E. C. CALAVAN and D. W. CHRISTIANSEN

Variability of Cachexia Reactions among Varieties of Rootstocks and Within Clonal Propagations of Citrus

ORLANDO TANGELO [*Citrus sinensis* (L.) Osbeck x *C. paradisi* Macf.] and sweet lime (*C. limettioides* Tanaka) have been useful indicators for cachexia (2, 4, 9) and are the principal plants employed for the detection of this virus in symptomless candidate trees enrolled in the University of California Citrus Variety Improvement Program (10). Previous work (2, 6, 7, 8) indicated some variation in incubation period and in symptoms of cachexia among plants of these and other indicators. Additional evidence was needed to determine the reliability of the indexing procedures for detection of cachexia virus infection. This paper reports the results of a 4-year field study of cachexia indexing and disease development in 850 trees of many stionic combinations, including 3 scion and 73 rootstock varieties.

Methods and Materials

Several scions from each of 3 cachexia-infected trees, Marsh grapefruit (*C. paradisi* Macf.), Willow-Leaf mandarin (*C. reticulata* Blanco), and Wekiwa tangelo were bud-propagated singly in the nursery on seedling rootstocks of 59 varieties, principally mandarins, tangelos, and sweet limes. In addition, 1 or 2 scions from the infected Marsh and Willow-Leaf trees were grown on seedling rootstocks of 14 other varieties. The Marsh grapefruit source was known to be infected with a virus causing xyloporosis in Palestine sweet lime and cachexia in Orlando tangelo (2). Exocortis virus, as determined recently by the Etrog citron test (3), was present in the 3 scion sources, but only the Wekiwa source consistently caused symptoms typical of exocortis on trifoliate orange [*Poncirus trifoliata* (L.) Raf.]. Two or more seedlings of each rootstock selection were retained as non-inoculated controls. All budlings and seedlings were planted in an orchard 1 year later and examined annually for symptoms by peeling strips of bark from scion and rootstock portions of the trunks.

Results

IN ROOTSTOCKS.—Numerous tiny pits in the wood and pointed pegs and gum in the bark were detected in 2 Parsons Special mandarin rootstocks with infected Marsh grapefruit tops 18 months after budding. After incubation periods of 2, 3, and 4 years, respectively, cachexia symptoms were found in 57 root stocks representing 23 varieties; in 213 representing 38; and in 281 stocks representing 40 varieties. Of the 281 affected rootstocks, 122 had mild, 75 moderate, and 84 severe symptoms. Great differences in reaction to cachexia infection from different inocula were apparent within most highly sensitive indicator rootstock varieties. Qualitative and quantitative differences in symptoms also occurred among trees of a single stionic combination and similar differences were noted at different sites on a single stock. Listed according to their approximate order of decreasing sensitivity within groups, the 40 rootstock varieties that reacted definitely to cachexia infection were: Owari, Batangas, Parsons Special, Oneco, Tien Chieh, Tankan, Willow-Leaf, Silverhill, Kinokuni, Roeding, Scarlet Emperor, Wilking, Cleopatra, Ponkan, and CPB (Citrus Physiology and Breeding, U. S. Department of Agriculture)-12080 mandarins; Philippines Red and Kusaie mandarin limes (*C. limonia* Osbeck); CRC (University of California Citrus Research Center, Riverside)-919, Columbian, CRC-1044, CRC-920, CRC-921, Palestine, and CRC-363 sweet limes; Wekiwa, Sunshine, Orlando, CPB-40119, Early, Seminole, Thornton, Yalaha, Sacaton, Ugli, Altoona, Pearl, and Clement tangelos; Millsweet sweet lemon [*C. limon* (L.) Burm. f.]; *C. macrophylla* Wester; and *C. depressa* Hayata. Plants of the last 3 species developed relatively mild symptoms.

