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Experimental Evidence That Cachexia and Xyloporosis Are Caused by the Same Virus

THE RELATION of cachexia to xyloporosis and evidence for considering the two names to be synonymous were reviewed in 1957 (4). Additional evidence for their synonymy has appeared since (1, 7).

In 1958, the synonymy of cachexia and xyloporosis was less widely accepted than it is today. At that time, G. G. Norman of the Florida Department of Plant Industry requested an experimental investigation of their relationship and offered in 1954 index plots of the Florida Citrus Budwood Program for experimental use. The results of that investigation are reported here.

The xyloporosis syndrome originally described by Reichert and Perlberger (9) was later broadened (10) to include symptoms previously attributed to little leaf disease (8) and stubborn disease (6). In this paper the term cachexia signifies the syndrome normal to that virus in Orlando tangelo (*Citrus paradisi* Macf. x *C. reticulata* Blanco) (2, 3) and the name xyloporosis signifies symptoms of that disease in sweet lime [*C. aurantifolia* (Christm.) Swing.], as originally described (9).

Materials and Methods

Each candidate parent tree in the Florida Citrus Budwood Program is examined for psorosis leaf symptoms during two spring flushes of growth and indexed for tristeza virus on West Indian lime [*C. aurantifolia* (Christm.) Swing.]. If both tests are negative, buds from the candidate tree are worked on three Orlando tangelo and on three sweet orange [*C. sinensis* (L.) Osbeck] seedlings planted alternately in rows in the test area. Each plot consists of six trees—three Orlando tangelo and three sweet orange—and is numbered with the accession number of the parent tree. The trees in the plots offered for our use were budded in 1954 and examined through 1958, a period of five years. Thus, the trees infected with psorosis virus and those infected with cachexia virus were known. Trees infected with psorosis virus were not used in this experiment. The exocortis tests, performed in another block, were too young to reveal the incidence of exocortis virus in trees of the 1954 block. Most of the candidate trees were old-line varieties in commercial orchards, and such trees usually carry several viruses. Consequently other viruses, besides the four indexed, could be present in these tests.

There were 268 plots in the 1954 block. Most of the parent trees were varieties of sweet orange but a scattering of grapefruit (*C. paradisi* Macf.) and mandarin (*C. reticulata* Blanco) varieties also were present. In 78 plots, all three Orlando tangelo trees were positive for cachexia; in 68 plots, two of the three Orlando tangelo trees were positive; in 33 plots, one of the three was positive; and in 89 plots, all three were negative. For use in the experiment, 58 cachexia-positive (3 of 3) plots and 67 cachexia-negative (0 of 3) plots were selected. Plots of intermediate rating were not used.

When this experiment was commenced in 1958, the cachexia-infected Orlando tangelo trees in the test block were in various stages of decline. For that reason, the Orlando tangelo trees were not used. The sweet orange seedlings, budded from the same sources, had made uniform growth despite the presence of cachexia or other viruses, and they were chosen for experimental use.

Design of the Experiment

One aspect of the similarity between cachexia and xyloporosis is that their susceptible-host ranges are alike. That being so, differences that might exist between them would presumably show most strongly in the reaction of citrus varieties of diminished or marginal susceptibility. Thus, seven varieties of citrus were selected to provide a range of susceptibility to cachexia and xyloporosis as follows: tangelo varieties, Sunshine and Nocatee, sweet lime varieties, Columbia, Butwal, and Ward's, a sweet lime of Palestine type propagated by a Florida nurseryman, and mandarin lime (*C. aurantifolia*) varieties Rangpur and Kusaie.

In this experiment, each sweet orange tree was budded with one Orlando tangelo bud and with one of the test buds. The first tree was budded with Orlando tangelo and with Sunshine tangelo, the next tree with Orlando tangelo and Nocatee tangelo, and the third tree with Orlando tangelo and Columbia sweet lime. Thus, in the first plot of three sweet orange trees, inoculated in 1954 from the same parent tree (Hamlin orange No. 23-1-11) and rating three out of three negative for cachexia in 1959, there were two types of comparisons possible: (a) the reaction of Orlando tangelo compared with that of three of the seven test varieties, and (b) a comparison between the reactions of the three test varieties by using the Orlando tangelo as a reference. In one plot, only three of the test varieties could be compared. Thirty-five three-member combinations of the seven test varieties are possible, however. Each combination was used three or four times in the experiment.

