Effect of Citrus Viroids and the Influence of Rootstocks on Field Performance of Navel Orange

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ABSTRACT. Studies continue on testing the field performance of Washinton navel orange trees on sour orange and Troyer citrange rootstocks inoculated with citrus exocortis viroid (CEV) as well as several citrus viroids and mixtures characterized by mild to moderate reactions in citron. Tree size, fruit production, color development and fruit quality were evaluated during 10 fruiting seasons at the Lindcove Research Field Station in the Central Valley of California. The presence of CEV and combinations of citrus viroid-Ia, -IIa and -IIIb reduced tree size and yield. Navel orange on Troyer citrange rootstock continued to outyield trees on sour orange rootstock. The retardation of color development noted in 1985 was not observed in the 1988 or 1989 seasons. Fruit quality was not affected by presence of viroids. However, fruit from trees on Troyer citrange rootstock continued to show earlier higher sugar-acid ratios.

In 1977, an experiment was designed to test the effect of CEV and a number of "mild" isolates of CEV on the performance of navel orange trees grafted on two rootstocks: sour orange and Troyer citrange. At the inception of this experiment certain isolates of CEV were judged as "mild" based on intensity of bark cracking symptoms on trifoliate rootstocks and also on mild symptoms induced on greenhouse-indexed citron plants. The range of symptoms induced on citron by these "mild" isolates were all less severe than those caused by CEV. Several years after the initiation of this experiment, these milder reactin "exocortis isolates" were shown to be a series of distinct citrus viroids (CV) in pure form or in various mixtures containing CV-Ia, -IIa or -IIIb (3, 4, 5, 10, 13).

Nauer *et al.* (8) reported that after six fruiting seasons (1980-1985), presence of CEV significantly reduced fruit yield and tree size. Also, in the fruit color evaluation of 1985, presence of CEV induced a slower color development of navel fruit on trees on both rootstocks when compared to fruit on viroid-free trees. The presence of certain mixtures of citrus viroids were also shown to have an effect on retarding fruit coloring during this one season. Although some trends were noted on the effects of CV on reduced tree size and fruit maturity, they were not significant statistically. There were no effects on fruit shape, rind thickness or juice percentage. A major finding of this first report was the superiority in performance of navel orange trees on Troyer citrange rootstock in fruit yield, size and color development when compared to trees on sour orange rootstock (8). This rootstock difference was independent of viroid content.

This paper reports the results of continuing studies over four additional fruiting seasons on the effects of CEV and CV on tree size, fruit yield, fruit size, fruit coloring and sugar-acid ratios for navel oranges on sour orange and Troyer citrange rootstocks.

MATERIALS AND METHODS

Old-line Parent and Atwood navel orange buds containing various citrus viroids and free of all other known viruses were grafted on sour orange and Troyer citrange seedlings in 1977. The citrus viroids present and the citron reaction induced are given in Table 1. Details of the background and history of the inoculum sources were presented in the previous report (8). All inoculum sources had been previously indexed to appropriate indicator plants and were found free of all other known graft transmissible

TABLE 1
CITRUS VIROIDS PRESENT IN EXPERIMENTAL TREATMENTS, VIROID SOURCES AND THEIR REACTIVITY TO CITRON

Treatments	Citrus viroids (CV)	Viroid code	Navel scion variety	Reactivity in citron[z]
A	IIa	E-829	Parent	0.5
B	IIa	E-818	Parent	1.5
G	IIa	E-818	Atwood	1.5
C	IIa, IIIb	E-805	Parent	3.0
D	Ia, IIa, IIIb	E-803	Parent	4.0
E	CEV, IIa, IIIb	E-800	Parent	10.0
F	STG[y]-Neg	—	Atwood	0

[z]Reactivity in citron: 0 = negative; 1 = very mild; 2-3 = mild; 4-6 = moderate; 7-8 = severe; 9-10 = very severe.
[y]Shoot tip grafted negative control.

pathogens. Trees were planted in 1978 in a completely randomized block at the Lindcove Field Station located in the Central Valley of California. There were 10 replicates for each of the seven treatments respectively on two rootstocks (sour orange and Troyer citrange) totaling 140 trees. Trees were examined annually.

Fruit yield by weight was obtained annually for each tree over ten fruiting seasons from 1980 through 1989. Tree volume was calculated from height and width measurements in 1985 and 1989. Fruit rind color readings were obtained only in 1985, 1988, and 1989. Two observers examined fruit on each side of the tree during the period of color break, usually in October or November. The color ratings varied from deep green to orange and were rated on a scale of 1 to 5 based on a color photograph of five fruit in each of the five color categories. Fruit size was obtained by caliper measurement of twenty fruit selected at random around each tree. A 20-fruit sample collected from each tree was analyzed (8) for juice quality in 1988 for comparison with earlier analysis.