Mild indefinitive reactions, mostly pitting and pegging, were noted in 14 additional varieties of rootstocks: *C. limetta* Risso var. *aromatica;* Kansu ichandarin (*C. junos* Siebold); Ponderosa lemon; Egyptian lime [*C. aurantifolia* (Christm.) Swing.]; Hung Kat and Tim Kat mandarins; *P. trifoliata;* Faris sweet lemon; San Jacinto, Sunrise, Webber, and Williams tangelos; Dweet and CRC-2970 tangors (*C. sinensis* x *C. reticulata*). No reaction was found in 19 varieties of rootstocks: Morton citrange; Yuzu ichandarin; CPB-10174 and Laranja crave mandarins; Australian red, Borneo, CRC-2451, CRC-2693, Japanische citroen, Rangpur, and Santa Barbara red mandarin limes; CRC-3178 Satsuma (*C. unshiu* Marcovitch); sunki (*C. sunki* Tanaka); Bishop, CRC-2609, CRC-2791, Sexton, Siamelo, and Wilsh tangelos.

The first symptoms in most rootstocks were tiny pits in the wood with corresponding pegs in the bark. As these pegs enlarged they often became sharp- or blunt-tipped cones flattened laterally so that the vertical axis considerably exceeded the width. Sizes and shapes of individual bark pegs varied greatly, even in the same plant. In 3- to 4-year-old bark of most mandarin and sweet lime and several tangelo varieties, including Orlando and Sunshine, most pegs were less than 1.5 mm in any dimension. Larger pegs usually developed in Wekiwa and CPB-40119 tangelos, Owari Satsuma and Tankan tangor. However, size and concentration of the pegs in the rootstock bark varied considerably according to scion; for example, the pegs in Wekiwa stocks with grapefruit tops were smaller and more densely concentrated than in Wekiwa stocks with Wekiwa tops. Scattered pegs of various sizes occurred in Palestine sweet lime bark; heavy concentrations of pegs developed near the bud-union in many sweet lime stocks. Gum usually was present within the larger pegs and in the middle and inner portions of the bark of many severely affected stocks, particularly tangelos and mandarins. In some cases gum was found in the cambial zone and in rings and pockets in the wood. The amount of fresh gum fluctuated seasonally in many trees.

Rough flattened areas were found on severely affected trunks of several varieties. Small round to oval holes (inverse pitting), usually less than 0.5 mm in diameter, were distributed irregularly on the cambial face of the bark in some severely lesioned areas; corresponding pegs, blunt- or sharp-pointed, protruded from the wood. Symptoms found in plants of several rootstock varieties are shown in Figures 1 and 2.

The most severe rootstock reactions occurred in Parsons Special mandarin, Owari Satsuma, and Sunshine and Wekiwa tangelos; of these, only the Owari responded to inoculation from Willow Leaf and its response was mild. However, Batangas mandarin reacted fairly well to inoculations from each source but non-inoculated Batangas seedlings developed small scattered pits when about 4 years old. Columbian and CRC-919 sweet limes reacted mildly to inoculation from Wekiwa tangelo and moderately or severely to other inoculations. The reaction of Palestine sweet lime was mild except under grapefruit tops. Orlando tangelo reacted strongly to inoculation from cachexia-infected Marsh, moderately to inoculation from Wekiwa, and rarely and very mildly to inoculation from Willow Leaf. Severe decline and chlorosis occurred in

