Observations on the Effect of Viroid Inoculation of Grapefruit Grafted on Carrizo Citrange Planted at High Density

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ABSTRACT. Young budlings of Star Ruby grapefruit grafted on Carrizo citrange rootstock were inoculated in the nursery with a citrus exocortis viroid (CEVd) source. One year later, inoculated and noninoculated plants were established in the field at a 2.25 × 3.25 m spacing (1,367 plants/ha). Side plots of Star Ruby grapefruit on siamelo and Flying dragon were also established for comparison. The growth, canopy volume, cumulative fruit yield and fruit quality were evaluated after 4 yr. CEVd inoculation of grapefruit on Carrizo citrange rootstock resulted in a 10% reduction of tree height and trunk diameter and a 30% reduction in canopy volume in comparison with uninoculated control plants and the infected trees showed higher yield efficiency than healthy control plants. Fruit quality was not affected by viroid inoculation and the infected plants did not show symptoms. The lowest mean values of tree height, trunk diameter and canopy volume, and the highest yield efficiency were obtained from trees grafted on Flying dragon; whereas the largest tree height and canopy volume and the lowest yield efficiency were from trees grafted on siamelo.

In 1968, Cohen in Florida first reported on the possible use of exocortis to produce dwarf trees (5), but Australia was the first country to conduct large-scale tests of high density plantings using trees on trifoliate orange dwarfed by graft-transmissible agents (10). At that time the dwarfing factor was unknown, although the possibility that viroids might be involved was suggested (3). Several papers have discussed the beneficial aspects of the dwarfing technology using infectious agents (1, 4, 7, 11, 12). However, it was only after development of sequential polyacrylamide gel electrophoresis (sPAGE) for viroid identification could the effect of specific viroids or mixtures on infected plants be properly evaluated (7, 9, 13). These data suggested that the rootstocks most suitable for induction of dwarfing through viroid inoculation were trifoliate orange and citranges.

Because most reports of tree dwarfing are based on a small-scale plots where inoculation was not done purposely and a mixture of viroids were present in the field plots (14), we must be cautious in interpretating previous results. If viroids are chosen as a dwarfing agent (2), certain "safety rules" should be followed (8) and only identified sources of viroids should be used.

In this paper, we report preliminary observations on growth and production of grapefruit trees propagated on several rootstocks among which was Carrizo citrange and was dwarfed with CEVd and planted at high density in a large-scale plot.

A CEVd isolate inducing severe epinasty, stunting and leaf curl on Etrog citron Arizona 861-S1 was phenol-extracted from young bark, partially purified by CF11 cellulose column chromatography (6), and then separated in a 5% polyacrylamide gel. The CEVd band was eluted from the gel and slash inoculated onto trifoliate orange and Troyer citrange, which served as donor plants for further inoculation.

One-yr-old Star Ruby grapefruit propagated on Carrizo citrange (2,447 trees) were graft-inoculated in 1991 with two bark patches from donor plants and planted in 1992 following a 3.25 m × 2.25 m spacing (1,367 trees hectare) in Calabria in southern Italy. Ten percent of the plants were left as uninoculated controls randomly distributed in the experimental plot. Six rows of grapefruit plants propagated on siamelo and three on Flying dragon trifoliate orange were also planted to compare performance of CEVd-inoculated grapefruits with similar plants on a vigorous or a dwarfing rootstock. All together, about 4,000 trees were established in a sandy clay loam soil with pH 7.25 and low electric conductivity (0.63 dS/m).

Tree height and rootstock trunk diameter 30 cm above the ground were measured annually, and the canopy volume was estimated in 1995. The cumulative yield, fruit size and juice quality were also evaluated. The viroid content of inoculated and uninoculated trees was analyzed by sPAGE (15) in 1994 and 1995.

Table 1 presents the average size and cumulative yield of trees in each treatment after 4 yr. Inoculation of grapefruit trees on Carrizo citrange with CEVd resulted in 10% reduction of tree height and trunk diameter and 30% reduction of the canopy volume in comparison with uninoculated control trees. The lowest mean values of tree height, trunk diameter and canopy volume were obtained in grapefruit trees grafted on Flying dragon; the largest trunk diameter in trees on Carrizo citrange without CEVd inoculation; and the largest tree height and canopy volume values were trees on siamelo. The highest cumulative yield per tree was obtained from uninoculated plants grafted on Carrizo citrange, followed by those on Flying dragon, CEVd inoculated plants on Carrizo citrange and plants on siamelo; whereas the highest yield efficiency was observed on grapefruit plants grafted on Flying dragon, followed by CEVd inoculated plants on Carrizo citrange, uninoculated plants on the same rootstock and plants grafted on siamelo. CEVd inoculation of plants on Carrizo citrange did not affect fruit quality in comparison with viroid-free control plants. Viroid analysis of bark extracts by sPAGE confirmed the inoculum stability in the infected plants, and the absence of contaminations in the uninoculated plants (data not shown).

These preliminary results indicate that grapefruit trees on Carrizo citrange grew well and did not show symptoms 4 yr after inoculation with CEVd. They were not uniform but, presently, the average yield is satisfactory. These findings agree with previous reports that CEVd-infected trees on Carrizo citrange may remain symptomless after 25 yr (1). Moreover, citrange is the only rootstock suitable for dwarfing purposes in Italy, as few soils allow growing trifoliate orange or Flying dragon.

Viroid inoculation seems to offer good possibilities to obtain trees with reduced size and good yield efficiency which are suitable for high density plantings. However, additional information on the possibilities of viroid dissemination through pruning tools under field conditions is still needed. A careful risk assessment with evaluation of the cost-benefit ratio should be done before massive release of a pathogen for horticultural purposes is allowed. With this aim at least the following points should be considered:

- A reasonable long-term observation is needed to assess the stability of the pathogen to fully diagnose undesirable side effects of the inoculation.
- Only thoroughly characterized viroids (nucleotide sequence, host range, type of symptoms) should be used.
- Inoculated budwood should be free of other graft-transmissible disease agents.
- To achieve closer control of the pathogen, only budwood source trees should be inoculated and the stability of the pathogen periodically checked.
- Only viroid sources tested for specific varieties under local conditions should be used, as environmental conditions and culture

TABLE 1

GROWTH AND CUMULATIVE YIELD OF 4-YR-OLD STAR RUBY GRAPEFRUIT GRAFTED ON CARRIZO CITRANGE ROOTSTOCK WITH AND WITHOUT CEVd INOCULATION COMPARED WITH SIMILAR TREES ON A VIGOROUS (SIAMELO) AND A DWARFING (FLYING DRAGON TRI FOLIATE ORANGE) ROOTSTOCK

	Canopy volume m^3	Area under canopy m^2	Trees/ha[z]	Tree height cm	Trunk diameter cm	Cumulative yield (1993-95) Kg/tree	Yield efficiency	
							Kg/m^3	Kg/ha
Carrizo + CEVd[y]	3.33	2.69	3.7	177	6.5	48.5	14.6	24.6
Carrizo[x]	4.82	3.60	2.8	195	7.2	57.6	11.9	21.87
Flying dragon[w]	2.02	1.98	5.1	156	6.0	51.1	25.3	32.27
Siamelo[v]	7.93	4.66	2.1	252	6.9	41.7	5.2	12.16

[z]Estimated number of trees according to the actual area under canopy
[y]Mean values from 120 trees
[x]Mean values from 120 trees
[w]Mean values from 40 trees
[v]Mean values from 100 trees

techniques may alter the effects observed in other conditions.

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