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Evaluation of Seedlings of 63 Citrus Cultivars for Cachexia Detection

E. M. Nauer and C. N. Roistacher

ABSTRACT. The detection of cachexia (xyloporosis) by indicator plants takes 6 months to 1 year or longer using the current indicator cultivars of Orlando tangelo or Parson's Special mandarin. This study evaluated seedlings of 63 mandarin and mandarin hybrids present in the citrus variety collection at the University of California Citrus Research Center at Riverside as cachexia indicators. Sixteen seedlings of each cultivar were inoculated with two severe isolates of cachexia by leaf disc and bud grafts. Plants were held under two temperature regimes in glasshouses at two separate locations in California. All seedlings were periodically observed and evaluated for symptoms over a 1-year period, after which the bark was peeled and observations made for gumming or pitting. The results showed that none of the 63 cultivars tested showed significant symptom development necessary to be considered as an indicator for cachexia.

Index words. Cachexia, xyloporosis, mandarins, indexing.

Most citrus virus and virus-like diseases can be detected by biological assay on selected susceptible cultivars in a relatively short period of time (3, 7, 13). Symptoms can appear within 3 to 4 weeks following inoculation of the assay plants which may be either nucellar seedlings or clonally propagated indicator cultivars on appropriate rootstocks. Seldom does detection of citrus psorosis, tristeza, vein enation, tatterleaf, or exocortis require more than 2 to 3 months for definitive symptom expression under optimum growing conditions.

Cachexia (xyloporosis) reactions and symptoms in susceptible citrus trees tend to be variable and slow to appear (2, 6). The shortest time required for cachexia detection by biological assay methods utilizing Parson's Special mandarin is about 6 months to 1 year depending on greenhouse conditions (8, 12). Results with this method are somewhat variable and appear to be considerably influenced by temperature and possibly by other environmental factors. Another method reported in 1965 (11) utilizing Orlando tangelo produced some cachexia symptoms in 10 months, but in other tests symptom expression required as long as 24 months. Since citrus cachexia is an important gumming and stem-pitting disease of certain mandarins, tangelos, and other citrus throughout the world, and it is highly transmissible mechanically by tools (10), there is a need for a quicker and more reliable method of detection. This paper reports investigation of 63 citrus cultivars as possible biological assay indicators for citrus cachexia.

MATERIALS AND METHODS

Beginning in 1976, seedlings of 84 citrus cultivars were evaluated as rapid test indicators for citrus cachexia disease. All were mandarins, mandarin hybrids, or species closely related to the mandarins, and represented most of the cultivars in this group that were available in the variety collection at the University of California Citrus Research Center at Riverside. Twenty-one of these cultivars were eliminated because of poor seed germination, excessive seedling variability, or inherent weakness of the seedlings. The balance of the 63 cultivars is listed in table 1.

Seed was sown in flats in a UC

TABLE 1
CULTIVARS EVALUATED FOR CITRUS CACHEXIA SYMPTOM EXPRESSION

Name	CRC No.*	Name	CRC No.*
1. Altoona tangelo	2792	33. Mandarin (unnamed)	3086
2. Beauty of Glen Retreat mandarin	3279	34. Minneola tangelo	3145
3. Belady mandarin	3363	35. Nova mandarin	3615
4. Bishop tangelo	2782	36. Oneco mandarin	263
5. Bower mandarin	3649	37. Ortanique tangor	3400
6. *Citrus benikojii*	3149	38. Owari mandarin x Imperial grapefruit	2871
7. *C. depressa*	2448	39. Pearl tangelo	2849
8. *C. sunki*	2868	40. Ponkan mandarin	2329
9. *C. tachibana*	3150	41. Robinson mandarin	3644
10. *C. yatsushiro*	3466	42. Roeding mandarin	1200
11. Clementine x Silverhill mandarin	3731	43. Sampson tangelo	312
12. Cleopatra mandarin	270	44. Scarlet Emperor mandarin	3326
13. Dancy mandarin	3026	45. Sexton tangelo	2543
14. De Ba Ahmed mandarin	3369	46. Siamelo	2586
15. Dweet tangor	3018	47. Soh Niamtra mandarin	3260
16. Early tangelo	2560	48. Solid Scarlet mandarin	3328
17. Empress mandarin	3613	49. Sumwui Kom mandarin	3081
18. Fairchild mandarin	3559	50. Sunrise tangelo	2606
19. H-56 tangor	3096	51. Sunshine tangelo	2788
20. Hill mandarin	3478	52. Szinkom mandarin	3085
21. Honey mandarin	3177	53. Tangelo hybrid (open pollinated)	3331
22. King tangor	303	54. Tankan tangor	2224
23. Kinnow mandarin	3021	55. Thornton tangelo	2013
24. Kunembo mandarin	3077	56. Tien Chieh mandarin	2376
25. Lee mandarin	3614	57. Tim Kat mandarin	2692
26. Mandarin hybrid (open pollinated)	3232	58. Webber tangelo	2746
27. Mandarine sanguine	3367	59. Willowleaf mandarin	3362
28. Mandarinette mandarin	3405	60. Willowleaf x blood orange	2870
29. Mandarin (unnamed)	3367	61. Willowleaf x Imperial grapefruit	2872
30. Mandarin (unnamed)	3405	62. Wilsh tangelo	2843
31. Mandarin (unnamed)	2377	63. Yalaha tangelo	2559
32. Mandarin (unnamed)	2869		