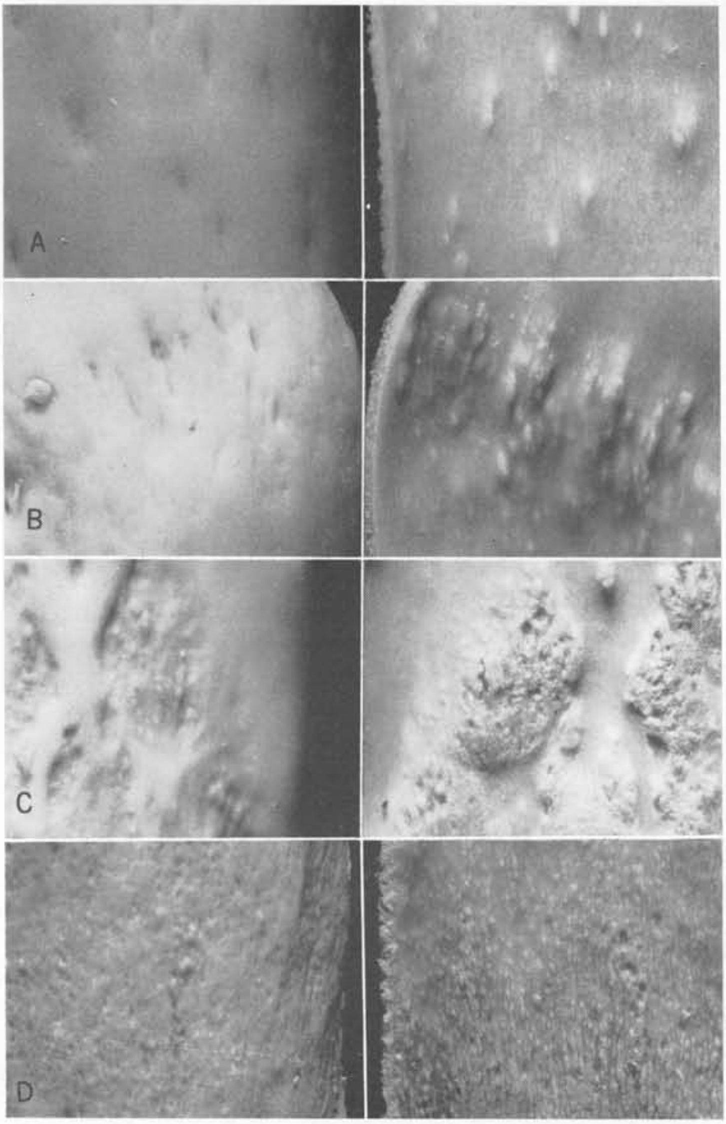

FIGURE 1. *Cachexia symptoms on cambial faces of wood (left) and bark (right) of 4-year-old rootstocks: A, Batangas mandarin (under Wekiwa); B, CRC-919 sweet lime (under Willow Leaf); C, Parsons Special mandarin (under Wekiwa); D, Sunshine tangelo (under Marsh).*

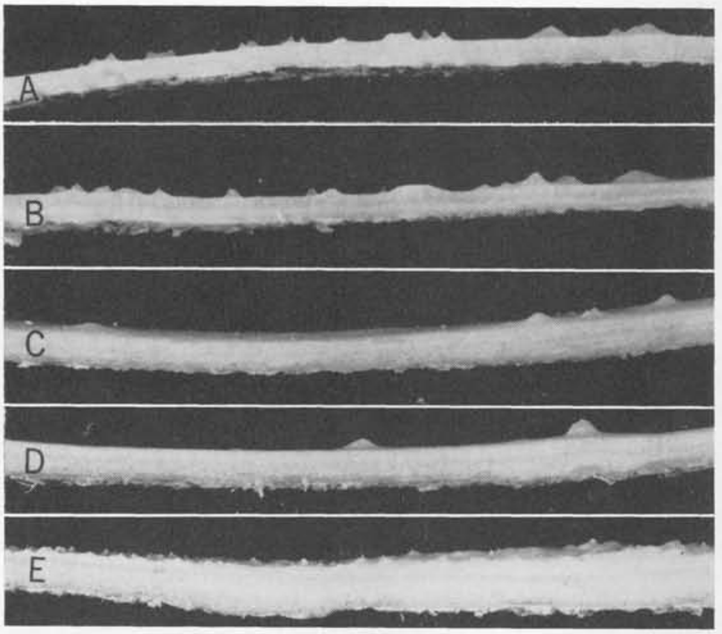

FIGURE 2. *Radial sections of bark showing a variety of cachexia-induced pegs in 5 varieties: A, Oneco mandarin; B, Batangas mandarin; C, CRC-919 sweet lime; D, Palestine sweet lime; E, Sunshine tangelo.*

the grapefruit trees on Parsons Special and Sunshine rootstocks but very few trees of other stionic combinations were similarly affected, although many with severe cachexia developed some winter chlorosis.

