

# Growth and Yield of Young Sweet Orange Trees on Swingle Citrumelo Rootstock Inoculated with Citrus Viroids\*

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**ABSTRACT.** Hamlin sweet orange trees budded to Swingle citrumelo were planted in a commercial grove and inoculated 6 months later with four isolates of exocortis viroid characterized by their citron indexing reaction as either mild or severe. The inoculum sources were apparently free of xyloporosis viroid. The randomized complete block experiment consisted of two-tree plots, six replications, and an uninoculated control. Trees on Swingle citrumelo are apparently tolerant of exocortis viroid in terms of bark scaling on the rootstock but their growth and yield were affected depending on the isolate. After 9 yr, no bark scaling below the bud union had occurred. Scion trunk cross sectional areas, tree heights, and canopy volumes were generally smaller in those trees inoculated with a severe isolate. There were no differences among treatments in fruit size or juice quality. Uninoculated trees had a mean 5-yr cumulative yield of 447 kg that was significantly higher than for trees inoculated with a severe isolate which had cumulative yields of less than 375 kg/tree. Trees with a mild isolate were intermediate in size and yield.

*Index words.* citrus exocortis viroid, sequential PAGE, citrus viroids.

Trifoliolate orange is well-known for its susceptibility to exocortis disease as are some of its sweet orange hybrids such as Carrizo and Troyer citranges (2, 7, 9). Less is known about the effects of this disease on hybrids of grapefruit with trifoliolate orange. One such hybrid, Swingle citrumelo, has become a popular commercial rootstock in Florida (8); however, its use has not been restricted by any concern about exocortis because many scion cultivars are propagated from registered mother trees tested free of citrus exocortis viroid (CEV).

Biological indexing of citrus trees for CEV has commonly been done using citron plants. This method is now considered less reliable because recent reports have clearly shown that other viroids can be present in citrus trees (1, 10, 11, 12, 13, 14, 18, 19, 20). These viroids, perhaps alone or in various combinations, appear to influence the expression of exocortis symptoms and may have other presently unknown independent effects on tree behavior (10, 13, 16, 20). Therefore, our objective was to determine citrus viroid effects in sweet orange trees on Swingle citrumelo.

## MATERIALS AND METHODS

Registered Hamlin (clone: H-3-28-5-XE) sweet orange trees on Swingle citrumelo were obtained from a commercial nursery and planted in December 1979 in a commercial grove near Fort Pierce, Florida. The soil in this area is bedded because of naturally poor drainage. The trees were arranged in randomized complete blocks on a double-row bed at a spacing of 5.5 × 7.3 m with two-tree plots across the bed and six replications.

The treatments, summarized in Table 1, were isolates presumed in 1979 to contain CEV because they caused a reaction in citron and were classified according to that response. When our experiment was initiated, the complete viroid content of each field source was unknown. Each isolate is part of a CEV collection and was designated with an 'E' number that is used herein.

Approximately 6 months after planting, the trees were inoculated by inserting three bark chips per tree into the trunk. The original field tree was used as the inoculum source for treatments E-1 and E-4. Trees from another experiment which had been inoculated

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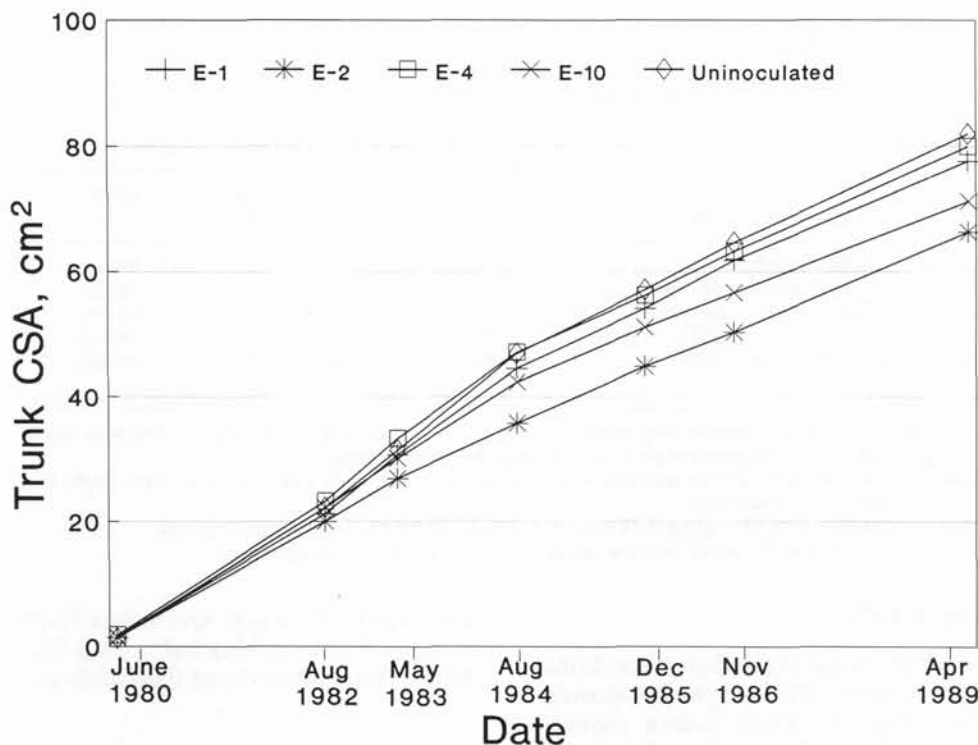


