The Effect of Citrus Exocortis Viroid (CEV) and Related Mild Citrus Viroids (CV) on Field Performance of Washington Navel Orange on Two Rootstocks

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ABSTRACT. Washington navel orange trees were inoculated with one citrus exocortis viroid (CEV) and three citrus viroid (CV) isolates with shoot-tip-grafted viroid-free trees as controls. Two rootstocks were evaluated: sour orange and Troyer citrange. Trees were planted in 1978 in a replicated block in the Central Valley of California. CEV significantly reduced fruit yields of trees on Troyer compared to all other treatments and of trees on sour orange when compared to shoot-tip-grafted control trees. CEV also induced significantly poorer color of fruit for trees on sour orange or Troyer rootstocks. Tree size was significantly reduced for trees on sour orange and Troyer in the presence of CEV. The three CV isolates had no significant effect on tree size. There were also no significant effects by CEV or CV on sugar-acid ratio, rind thickness, fruit shape, or juice percentage. When sour orange was compared to Troyer citrange as rootstocks with or without viroids, trees on Troyer produced larger fruit, increased yield, and in three out of four years, produced fruit with significantly better rind color. There was no significant difference in the size of trees on sour orange compared to Troyer citrange.

Index words. Dwarfing, sour orange, stunting, Troyer citrange, Washington navel orange, citrus exocortis viroid, viroids, citrus viroids.

Citrus exocortis, now known to be caused by the citrus exocortis viroid, was first reported to induce stunting of grapefruit, navel, and Valencia oranges on trifoliate orange rootstock. (2, 9). Calavan *et al.* (2) showed that exocortis was responsible for reduction in growth and fruit production of Valencia orange trees on trifoliate orange rootstock, but that in some years it induced larger fruit size. Also, the tendency toward alternate bearing was absent or less prevalent in infected trees. The concept of using exocortis for dwarfing trees was first proposed by Cohen (4). Since then, other studies using CEV for dwarfing have been made (1, 3, 5, 6,).

For many years exocortis was believed to be a virus disease, but was later shown to be a pathogenic low molecular weight RNA or viroid (19). Recent studies have shown that the milder reacting isolates on citron, classified as mild CEV isolates, represent distinct viroids with molecular weights of 311 to 355 nucleotides, smaller than the 371 nucleotide CEV (7, 8, 20). These citrus viroids do not multiply in gynura nor induce the intense epinasty, vein browning and stunting of citron plants and leaves as does CEV. The general classification of "citrus" viroids has been proposed for these low molecular weight RNAs (8).

With the advent of shoot tip-grafting *in vitro*, (14, 16), CEV and other viroids could be eliminated from old line citrus, thus bypassing the long-term nucellar seedling process, the only other known method of eliminating CEV from infected budlines. It then became possible to study the effect of CEV directly on the performances of sweet orange trees and fruit by removing CEV from old line citrus or by inoculating CEV back into CEV-free lines.

An experiment was designed in 1977 to study the effect of CEV and mild reacting isolates on citron, now known as CV isolates, on the performance of navel orange trees on two rootstocks. In addition, a shoot-tip-grafted virus and a viroid-free control were included for comparison.

MATERIALS AND METHODS

Greenhouse-grown seedlings of sour orange and Troyer citrange were

budded in March 1977. Scions used were an old budline Parent navel orange, an old budline Atwood navel which had been shoot-tip grafted to eliminate a very mild isolate of a CEV-like viroid (now identified as citrus viroid (CV) IIa). and an Atwood navel, not shoot-tip grafted containing CV IIa. The Atwood navel is virtually indistinguishable in tree and fruit characteristics from Parent (10, 12). The Parent navel orange selection, VI 12, was believed to be free of viruses and CEV in 1977 based on the best available index procedures at that time. However, it was later found to contain the mild CV IIa by use of a more sensitive citron indicator (17). Rootstocks were sour orange and Troyer citrange.

Trees of Parent navel were inoculated in June 1977 by inserting two buds from a known viroid source plant, one bud each into the scion and rootstock. Treatments and viroids used as inoculum are given in table 1. Treatments were as follows:

A. Control. Inoculated with Troyer seedling buds for wounding effect. Later, all Parent navel control plants were found to contain CV IIa and this budline was subsequently coded as source E-829. Source E-829 (CV IIa) induces only a very mild leaf-tip browning symptom when inoculated into 861 S-1 citron (17) and may cause mild to moderate bark cracking of trifoliate rootstock.

B. Inoculated with buds of Atwood navel viroid source E-818. Reaction on 861 S-1 citron is mild, but stronger than the E-829 infected Parent, even though E-829 and E-818 have been found to contain CV IIa (table 1). The viroid from this source also induces mild definitive bark cracking of trifoliate rootstock.

C. Inoculated with buds from viroid source E-805, in Bearss lime. This viroid from E-805 induces mild to moderate leaf epinasty on citron and mild to moderate bark cracking on Rangpur lime and trifoliate rootstocks. Source E-805 contains CV IIIb (Table 1).

