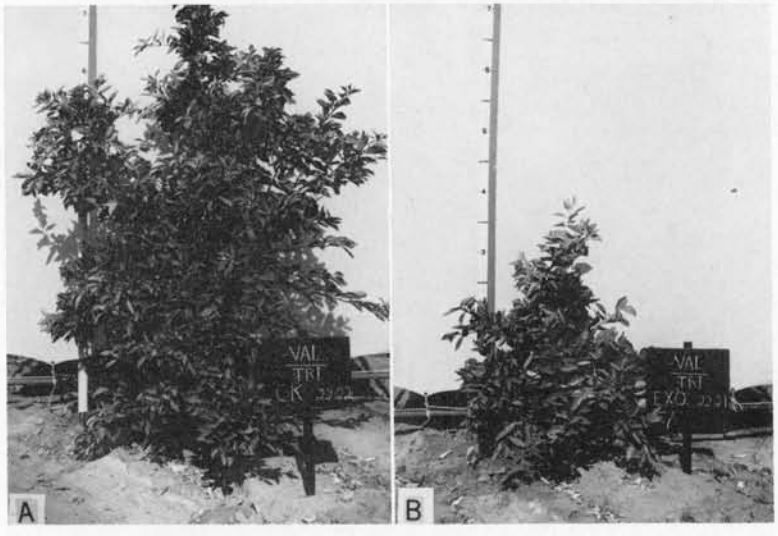

FIGURE 1. *A. Noninoculated control tree of Valencia orange/Trifoliate orange. B. Inoculated tree of same combination stunted by exocortis virus. Photographed 30 months after planting.*

Budling controls having postpropagative grafts from trees free of exocortis virus were not stunted.

Stunting of the same order of magnitude as that illustrated in Figure 1 occurred in the tops of inoculated navel orange and grapefruit (*C. paradisi* Macf.) on trifoliate orange stocks; the degree of stunting was slightly less for grapefruit on Rangpur lime stocks, much less for navel orange on Rangpur lime stocks.

STRAINS OF EXOCORTIS VIRUS.—Indicator trees inoculated with buds from some exocortis sources gummed and developed bark cracking and shelling more quickly and severely than trees inoculated with buds from other sources. Since the time required for symptoms to develop, as well as the severity of these symptoms, was consistent for each exocortis source, it is concluded that different sources carry different strains of exocortis virus. A strain carried by the old-line CES Eureka lemon is

FIGURE 2. *Comparative reactions of Morton citrange stocks (under calamondin) to different strains of exocortis virus. A. Severe exocortis on tree inoculated with severe strain. B. Mild exocortis (arrow) just below original soil line on tree inoculated from mild strain. Photographed 5 years after inoculation, in Plot 4.*

especially virulent and has a shorter incubation period in most exocortis indicators, 2 years or less (15), than the strains carried by many other sources (Fig. 2); some strains of exocortis virus have an incubation period of 4 years or more. A strain of virus carried by the CES Eureka lemon causes more stunting and more seasonal chlorosis of trees having sensitive rootstocks than some other strains studied. Similar results were obtained in several field plots with trees having Cuban shaddock (a lemon hybrid), Morton citrange (*P. trifoliata* x *C. sinensis*), Palestine sweet lime, Rangpur lime, or trifoliate orange rootstocks.

Discussion

The early reports of stunting by exocortis were based mostly on observations in rootstock plots and commercial orchards. Some recent reports compare infected trees with trees containing no exocortis virus; others are based on observations of trees having mixtures of viruses, or on trees not indexed for viruses. An association between exocortis and stunting of some trees having indicator rootstocks already has been clearly established, but it has not been previously determined whether exocortis virus alone caused the stunting. Even those experiments based on inoculated trees and comparable controls apparently were done without indexing to determine whether other detectable viruses were present in the experimental trees.

Measurable stunting in most of the inoculated trees in our experiments is attributed to exocortis and to no other virus. The influence of viruses other than exocortis on the stunting indicated by data in Table 1 is assumed to have been small for the following reasons: less than 1/3 of the exocortis sources used contained any other detectable virus, as judged by the results of indexing (14); few calamondin (*C. mitis* Blanco), lemon, and grapefruit (*C. paradisi* Macf.) trees became infected with vein enation virus, none with tristeza virus; fewer than half the Valencia orange trees acquired vein enation virus and less than 1/5 acquired tristeza virus; no effect of tristeza or vein enation viruses on the growth of the experimental trees could be detected.

Exocortis lesions and associated gum may directly affect the functions of the vascular system and reduce growth. Stunting, however, is sometimes evident before exfoliation occurs (4), and exocortis often can be detected before shelling appears (3). We have observed milky or water-soaked plaques of tissue developing near the cambium in the bark of exocortis indicator stocks before the bark cracks and exfoliates. No

specific symptoms of exocortis developed in 3 nonindicator stocks (grapefruit, sour orange, and sweet orange) having Eureka lemon tops stunted by exocortis virus, nor did external symptoms develop in most of the stunted trees of Lisbon lemon on Troyer citrange stock. It is assumed, therefore, that in many nonindicator plants exocortis virus causes inconspicuous internal changes which lead to stunting without specific symptoms or any severe effect.

Fraser and Levitt (4) suggested that exocortis virus exists in a number of strains. They noted variations in degree of stunting, location and amount of bark scaling, and in rate of external symptom development which might be related to differences in strains or mixtures of virus. Although the same kinds of variations occur in California, only those involving conspicuous differences in incubation period (15) and severity of symptom development in genetically uniform indicator plants grown in similar environments have proved to be due to differences in virus strains. Other factors also affect exocortis symptom development. The influence of nutritional factors upon exocortis symptoms has been demonstrated by Weathers (13); high levels of nitrogen and phosphorus reduced the incubation period of exocortis to less than 1 year in some cases, whereas low levels of nitrogen and phosphorus favored stunting without scaling. Variable incubation periods and stunting without scaling in trees infected with exocortis virus may be due to environmental factors and are not necessarily dependent upon variations in host or in virus strains.

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