Fig. 1. Changes in trunk cross-sectional areas (CSA) of Hamlin sweet orange trees on Swingle citrumelo inoculated with sources giving a severe CEV reaction (E-1, E-2) in citron, a mild reaction (E-4, E-10), or uninoculated.

with bark chips from a commercial Pineapple sweet orange (clone: Pi-55-44-19-X) mother tree and the original field tree were the sources for E-10 and E-2, respectively. Inoculation was considered successful if two chips remained alive; if only one survived, the tree was reinoculated.

Five yr after planting, all the experiment trees were indexed with citron. The inoculated trees gave a positive response for CEV and the control trees were negative. The source trees were also indexed with Parsons Special mandarin for xyloporosis and were negative after 2 yr.

Trunk circumference was measured periodically 15 cm above the bud union and data converted to cross-sectional area. Tree canopy width and height were measured in 1989. Fruit samples were collected annually for juice analysis and yield per tree was measured during the 5-yr period from 1984-85 to 1988-89.

Viroid content was determined by collecting budwood from four replications of the experiment which was then used to graft inoculate citron 861 plants. After the citron plants were systemically infected (3 months or longer), bark was stripped from young growth flushes, weighed into 8-g aliquots, and viroids extracted and purified using the procedure described by Duran-Vila *et al.* (11). The purified viroids were characterized for relative size by sequential polyacrylamide gel electrophoresis (sPAGE) (17).

The presence of citrus tristeza virus (CTV) was determined in the citron plants inoculated from the experiment trees by double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) (3). Polyclonal antisera prepared against whole, unfixed CTV isolate T-26 was used in DAS-ELISA.

Data were subjected to an analysis of variance with mean separation by Duncan's multiple range test.

TABLE 1  
CITRUS EXOCORTIS AND RELATED VIROID EFFECTS ON THE GROWTH AND  
YIELD OF 10-YEAR-OLD TREES OF HAMLIN SWEET ORANGE ON  
SWINGLE CITRUMELO ROOTSTOCK

Isolate	Original host <sup>z</sup>	Citron reaction <sup>y</sup>	Bark scaling <sup>x</sup>	Cum. yield (1984-89) (kg/tree)	Canopy vol. (m <sup>3</sup> )	Treeht. (m)	Increase in trunk cross-sectional area (1980-89) (cm <sup>2</sup> )
—	Uninoculated	—	—	447 a <sup>w</sup>	16.9 a	2.7	80.6 a
E-1	Marsh/Rangpur	Severe	+	357 c	16.3 a	2.7	76.2 ab
E-2	Val/trifoliolate	Severe	+	365 bc	12.7 bc	2.5	64.9 b
E-4	Val/trifoliolate	Mild	—	422 ab	11.2 c	2.4	78.1 ab
E-10	Pine/Rangpur	Mild	—	373 bc	14.7 ab	2.8	69.6 ab
						ns	

<sup>z</sup>Host from which the isolate was obtained. Marsh = Marsh grapefruit; Val = Valencia sweet orange; trifoliolate = trifoliolate orange; Pine = Pineapple sweet orange.

<sup>y</sup>Severe reaction gives severe leaf epinasty and vein necrosis; mild reaction gives very slight leaf epinasty without vein necrosis.

<sup>x</sup>Based on observation of original field source tree from which the isolate was obtained.

<sup>w</sup>Mean separation by Duncan's multiple range test, 5% level; ns = nonsignificant.