Many Wekiwa and Willow-Leaf trees and some seedling controls were infected naturally by tristeza virus, whereas most grapefruit trees remained tristeza-free. Tristeza infection caused pitting in the wood of some seedling plants of several varieties, including sweet lime and Orlando tangelo. Stem pitting caused by tristeza was usually mild in Orlando and in most other good indicators of cachexia, but was severe in *C. macrophylla* (Fig. 3).

The results show that the rootstocks (Owari Satsuma, Parsons Special mandarin, Wekiwa, Sunshine, and Orlando tangelo) most sensitive to cachexia virus from certain inocula were not the best indicators for virus from all 3 sources. The over-all value of Batangas mandarin and sweet lime indicators was tempered somewhat by their tendency to be pitted mildly from tristeza infection and from a still undetermined cause. Varietal differences in reaction to cachexia infection appeared

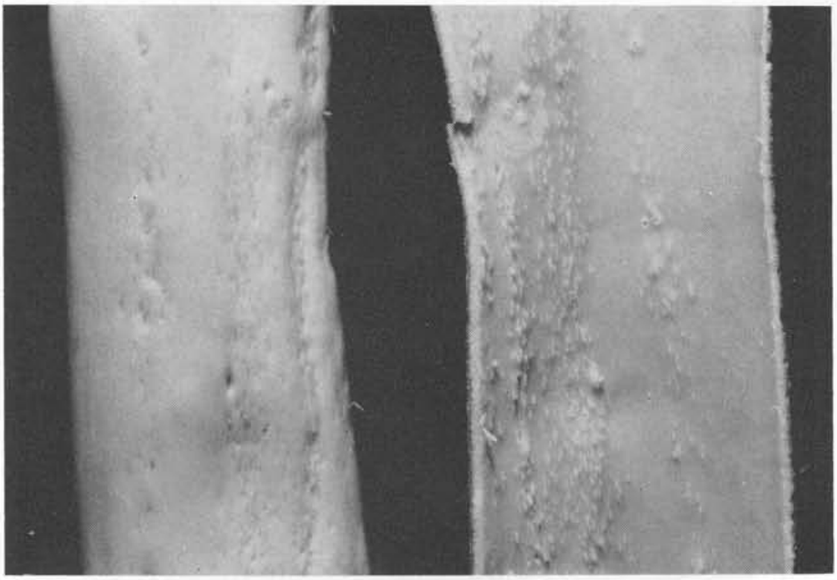

FIGURE 3. *Pit and peg symptoms of tristeza in wood (left) and bark of 4-year-old C. macrophylla seedling.*

to be due mainly to differences in host sensitivity. Some influence of scions on symptom development in the rootstocks was apparent but the variable development of cachexia within trees of single stionic combinations having mostly nucellar rootstocks suggested variation in the pathogen.

IN SCIONS.—Marsh grapefruit scions developed no cachexia symptoms. Wekiwa tangelo scions were 47 per cent, Willow-Leaf mandarin 93 per cent, symptomless at 4 years of age. Some scions of Wekiwa tangelo and Willow-Leaf mandarin developed symptoms similar to those found in the same varieties of rootstocks. The numbers of affected trees and scions of these 2 varieties after 2, 3, and 4 years of growth are shown in Table 1. Wekiwa scions and their rootstocks were affected sooner and more severely, on the average, than Willow-Leaf scions. Among the affected Wekiwa scions 4 years old, 70 had mild, 25 moderate, and 37 severe symptoms; while 18, 1 and 0, respectively, of the Willow-Leaf scions were similarly affected. Marked differences in symptoms were noted even among severely affected Wekiwa scions (Fig. 4). Sections cut through severely pitted Wekiwa trunks revealed that gum layers and severely pitted and pocketed areas of wood were

