Field Evaluation of Dwarfing Effect of Two Combinations of Citrus Viroids on Different Citrus Species

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ABSTRACT. Seedlings of trifoliate orange, Troyer citrange and sour orange, and trees of sweet orange and clementine grafted on the above rootstocks, have shown a different reduction in size when inoculated with two combinations of citrus viroids. Inocula used were: ARA, a source containing citrus exocortis viroid (CEVd) plus two additional citrus viroids (CVd), that induced severe stunting on trifoliate orange and citrange (as seedlings or rootstocks); CMC, a source having a viroid RNA CVd-III group, that induced mild dwarfing. With both viroids sources 5 yr after inoculation, no effect was observed on sour orange and fruits showed no differences in quality. More yield was noted in Moro sweet orange grafted on trifoliate orange and citrange inoculated both with ARA and CMC inocula than uninoculated trees. Electrophoretic profiles of extracts from different seedlings and trees showed no modification of the pattern of viroids during 5 yr of observations.

Since Cohen (8) proposed the use of exocortis to obtain dwarfed trees, many studies have evaluated stunting effects of strains or sources of viroids on different stock-scion combinations (4, 5, 6, 7, 9, 10, 13, 14). Recent studies have shown that many citrus viroids (CVd), distinct from CEVd, are present in commercial plantings often as a mixture. Some of them have been found separately or inoculated on susceptible species where they induce mild symptoms of exocortis (1, 11) or xyloporosis diseases (12). Their effects on commercial stock-scion combinations have been investigated by different authors (6, 12, 14).

In this paper we report the results of the field evaluation of two inoculum sources to limit the tree size, and of the biochemical analysis to determine any change in viroid content during 5 yr of observation. Preliminary results have been published elsewhere (2).

MATERIALS AND METHODS

Six-month-old seedlings of trifoliate orange, Troyer citrange and sour orange, grown in the greenhouse, were planted in the field in the spring of 1983 at a spacing of 1 x 1 m. Twenty-four trees of each species were budded to Moro sweet orange and SRA]63 clementine in June, 1984. Moro scions were taken from virus-free nucellar trees, whereas clementine were from an old virus-free line from Corsica. The same number of seedlings of each stock were left ungrafted.

Inoculation was done in the summer of 1984, by insertion of two pieces of bark into each trunk. Two different inoculum sources were used (eight trees per treatment). The ARA source was a clementine grafted on sour orange which induced a severe reaction on Etrog citron. Polyacrylamide gel electrophoresis (PAGE) revealed the presence of CEVd and two other bands belonging to the CVd-II and CVd-III groups (11). The CMC source was a clementine on alemow and gave a mild reaction on Etrog citron. Extracts of this source showed in PAGE a single band belonging to CVd-III group. Both sources were free from tristeza, psorosis and citrus variegation.

Eight uninoculated trees were left as healthy controls.

Field observations. Five years after inoculation, the trunk circumference above and below the budunion for budded trees and at 30 cm above the ground for seedlings and the yield were measured. The presence of symptoms was checked every year. Treatment means were compared using Duncan's multiple range test.

Electrophoretic and hybridization tests. Nucleic acids were extracted from field samples collected each year in summer. The method of extraction and the electrophoretic and hybridiza-

TABLE 1
CIRCUMFERENCE OF SEEDLINGS, MORO SWEET ORANGE AND CLEMENTINE SRA 63 FIVE YEARS AFTER INOCULATION

Rootstocks and inocula	Seedlings[z]	Moro		Clementine[y]	
		stock	scion	stock	scion
Troyer citrange					
ARA[x]	23.5b[w]	19.3b	17.1c	23.2a	16.5b
CMA[x]	26.4ab	24.5b	22.7b	21.0a	17.2b
Healthy	29.5a	29.5a	27.7a	25.0a	22.5a
Trifoliate orange					
ARA	12.9c	15.0b	14.0b	16.8c	14.1b
CMC	15.8b	16.5b	13.2b	17.7b	14.5b
Healthy	22.2a	22.3a	17.6a	23.0a	19.0a
Sour orange					
ARA	22.3b	22.5a	23.5a	21.8a	22.0a
CMC	22.9a	22.6a	22.6a	21.5a	21.5a
HEALTHY	23.0a	22.5a	23.0a	21.7a	21.8a

[z]trunk circumference at 30 cm above the ground.
[y]trunk circumference at 4 cm below budunion (stock); trunk circumference at 4 cm above budunion (scion).
[x]ARA = severe strain; CMC = mild strain.
[w]Mean separation by Duncan's multiple range test, ≤ 0.05.

tion tests utilized were as described (3). CEVd and hop stunt viroid (HSVd) probes were used in molecular hybridization tests.