D. Inoculated with buds from viroid source E-803 in Frost nucellar navel orange. This inoculum contains CV I, IIa, and IIIb (8, 20), and reacts moderately in citron, inducing leaf epinasty and stem browning, but only slight stunting.

E. Inoculated with buds from source E-800 from a field lemon budded on trifoliate stock which shows severe exfoliation on the trifoliate and severe stunting of the tree. In addition to CEV this inoculum contains CV IIa and IIIb. Reaction on citron was very severe with intense stunting, severe leaf epinasty, and severe stem cracking and browning.

F. The shoot-tip grafted control. An Atwood navel which had been shoot-tip grafted and contained no viruses or viroids.

G. The Atwood navel that was the source of E-818 which was used as inoculum in treatment B and contains CV IIa.

All inoculum sources and all scion sources were tested and found free of tristeza, psorosis, concave gum, vein enation, and cachexia by the methods employed in the Citrus Clonal Protection Program of the University of California (13).

These trees were planted in the field at the Lindcove Field Station in the Central Valley of California in the spring of 1978. There were five replicates in a randomized block; each replicate consisting of two trees each of the seven scion treatments on two rootstocks for a total of 70 trees.

RESULTS

To date, after 8 yr in the field, tree size, fruit production, and quality data show little effect of CV infection, regardless of severity of the isolate. Several significant differences were found when sour orange and Troyer citrange rootstocks were compared, and between CEV-infected trees and non-CEV-infected trees. These results are summarized in table 2.

Total yield over six harvest seasons from 1980/81 through 1985/86 is

TABLE 1.
EXPERIMENTAL TREATMENTS SHOWING SCIONS USED, CITRUS EXOCORTIS VIROID (CEV) AND CITRUS VIROIDS
USED AS INOCULUM OR PRESENT IN SCION WOOD

Treatment	Navel scion used	Budwood source code	Inoculum source	Citrus viroids[z]				Reaction[y] on citron
				CEV	I	IIa	IIIb	
A	Parent	E-829	Non-inoculated	−	−	+	−	0.5
B	Parent	E-818[x]	Atwood navel	−	−	+	−	1.5
C	Parent	E-805[x]	Bearss lime	−	−	−[x]	+	3.0
D	Parent	E-803[x]	Frost navel	−	+	+	+	4.0
E	Parent	E-800[x]	Eureka lemon	+	−	+	+	10.0
F	Atwood	—	Non-inoculated (STG)[w]	−	−	−	−	0.0
G	Atwood	E-818	Non-inoculated	−	−	+	−	1.5

[z]See Duran-Vila *et al*. (7, 8) and Semancik *et al*. (20) for explanation.
[y]Reaction rating on 861-SI citron: 0 = none, 1 = very mild, 2 = mild, 4 = mild-moderate, 6 = moderate, 8 = severe, 10 = very severe.
[x]Treatments B, C, D, and E also contained the citrus viroid IIa which was present in Parent navel scion source E-829.
[w]STG = shoot-tip grafted.

TABLE 2
EFFECTS OF VIROID TREATMENTS ON YIELD, RIND COLOR, FRUIT SIZE, AND TREE SIZE OF NAVEL ORANGES

Treatment[z]	6-yr total yield (kg/tree)		Rind color rating Nov. '85		Fruit diameter (cm) Nov. '85		Tree height (m) Nov. '85		Tree width (m) Nov. '85	
	Sour	Troyer	Sour	Troyer	Sour	Troyer	Sour	Troyer	Sour	Troyer
A	246bcd[y]	327a	3.08bc	3.23abc	7.69cd	7.84bcd	2.62abc	2.61abc	2.39abc	2.38abc
B	257bc	341a	3.38abc	3.23abc	7.61d	7.96bcd	2.58abc	2.79a	2.27bcd	2.38abc
C	217cd	306a	2.85bcd	3.15abc	7.86bcd	8.05bc	2.64abc	2.42bcd	2.13de	2.18cde
D	213cd	307a	2.83cd	3.05bc	7.62d	8.12b	2.41cd	2.47bc	2.18cde	2.12de
E	203d	224bcd	2.25d	2.78cd	7.75bcd	8.49a	2.21de	2.01e	2.00ef	1.91f
F	263b	331a	3.25abc	3.83a	7.79bcd	7.78bcd	2.70ab	2.82a	2.45ab	2.55a
G	204d	309a	2.80cd	3.53ab	7.94bcd	8.05bc	2.55abc	2.77a	2.27bcd	2.50a
Average	229	306	2.92	3.26	7.75	8.04	2.53	2.56	2.24	2.29

[z]See Table 1.
[y]Mean separation within measurements by Duncan's multiple range test, 5% level.

shown in table 2. All treatments on Troyer rootstock, except for treatment E (trees inoculated with CEV), bore significantly larger crops than those on sour orange rootstock. There were no significant yield differences among viroid-free and CV-inoculated trees on Troyer, whereas CEV-inoculated trees bore significantly less fruit than all other treatments. On sour orange, Parent navel trees with CEV bore significantly less fruit than trees infected with mild CV. Trees of shoot-tip-grafted, viroid-free Atwood navel on sour orange yielded significantly larger crops than the original Atwood budline containing the very mild CV.