Before budding, all trees in the block were cut back to a height of about 1½ meters. The wounds were painted, but the trees were not whitewashed. Budding commenced in February, 1959, and the last rebudding was done in February, 1960.

Results and Discussion

Failure to whitewash the trees resulted in considerable sunburn, which weakened many trees. Many buds died, made weak growth, or failed to grow. About 70 per cent reached measurable size by 1963. A survey was made in June, 1961, and 46 plots were examined in detail in September of that year. All plots were examined in June, 1962, and again in June, 1963.

Examination consisted of measuring in millimeters with a small steel tape the circumference of each bud sprout immediately above its union with the sweet orange tree and examining the cambial face of the wood and bark for pitting and gum impregnation. A strip of bark 5-25 mm wide by 25-75 mm long, depending on the size of the twig, was cut across the union. Severity of pitting and gumming was rated on a scale of 0 to 10. The excised bark specimens were labeled and preserved in F.A.A. solution for possible future microscopic examination. Specimens of bark with indicated ratings are shown in Figure 1.

COMPARATIVE GROWTH OF ORLANDO TANGELO AND TEST BUDS.—On first examination, three years after initial budding, the Orlando tangelo sprouts had made considerably better growth as determined by their circumference at the union than the sprouts of seven test varieties (Fig. 2). This pattern of growth continued in both the cachexia-negative and the cachexia-positive groups through 1963 (Table 1). After five years the average circumference of the bud-unions on cachexia-negative trees in decreasing order of size were as follows: Orlando tangelo, Sun-

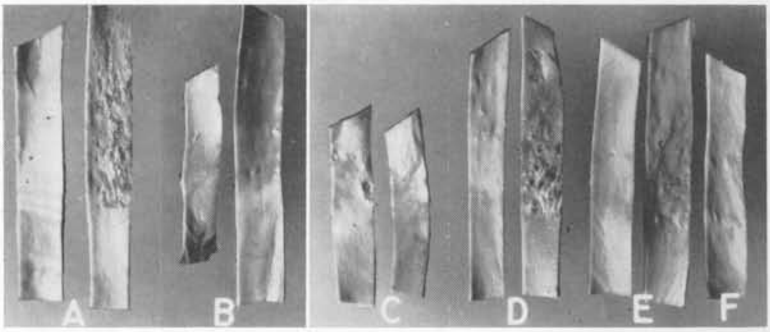

FIGURE 1. *Rating of pitting and gumming on several varieties: A. left, Ward's sweet lime—0; right, Orlando tangelo—10. B. left, Kusaie lime—0; right, Orlando tangelo—2. C. left, Sunshine tangelo—3; right, Orlando tangelo—1. D. left, Nócatee tangelo—4; right, Orlando tangelo—10. E. left, Columbia sweet lime—0; right, Orlando tangelo—7. F. left, Butwal sweet lime—1; right, Orlando tangelo—0 (dead, no bark specimen).*

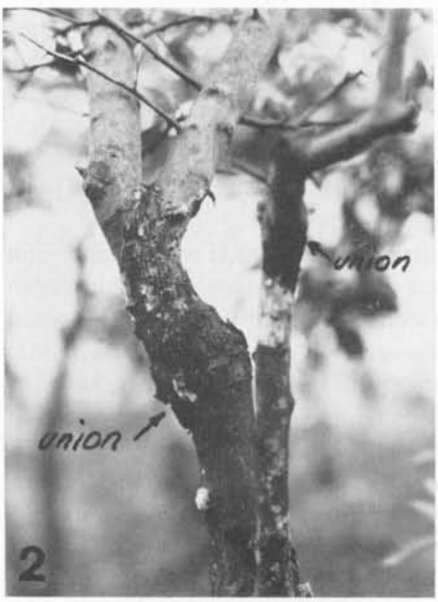

FIGURE 2. *Orlando tangelo sprout, left, and Rangpur lime, right, showing the different rates of growth.*

shine tangelo, Ward's sweet lime, Columbia sweet lime, Kusaie lime, Butwal sweet lime, Nocatee tangelo, and Rangpur lime. Among the cachexia-positive trees the order was similar except that Kusaie lime fell fifth to last place.