RESULTS

Yield-rootstock effect. In the previous report (8), results of the first 6 yr of fruiting (1980-1985) had shown significantly larger yields for navels on Troyer citrange rootstock compared to sour orange rootstock. Only treatment E containing CEV deviated from this observation and displayed a reduced yield for navel on Troyer. This trend of comparatively larger yields for trees on Troyer citrange rootstock continued during the four addition seasons from 1986 through 1989 (Table 2).

Yield-viroid effect. The effect of the various viroids on yield for the ten seasons is shown in Table 2. Citrus viroids appear to reduce yield in proportion to their reactivity on citron. Treatments D and E with reactivity ratings to citron of 4 and 10 (Table 1), reduced yields in comparison to the shoot tip grafted (STG) negative controls by 17 and 23% for navels on sour orange rootstock and 21 and 34% respectively for navels on Troyer citrange rootstock. Treatment C with a reactivity rating of 3 reduced yield by 18% significant only for navels on Troyer citrange rootstock compared to the STG control. Presence of CV-IIa in treatments A, B, and G with a reactivity rating of 0.5 to 1.5 had no effect on yield reduction in comparison to the STG control treatment F. The presence of CEV in treatment E reduced fruit yield of trees on Troyer citrange rootstocks compared to all other treatments except treatment D on Troyer.

Certain treatments appear to enhance the rootstock effect on yield. Yield was reduced for fruit on sour

TABLE 2
THE EFFECT OF CITRUS VIROIDS AND ROOTSTOCKS ON FRUIT YIELD OF NAVEL ORANGE

Treatment	Citrus viroids	Ten-year average yeild in kg/tree			
		Sour orange		Troyer citrange	
A	IIa	697.3 a[z]	A[y]	757.8 ab[x]	B[y]
B	IIa	658.3 ab	A	837.3 a	B
G	IIa	605.3 abc	A	751 abc	B
C	IIa, IIIb	627.6 abc	A	674.8 bc	B
D	Ia, IIa, IIIb	573.0 bc	A	651.6 cd	B
E	CEV, IIa, IIIb	542.3 c	A	542.5 d	A
F	STG—Neg.	691.3 a	A	824.4 a	B
	Mean	627.9	A	719.9	B

[z]Mean separation within measurements by Duncan's multiple range test, 5% level for *yield vs treatment*.
[y]Mean separation within measurements by Duncan's multiple range test, 5% level for *yield vs rootstock*.

orane rootstock compared to Troyer citrange for treatments B and G containin CV-IIa, and for treatment F (the shoot tip grafted control). The average accumulated total yield per tree for the ten fruiting seasons for treatments B, G and F on sour orange rootstock was 658, 692 and 605 kg respectively compared to 837, 824 and 751 kg for trees on Troyer rootstock. This represented a significant reduction in yield for trees on sour rootstock of 21.4, 16.1 and 16.4% respectively. Reduction of yield for navels on sour compared to Troyer for treatments A, C, D, and E were 8.0, 7.0, 12.2 and 0.0% respectively.

Tree volume in relation to viroids and rootstocks. Tree volume measurements made in 1985 showed that presence of CEV and certain CV reduced tree size for treatments A, B, C, D and E (8). Tree volume measurements made in 1989 are given in Table 3. Presence of CV-IIa in treatments A, B and G had no effect on tree volume regardless of rootstock. However, presence of various combinations of CV-Ia, -IIa or -IIIb in treatments C and D, or CEV in treatment E reduced tree volume for trees on Troyer citrange rootstock by 46.7, 51.0 and 60.2% respectively compared to the viroid-free control.

There appears to be a correlation between the reaction induced by CV on citron indicator plants (Table 1) and reduction in tree volume on Troyer rootstock. Also, volume reduction of these citrus viroids was greater for trees on Troyer citrange rootstock than on sour orange rootstock. Presence of CEV in treatment E reduced volume 36% for trees on sour orange rootstock compared to the STG control (treatment F) or treatments A, B, and G which contained only CV-IIa.

Fruit color break. In the 1988 and 1989 seasons, readings made for color break showed no differences between treatments. This was in contrast to the 1985 readings (8) in which treatments C, D and E affected color break when compared to the STG control.

In the November, 1988 color readings there were no differences between rootstocks on fruit color development. However, there was a rootstock effect on color break on fruit for the *early* readings taken on October 16, 1989. All fruit on trees with sour orange rootstock showed slower color development for all treatments (Table 4). However, color readings taken two weeks later did not show this rootstock effect.