* Citrus Research Center accession numbers assigned in approximate order of acquisition beginning in 1909.

potting mix (4). When the seedlings were about 10 cm high, 24 of the most uniform were selected and transplanted into 4-liter plastic containers, 3 seedlings per container. Seedlings were then grown in a greenhouse until the lower portion of the stem was about 7.5 mm in diameter. Two seedlings in each container were inoculated while the third was left as a control. Two sources of cachexia in Valencia orange were used—Ca 902 and Ca 908; both had previously shown severe cachexia symptoms in the Parson's Special mandarin test, and both were negative for exocortis and other citrus pathogens. Two methods of inoculation were used: half the plants were inoculated by T-budding with two buds in each plant and the other half by the leaf-disc grafting method (1) with one leaf-disc in each of four leaves per plant. After inoculation, all plants were cut back at about 22 cm above the soil. For positive controls, 32 plants of Parson's Special mandarin on rough lemon rootstock were bud-inoculated with the same sources of cachexia.

Plants were held in a warm

greenhouse (38 C maximum day, 24 C minimum night) and observed for a period of one year following inoculation. Two locations were used: half the plants, consisting of both bud and leaf-disc inoculations, were kept in a greenhouse at Riverside; the other half were grown also under warm conditions in a greenhouse at Lindcove in the central valley of California. Periodic examinations and measurements were made in an attempt to detect any unusual symptoms in leaves or stems that might delineate cachexia. After 6 months of growth, plants were cut back to a height of about 15 cm, and the bark was peeled from the cut-off portions so the cambial area could be examined for discoloration, gumming, pitting, or any other symptoms of cachexia infection. Plants were allowed to grow for 6 additional months and were then harvested and the bark of the entire plant peeled for cambial observation. In each case where some obnormal growth symptom appeared, the test was repeated with another set of plants, either as new seedlings or clonally propagated from an explant freed of cachexia by shoot tip grafting *in vitro* (5).

RESULTS AND DISCUSSION

All positive control Parson's Special mandarin plants exhibited the strong gumming reaction typical of cachexia after one year. Inoculated seedlings of 61 of the 63 cultivars showed no growth or symptom differences from the controls. Only Bishop tangelo and Ortanique tangor appeared promising.

Bishop tangelo produced highly variable seedlings, several of which exhibited shoot tip distortion within 3 months following inoculation with cachexia. The two Bishop tangelo seedlings which showed the most severe shoot tip distortion were freed of cachexia virus by shoot tip grafting *in vitro* and were then increased clonally and retested by reinoculation with cachexia. In this second test, the uninoculated controls also showed as much shoot tip distortion as the inoculated plants. Therefore, Bishop tangelo was eliminated as a possible rapid cachexia indicator.

Ortanique tangor exhibited moderate to severe leaf chlorosis in several inoculated seedlings. Since the seedlings of Ortanique were highly uniform, they were presumed to be mostly nucellar, and this cultivar was retested by growing more seedlings. This second test was carried out under two temperature regimes in the greenhouse. In the cooler greenhouse room (28.3/19.8 C), there were no differences detected between cachexia-inoculated plants and controls. In the warmer greenhouse room (35.7/23.9 C), inoculated seedlings again exhibited considerably more leaf chlorosis than controls. A third test with Ortanique was initiated in the warm greenhouse room. In this test, both bud and leaf punch inoculations with three cachexia sources were used. Results were completely negative. Both inoculated plants and uninoculated controls exhibited the same degree of chlorosis, indicating that Ortanique cannot be used as a test plant for cachexia. This points out the importance of repeating a test to determine its reliability over a period of time.

Cachexia disease remains the only major citrus disease which cannot be detected by rapid seedling index. There is recent evidence that cachexia may be a viroid (9). If so, perhaps laboratory techniques can be developed for rapid detection. Studies are now under way using polyacrylamide gels (PAGE). It is apparent from our studies that short-term biological

detection of cachexia by use of indicator plants will be extremely difficult.

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