If there is a difference between the virus of cachexia and xyloporosis,

TABLE 1. Circumference of test bud sprouts on cachexia-free and cachexia-infected trees[a]

Stock infection	Tangelo		Sweet lime			Mandarin lime		Check
	Sun.	Noc.	Col.	But.	Ward	Rang.	Kus.	Orl.
Free								
1961[b]	—	75	104	74	120	86	82	123
1962	166	80	108	88	106	74	91	139
1963	128	84	113	88	111	75	90	158
Max. circ.	188	141	171	129	165	117	145	208
Infected								
1961[b]	83	60	88	75	93	70	65	100
1962	115	78	103	77	106	79	70	133
1963	128	91	100	83	112	75	69	145
Max. circ.	173	105	158	123	164	133	102	173

[a]Measurement in millimeters, just above union. [b]Incomplete data in 1961.

it might consist of more severe stunting produced by one or the other. If it is assumed that the largest union formed by each variety is the normal healthy size for that variety, it follows that the virus causing stunting is not present in that normal healthy plant. Thus, a comparison of maximum circumferences with the average circumferences should indicate the degree of stunting that occurred.

In the cachexia-negative group, the maximum circumferences fall into the same order of sizes as the average circumferences (Table 1). This fact suggests that the test variety was not stunted when the Orlando tangelo bud gave a negative test for cachexia.

In the cachexia-positive group, stunting was estimated by comparing the average circumference figure with the cachexia-negative figure for each of the seven test varieties. On that basis, percentage reduction in size in the cachexia-positive group was as follows: Kusaie lime—23, Sunshine tangelo—11, Columbia sweet lime—10, Orlando tangelo—8, Ward's sweet lime—7, Butwal sweet lime—6, Nocatee tangelo—5, and Rangpur lime—4. Thus, stunting of Kusaie lime was more than twice that of any other variety but only when cachexia symptoms were present in the paired Orlando tangelo sprout. Stunting of Orlando tangelo was intermediate between that in Columbia and Ward's sweet lime varieties.

Rating of cachexia and xyloporosis symptoms.—Symptom ratings of 0 to 10 were based on the occurrence of wood pitting (projections or pegs on the cambial face of the bark), their number and size, and on the amount of gumming present. In a given variety, the number and size of pits or pegs are less variable than the severity of gumming. Thus, when pits and pegs were very numerous but gumming was virtually absent, such a bark specimen was assigned a rating of 5 or possibly 6. If gumming was severe the specimen was rated 10. On the other hand, if gumming was severe, but the number and size of pits and pegs were only moderate, the specimen was rated 4 or 5 or possibly 6. In certain instances 25 mm or more of bark adjacent to the union was dead from the effects of the virus. Such instances were rated 10 even though the live bark above the dead area might rate only 5 or 6.

In the cachexia-negative series, about 95 per cent of the test buds developed no symptoms during the 5½-year period. However, 13 of the Orlando tangelo sprouts on sweet orange trees, previously rated negative for cachexia, developed cachexia symptoms ranging from 2 to 10 (average 7.6). If the Orlando tangelo bud in the cachexia-negative series showed cachexia symptoms, the test bud paired with it was not considered in the results. A few sprouts from test buds developed mild symptoms of cachexia when the paired Orlando tangelo buds did not (Table 2), possibly as a result of infection through root grafts with neighboring infected trees and because test buds were often lower on the sweet orange trees.

Occurrence of psorosis leaf symptoms on some of the Nocatee tangelo sprouts was reported by G. G. Norman. The five seedling Nocatee tangelo trees, source of the buds, were cut back two successive years to force psorosis leaf symptoms but none was found.

TABLE 2. Symptom occurrence and severity on Orlando tangelo and on seven test varieties budded on cachexia-infected and on cachexia-free trees, 1963

Stock infection	Test Varieties							
	Tangelo		Sweet lime			Mandarin lime		Check
	Sun.	Noc.	Col.	But.	Ward	Rang.	Kus.	Orl.
Free								
Sprouts	22	12	16	16	21	18	18	113
No. with symptoms	1	4	0	0	0	0	1	0
Avg. symptom rating[a]	1	3	0	0	0	0	1	0
Infected								
Sprouts	21	14	12	16	17	17	19	108
No. with symptoms	8	9	2	1	3	0	2	108
Avg. symptom rating[a]	1.6	1.8	1.5	1.0	2.0	0	1.0	7.1

[a]Only buds showing symptoms were averaged.