Fruit size and quality. Juice analysis made in October and November 1988 showed similar trends to that previously reported (8) in higher sugar-acid ratios from fruit on trees on

TABLE 3
THE EFFECT OF CITRUS VIROIDS AND ROOTSTOCKS ON TREE VOLUME OF NAVEL ORANGE TREES. MEASUREMENT OF OCTOBER, 1989

Treatment	Citrus viroids	Tree volume (m^3)			
		Sour orange		Troyer citrange	
A	IIa	28.87 a[z]	A[y]	26.64 ab[z]	A[y]
B	IIa	23.51 ab	A	27.66 ab	A
G	IIa	25.92 ab	A	29.15 ab	A
C	IIa, IIIb	20.52 bc	A	16.06 c	A
D	Ia, IIa, IIIb	20.21 bc	A	14.93 c	A
E	CEV, IIa, IIIb	16.46 c	A	12.12 c	A
F	STG—Neg.	25.78 ab	A	29.57 a	A
	Mean	23.04	A	22.30	A

[z]Mean separation within measurements by Duncan's multiple range test, 5% level for *volume vs treatment*.
[y]Mean separation within measurements by Duncan's multiple range test, 5% level for *volume vs rootstock*.

Troyer citrange rootstock compared with fruit from trees on sour orange rootstock (11.0 vs. 10.4 in October and 10.1 vs 9.7 in November respectively).

DISCUSSION

One unexpected, yet most significant development of these studies continues to be the lower yield of navel oranges on sour range compared to that on Troyer citrange rootstocks at the Lindcove Field Station in the Central Valley of California. This yield reduction in relation to rcotstocks was evident regardless of viroid content with the exception of treatment E which contained CEV.

Development of fruit color reported for the 1985 season could be associated with trees containing certain citrus viroids. However, there was little to no effects among different viroid-treatments during the 1988 and 1989 seasons.

A rootstock effect was detected with navel oranges on sour orange rootstock showing color retardation in the early 1989 season compared to fruit on trees on Troyer citrange rootstock. This was true for all treatments. In general, the fruit colored

TABLE 4
THE EFFECT OF CITRUS VIROIDS AND ROOTSTOCKS ON FRUIT COLOR DEVELOPMENT. MEASUREMENT OF OCTOBER 16, 1989

Treatment	Citrus viroids	Fruit color rating[z]			
		Sour orange		Troyer citrange	
A	IIa	2.15 a[y]	A[x]	2.75 a[y]	B[x]
B	IIa	2.25 a	A	2.60 a	B
G	IIa	2.17 a	A	2.45 a	B
C	IIa, IIIb	2.20 a	A	2.65 a	B
D	Ia, IIa, IIIb	2.30 a	A	2.55 a	B
E	CEV, IIa, IIIb	2.10 a	A	2.45 a	B
F	STG—Neg.	2.25 a	A	2.45 a	B
	Mean	2.20	A	2.56	B

[z]Fruit color rating range from: 1 = dark green to 5 = orange.
[y]Mean separation within measurements by Duncan's multiple range test, 5% level for *fruit color vs treatment*.
[x]Mean separation within measurements by Duncan's multiple range test, 5% level for *fruit color vs rootstock*.

better on trees on Troyer citrange rootstock than on sour orange rootstock for all seasons in which fruit color ratings were made. In 1985 only the STG control and treatment G showed significant rootstock effect on retardation of color development.

The presence of citrus exocortis viroid in treatment E was probably responsible for reducing tree size and yield regardless of rootstock. The reduction in yield for trees on Troyer citrange associated with the presence of citrus viroids or CEV was not in direct proportion to the reduction in tree size or canopy. This same trend was noted in 1985 but became even more apparent in 1989 as trees grew larger. This slower decline in yield in relation to decline in tree volume is in agreement with studies by Mendel (7) and Cohen (2).

Yield and tree size reduction may be correlated to the reactivity of viroids on citron. Note in table 1 that reactivity in citron for viroids in treatments C, D, and E are 3, 4 , and 10 respectively (on a scale of 0 to 10). These treatments all showed some yield and tree size reductions (Table 2 and 3). In contrast, CV-IIa, present in treatments A, B, and G, showed a very mild reaction in citron ranging from 0 to 1.5 and induced little or no reduction in yield or tree size.

Measurements of fruit size, rind thickness, fruit shape and juice percentage previously reported by Nauer *et al.* (8) over three seasons showed no effect by the presence of any of the viroids. The slightly higher sugar-acid ratios in fruit from trees on Troyer citrange rootstock when compared to fruit from trees on sour orange rootstock was noted for all three seasons but statistically significant for only one of three seasons in which juice analysis was made. A trend was again noted in higher sugar-acid ratios for fruit from trees on Troyer compared to sour.

This experiment was developed prior to our knowledge that the so-called "mild" strains of exocortis were in fact distinct citrus viroids which differ from CEV. In this study, insight is gained on the effects of some of these citrus viroids on commercial citrus. Some citrus viroids have been found,to affect fruit yield, fruit tree canopy, bark cracking and pitting in the trunk (unpublished, Roistacher *et al.*) and may affect fruit color development. More extensive research on these effects using known pure citrus viroids, individually and in known mixtures, would be helpful in illuminating the role that these unique transmissible entities play in affecting the quality and productivity not only of citrus but possibly other horticultural crops.

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