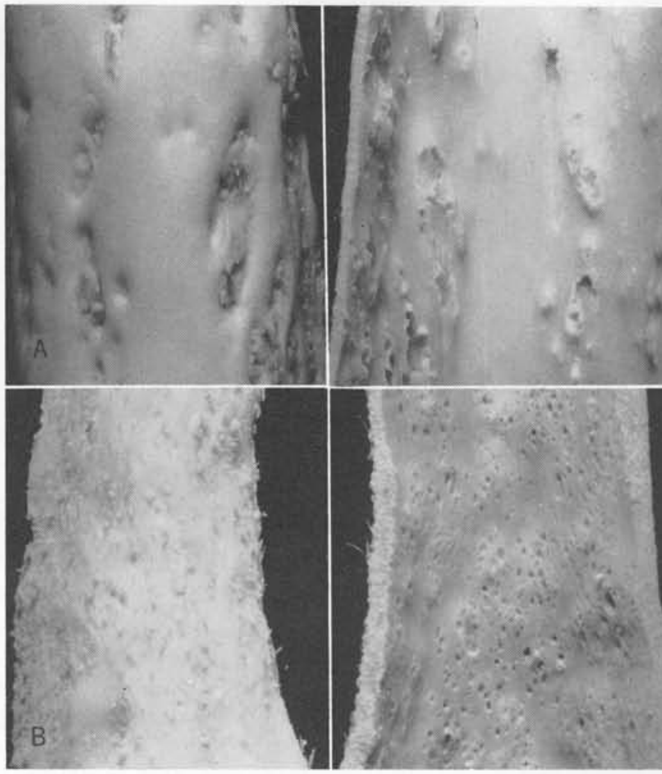

FIGURE 4. *Cambial faces of trunk wood (left) and bark of 2 Wekiwa tangelo trees propagated from the same cachexia-infected parent; A, trunk with scattered lesions containing clumped pits and pegs; B, trunk with small and large pits in wood and many round or oval pits in bark (inverse pitting).*

sometimes covered by nearly normal wood (Fig. 5). Additional evidence of seasonal variation in symptoms was obtained by frequent examinations of affected rootstocks of several varieties.

The data show that cachexia can develop independently in sensitive stocks and scions of certain trees, thus proving that many of the symptomless indicator scions are carriers of cachexia virus. The gradual incidence of disease in the scions implies that some of the indicator scions may require an additional 2 years, or more, to develop symptoms. Differences in cachexia development in scions could not be attributed solely to rootstock influence.

Discussion

Grant *et al.* (5) reported that in one experiment symptoms de-

TABLE 1. OCCURRENCE OF CACHEXIA SYMPTOMS AFTER STATED INCUBATION PERIODS IN TWO VARIETIES OF TREES PROPAGATED FROM CACHEXIA-INFECTED BUDWOOD SOURCES

Portion of tree affected	Total number of trees with indicated reaction					
	In Wekiwa scion group			In Willow-Leaf scion group		
	Incubation (yrs.)			Incubation (yrs.)		
	2	3	4	2	3	4
Scion only[a]	39	52	75	3	7	11
Rootstock only[b]	15	36	34	9	33	43
Scion and stock[b]	9	36	57	1	6	8
None[a]	184	123	81	267	234	218

[a]Based on 247 Wekiwa and 280 Willow-Leaf trees.

[b]Based on trees having cachexia-sensitive rootstocks, 177 Wekiwa and 170 Willow-Leaf.

veloped in inoculated Orlando tangelo but not in inoculated sweet lime. In the current work, almost the reverse situation occurred, for only 2 of 35 Orlando stocks inoculated with virus from Willow-Leaf mandarin have developed symptoms. Despite the variable responses in our experiments, no indication was found that cachexia and xyloporosis are caused by unrelated viruses.

Olson (6) mentioned 4 factors that may affect symptoms of cachexia: (a) mixtures of healthy and infected buds; (b) varietal differences in incubation periods; (c) genetic variants among seedlings of a variety; (d) interference from other disorders.