RESULTS

Field observations. Both inocula induced a significant reduction in size on seedlings and trees grafted on trifoliate orange or Troyer citrange. A dwarfing effect was also evident on sour orange seedlings inoculated with ARA (Table 1). That source induced a stronger stunting effect than CMC in all cases. The ARA inoculated seedlings were significantly smaller than healthy ones; differences were also observed between the two sources when inoculated on trifoliate orange. Moreover, ARA caused severe yellow blotching on trifoliate orange seedlings. A mild yellow blotching was also noted on a single Troyer citrange seedling inoculated with ARA. No symptoms were observed on species inoculated by CMC. None of the inoculated trees showed scaly-bark symptoms.

The fruit yield on all trees was too low to draw any definite conclusions. Moro sweet orange grafted on trifoliate orange or Troyer citrange showed earlier fruiting and yielded significantly more than uninoculated trees (Table 2). No difference was observed in clementine trees (Table 3). Fruit size, juice percentage, citric acid and total soluble solids showed no difference among treatments (Tables 2 and 3).

Electrophoretic and hybridization tests. Polyacrylamide gel electrophoresis (PAGE) profiles of nucleic acid extracted from the different inoculated species were the same as those extracted from the inoculum sources. ARA contained CEVd and two other bands belonging to the CVd-II and CVd-III groups; CMC inoculated trees showed only CVd-III RNA. Uninoculated control trees were free of viroid-RNAs in PAGE analysis. CEVd and HSVd specific DNA probes confirmed the presence of the two viroids in ARA extracts and their absence in CMC extracts.

DISCUSSION

Results show that the dwarfing effect varies according to inoculum

TABLE 2
YIELDS, FRUIT SIZES AND JUICE COMPONENTS OF MORO SWEET ORANGE ON DIFFERENT ROOTSTOCKS FIVE YEARS POST INOCULATION

Rootstocks and inocula	Yield/tree (Kg)	Fruit size (g)	Juice (%)	Citric acid (g/100 ml)	TSS[x] (%)
Troyer citrange					
ARA[z]	7.3b[y]	116.7a	47.7a	2.2a	11.9a
CMC[z]	7.0b	119.5a	43.8a	2.4a	11.9a
HEALTHY	4.4b	120.4a	46.4a	2.2a	11.9a
Trifoliate orange					
ARA	8.5b	114.5a	41.8a	1.9a	11.4a
CMC	9.4b	110.1a	40.0a	2.0a	12.0a
HEALTHY	6.2a	118.6a	41.6a	2.1a	12.5a
Sour orange					
ARA	7.8a	128.4a	43.7a	2.4a	12.3a
CMC	7.1a	120.5a	42.9a	2.3a	12.4a
HEALTHY	7.5a	119.8a	41.5a	2.1a	12.0a

[z]ARA = severe strain; CMC = mild strain.
[y]Mean separation by Dundan's multiple range test, P≤ 0.05.
[x]Total soluble solids.

source, citrus species and stock-scion combination. The ARA source appeared to effectively induce dwarfing. Trifoliate orange was very sensitive to both combinations of viroids. Reduction of trunk circumference was 42% and 28% when inoculated with the ARA source or the CM source, respectively.

The biometric measurements confirmed preliminary results obtained 4 yr after inoculation (2).

Due to the severe effects, ARA inoculum is likely an undesirable dwarf-

TABLE 3
YIELDS, FRUIT SIZES AND JUICE COMPONENTS OF CLEMENTINE SRA 63 ON DIFFERENT ROOTSCOCKS FIVE YEARS POST INOCULATION

Rootstocks and inocula	Yield/tree (Kg)	Fruit size (g)	Juice (%)	Citric acid (g/100 ml)	TSS[x] (%)
Troyer citrange					
ARA[z]	9.9a[y]	58.5a	46.8a	1.9a	13.6a
CMC[z]	7.6a	64.2a	46.6a	2.1a	13.9a
HEALTHY	8.9a	65.8a	47.2a	1.7a	12.8a
Trifoliate orange					
ARA	7.8a	70.1a	38.4a	1.6a	11.4a
CMC	7.9a	71.2a	39.3a	1.8a	12.6a
HEALTHY	83.a	73.1a	41.6a	1.6a	12.4a
Sour orange					
ARA	10.4a	64.6a	43.2a	1.9a	13.7a
CMC	11.7a	61.5a	42.1a	2.0a	14.0a
HEALTHY	12.3a	59.8a	45.5a	2.1a	14.0a

[z]ARA = severe strain; CMC = mild strain.
[y]Mean separation by Duncan's multiple range test, P ≤ 0.05.
[x]Total soluble solids.

ing factor on trifoliate orange. It may be useful in Troyer citrange unless, in further years, scaly-bark symptoms arise.

The CMC source has some promise because of its mild stunting effect on trifoliate (about 25%) and moderate stunting effect on citrange. No symptoms were observed on citrus inoculated with this source.

Sour orange grafted trees have not yet shown any reaction to the viroid inoculation. Since other authors (3) have reported stunting of this rootstock by viroids, we plan a long evalution.

The results of the electrophoresis and hybridization tests confirmed the stability of the inocula utilized over the past 5 yr, regardless of citrus species or combination.

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