## RESULTS

Differences in trunk cross-sectional area occurred within 2 yr after inoculation (Fig. 1). Each isolate reduced trunk growth below that of the uninoculated trees although the differences were small and often not significant (Table 1). The relative effects established early in the experiment persisted for the remainder of the trial. Trees inoculated with E-2 had the smallest change in trunk CSA after 9.5 yr but there were no significant differences among the isolates. Trees inoculated with E-2 and E-4 had the smallest canopies. There were no treatment effects on tree height or juice quality. The control trees had the highest cumulative yield, 447 kg/tree, and differed significantly from the E-1, E-2, and E-10 trees which had yields less than 375 kg/tree.

Analysis of the field sources of exocortis viroid by purification on CF-11 cellulose and separation on sPAGE gels indicated that each isolate used in the experiment contained viroid bands which corresponded to CEV and other viroids (Fig. 2). The presence of CEV was confirmed by dot hybridization assays with cDNA clones (T. O. Diener, personal communication). Isolates E-4, E-1, and E-2 each contained two smaller viroid-like bands in addition to

the larger CEV band, and isolate E-10 contained one additional viroid-like band. The smaller viroid-like bands are

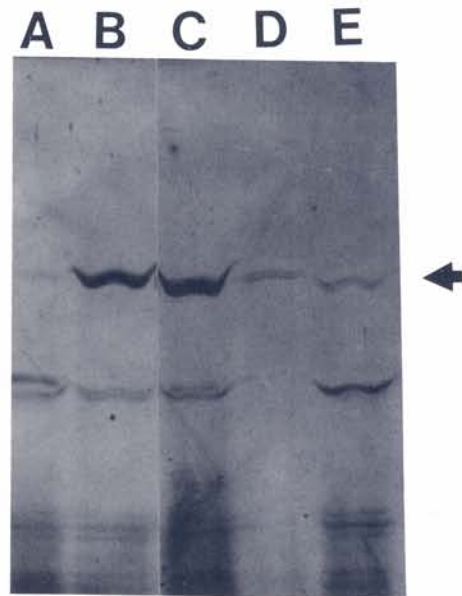


Fig. 2. Silver stained viroid and viroid-like RNAs after extraction from bark of inoculated citron plants, purification on CF-11 cellulose and sequential polyacrylamide electrophoresis on 5% gels containing 8M urea. Lane A is from E-4 infected citron; lane B is from E-1; lane C is from E-2; lane D is from chrysanthemum stunt infected Bonnie Jean chrysanthemum; and lane E is from E-10. The location of CEV is indicated by the arrow.

probably in the size range of the CV-II group as described by Semancik (18) although direct size comparisons were not made.

All citron plants inoculated with buds from the experiment trees were CTV infected as determined by ELISA and a severe strain of the virus was indicated by the degree of stem pitting in each citron plant.

## DISCUSSION

It seems reasonable to consider Swingle citrumelo as tolerant to CEV based on our results. The exocortis viroid was present in each inoculum source and, although tree growth was affected, it was only reduced to a small extent. Growth reductions have been reported for other tolerant rootstocks inoculated with CEV (16). Nevertheless, this classification of Swingle citrumelo is tentative because other viroids, such as reported herein, are found in citrus trees and can affect their behavior (13, 16). The individual field effects of these additional viroids are largely unknown, thus, their potential to interfere in the reaction of a citrus tree to CEV is also unknown. There is also evidence of interactions among these viroids (13, 19). Some of the symptoms in field trees and indicator plants assumed to be caused by CEV might be induced by other citrus viroids (10, 13, 20). The dwarfing of citrus trees on susceptible rootstocks, for example, was thought to be a CEV

reaction but it is likely that other viroids are involved (4, 5, 6, 13, 15).

There are indications in our results of possible tree responses to the viroids, other than CEV, detected in the inoculum sources. Isolates E-1 and E-2 caused a severe citron response and bark scaling in the original field sources but not in the experiment trees; however, if bark scaling on trifoliolate orange is a reaction to CEV, then that symptom would not be expected in trees on a tolerant rootstock like Swingle citrumelo as compared to the susceptible rootstocks of the host trees (Table 1).

The growth and yield of the experiment trees were not clearly divided according to the citron reaction of the four isolates. The E-1 isolate had virtually no effect on the three measures of growth but those trees produced the least amount of fruit. When the two "mild" isolates (E-4 and E-10) are compared, their effects on tree size and productivity were not identical.

We have provided preliminary evidence that Swingle citrumelo is tolerant to CEV; however, confirmation of this will require a more complete understanding of the disease and horticultural impacts of CEV and related citrus viroids on trees budded to Swingle and other citrumelos. Also, clarifying the apparent difference in sensitivity of trifoliolate orange and its sweet orange hybrids to CEV vs. its grapefruit hybrids may be useful in rootstock improvement programs.

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