In our experiment the differences in response to cachexia infection among rootstocks of different varieties probably was due largely to genetic differences in sensitivity to the infecting virus; moreover, variations

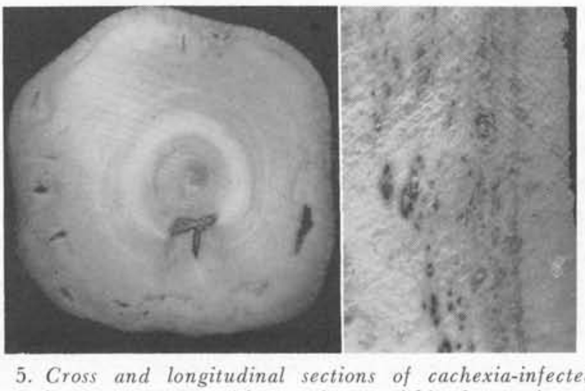

FIGURE 5. *Cross and longitudinal sections of cachexia-infected Wekiwa tangelo trunk showing pockets and layers of gum within the wood.*

were noted occasionally in off-type seedlings, and some buds from the infected scion sources may possibly have been free of cachexia virus. However, the mild and severe responses by Owari rootstocks under Willow-Leaf and Wekiwa tops, respectively, contrast sharply with the moderate to severe reactions in CRC-919 and Columbian sweet lime stocks under Willow-Leaf tops and the mild reactions in the latter 2 stocks under Wekiwa tops. The reason for this, and for the generally slower response of mandarins and tangelos to inoculations with virus from Willow Leaf than with virus from Marsh or from Wekiwa, may be due to differences in the virus complexes, to different concentrations of virus in the scions, or to direct effects of the scions on the rootstocks.

In another experiment by the authors, typical severe symptoms of cachexia appeared within 3 years on all 7 Orlando tangelo stocks inoculated from an exocortis-free, cachexia-infected Washington Navel orange, but it was not determined whether exocortis-free cachexia inocula would cause uniform reactions in larger numbers of plants or in indicators of other varieties. Although the effect of exocortis virus on responses of cachexia indicators to cachexia infection is unknown, it was determined in this experiment and by supplemental indexing that the average severity of exocortis reactions on exocortis indicators was greater with inoculum from Wekiwa than with inoculum from Marsh or Willow Leaf. This indicates that factors other than exocortis influenced the reactions, but does not eliminate the possibility that exocortis virus caused some interference in the experimental trees.

Because trees bud propagated from a single infected plant varied most noticeably in their reaction to cachexia, it may be assumed that the causal virus varied in composition, concentration, or both, in sensitive tissues. Mere differences in concentration of the virus within the tissues of the various buds used for propagation would seem unlikely to result in the great symptomatic differences observed 4 years later. If, however, cachexia virus exists in a complex of strains, competition might produce variations in relative concentrations of the component strains or their rates of movement, with resulting differences in effects on the host. Since interaction between unrelated viruses also is possible (1, 11), exocortis infection, concurrent with cachexia, in our experimental trees may have increased the variation in host reaction.

Conclusions

1. Absence of symptoms in a sensitive indicator plant 4 years old is inadequate evidence of freedom from cachexia virus.

2. The nature of cachexia makes it advisable to use 8 or more indicator plants of at least 2 very sensitive varieties for a minimum of 6 years to determine freedom from cachexia virus by the methods used here.

3. Symptoms and incubation periods of cachexia in indicator plants inoculated or propagated from individual source trees are likely to be highly variable.

4. The reasons for the observed variations in cachexia reactions are unknown, but the reactions appear to be influenced by host genetics, host physiology, environmental factors, and variations in the pathogen.

5. Indexing for cachexia should be done in tristeza-free situations, if possible, because tristeza infection causes varying amounts of wood pitting in many cachexia indicators. Where tristeza is unavoidable, the use of tristeza-infected cachexia-free controls is advisable.

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