In the cachexia-positive series, Orlando tangelo buds were free from cachexia symptoms on 15 trees. In two plots, two of three were negative and in two other plots, three of three were negative. The circumference of these buds ranged from 50 to 217 mm (average 163); consequently they were large enough to have developed symptoms if the cachexia virus had been present. Data from such trees were omitted from the results. In the remainder (Table 2), 22 per cent of the test buds had symptoms but wood pitting and gumming were much less pronounced in the test bud sprouts than in the paired Orlando tangelo sprouts. Thus 78 per cent of the test sprouts produced no symptoms in the 5-year period whereas all the paired Orlando tangelo buds developed symptoms, with an average rating of 7.1.

Relation of sprout size to symptoms.—From the time of the first examination, there seemed to be a relation between the size of the sprout and the severity of cachexia symptoms on it. Segregating the ratings given Orlando tangelo sprouts on cachexia-infected sweet orange trees according to the size of the sprout at the union shows that Orlando tangelo branches had an average cachexia rating of 4.2 in the 100-124 mm (circumference) class, of 6.3 in the 125-194 mm class, of 6.7 in the 150-174 mm class, and 7.6 in the 175-199 mm class (Fig. 3). The lower ratings of the seven test varieties may result in part from their less vigorous growth.

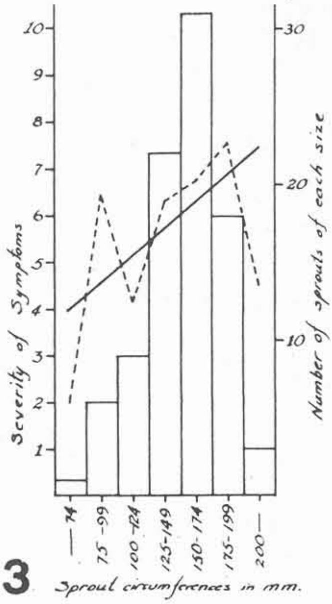

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Figure 3. *A graph showing the relation of symptoms to sprout size. Slope of the curve was calculated as the gradient of the area under it.*

Possible effect of exocortis virus on symptoms.—The lower ratings of the seven test varieties may also result in part from infection with exocortis virus. Sweet lime and mandarin lime plants react to exocortis virus (7) and preliminary results from the color test (5) indicate that approximately 50 per cent of the first 204 trees tested in the 1953 block were infected with exocortis virus. The better growth made by Orlando and Sunshine tangelo may result from their greater tolerance to exocortis virus.

If one overlooks Reichert's contention that little leaf symptoms are part of the xyloporosis syndrome (10), the main and perhaps sole argument that cachexia and xyloporosis are caused by different viruses rests on differences in symptoms produced by Orlando tangelo and those produced by sweet lime. The only difference that can be demonstrated is that symptoms on Orlando tangelo characteristically include pronounced gumming whereas the symptoms on sweet lime characteristically do not. However, gumming on Orlando tangelo may be mild. Some of the low ratings for cachexia symptoms on Orlando tangelo in this experiment are of that type. Moreover, the pronounced gumming characteristic of cachexia symptoms on Orlando tangelo occurs also on sweet lime (5) but only after a much longer time. In this experiment, symptoms appeared so much more slowly on sweet lime than on Orlando tangelo that in a number of instances bark of the Orlando tangelo sprout was killed near the union whereas the paired sweet lime sprout was only commencing to show mild symptoms.

Conclusions

These tests indicated that the seven test varieties—Sunshine and Nocatee tangelo, Columbia, Butwal, and Ward's sweet lime, and Rangpur and Kusaie mandarin lime—grew less vigorously than Orlando tangelo.

The stunting of the seven test varieties on cachexia-negative trees suggests that their less vigorous growth is normal and characteristic. The stunting of Kusaie lime sprouts on cachexia-positive trees, however, suggests that it is a feature of the reaction of Kusaie lime to cachexia.

The correlation between the size of bud sprouts and the severity of cachexia ratings in Orlando tangelo suggests that the severity of the symptoms reflects the physiology of the host plant.

That the symptoms produced by Sunshine and Nocatee tangelos, Columbia, Butwal, and Ward's sweet lime, and Rangpur and Kusaie mandarin lime are milder and develop more slowly than those produced