Fruit rind color was evaluated early in the harvest season by comparing laboratory fruit samples with a color chart and by evaluating color in the field. In four seasons, rind color of fruit from trees on Troyer citrange was slightly better (darker orange, less green) than that of fruit from trees on sour orange. In 1985-86, color was evaluated by walking through the orchard and rating color on two sides of the tree on a scale of 1 to 5 with 1 being the greenest and 5 the best colored fruit. The evaluator did not know which treatment was being rated. In this case, direct comparisons between Troyer and sour orange showed Troyer producing slightly better color than sour orange in six of seven treatments, but this difference was statistically significant only with treatment G (table 2).

We noted a poorer color with increasing severity of CV isolates on sour orange in 1982-83 and on both rootstocks in 1985-86, but this trend was not significant. The effect of CEV on rind color varied. Generally, CEV-inoculated trees on sour orange and Troyer produced fruit with poorer color than CV-infected trees, but in 1983-84, trees on Troyer with severe CEV produced the best colored fruit.

Fruit size effects are difficult to evaluate because of interactions between numbers of fruit and fruit size; generally, trees producing larger crops produce smaller fruit. Despite the fact that trees on Troyer produced more total fruit than trees on sour orange, fruits from trees on Troyer were slightly larger in most seasons. In 1985-86 (table 2), trees on Troyer produced larger fruits in six out of seven treatments, and this difference was significant in two treatments. There was no apparent effect of CV inoculations on fruit size. Neither was there any apparent effect of CEV on fruit size with sour orange rootstock. Trees on Troyer inoculated with CEV produced slightly, but not significantly, smaller fruit in 1983-84 and 1984-85, and significantly larger fruit in 1985-86 than CV-inoculated trees; however, in 1985-86, these trees produced only about 60% of the crop average of the other Parent navel treatments, so large-size fruit could be accounted for by low production.

Tree height and width were measured in November 1985. CEV infection produced significantly smaller trees on sour orange and Troyer rootstocks (table 2). Trees on Troyer inoculated with a moderate CV (treatment D) were significantly smaller in width measurement than viroid-free trees and very mild CV-infected trees on Troyer (treatments A, B, F, G). There is a slight indication of reduction of tree size by inoculation with mild and moderate CV isolates, but, to date, this reduction is too small to be promising on a commercial basis. Future tree size measurements will be taken on this block to further evaluate the possibility of using CV inoculation to reduce tree size in commercial orchards.

Analysis of juice samples showed slightly higher sugar-acid ratios in fruit from trees on Troyer when compared with trees on sour orange for all three seasons when such analyses were made. This result was statistically significant in only one season. There were no effects of CEV or of CV inoculation on juice sugar-acid ratio.

Other measurements taken were rind thickness, fruit shape, and juice

percentage. None of these characteristics were affected by viroid inoculation or rootstock treatments.

DISCUSSION

The mild CV initially selected for this experiment were believed to be isolates of CEV. They were known to induce mild bark cracking in trifoliate rootstocks (17), and some induced stunting of trees on trifoliate. However, recent studies show that these are distinct citrus viroids,and must be considered as separate pathogens. Therefore, the results of this study not only reflect the effect of CEV but also include the effects of these newly discovered citrus viroids (7, 8, 20) on tree and fruit production and quality.

One of the most significant results obtained thus far was the comparative reaction of navel orange on sour orange vs. Troyer citrange for the particular soil and enviromental conditions in the Central Valley of California. Trees of all treatments on Troyer citrange stock (except those inoculated with CEV), bore significantly more fruit, had better rind color, and larger fruit than treatments on sour orange stock.

The CEV source inoculum came from a severely affected lemon on trifoliate and when indexed induced a very severe reaction in citron indicator plants. The presence of this inoculum in trees on Troyer citrange reduced yield significantly when compared to all other treatments. At this time, no comparative evaluation or projection is given for the yield per unit area where the CEV-infected trees were close planted. In general, and with one year's exception, the presence of CEV in trees on Troyer and sour orange resulted in fruit color inferior to those without CEV. The effect of CEV on fruit size could not be effectively analyzed since crops were significantly reduced by CEV, and smaller crops tend to produce large fruit. However, there appeared to be no apparent effect of CEV on fruit size on trees on sour orange stock.

A significant effect of the presence of CEV was the stunting of all trees on sour orange as well as those on Troyer citrange. Trees on sour orange showed a 19% reduction in height and width compared to the shoot-tip-grafted controls. Similarly, trees on Troyer citrange infected with CEV showed a 29% reduction in height and 25% reduction in tree width. The effects of CEV on trees on citrange, and trifoliate orange are well documented (2, 3, 4, 5, 6, 15). However, our studies show the effect of a specific CEV isolate plus pure and mixed CV isolates in a tristeza-free environment on trees growing in the absence of any other known virus or pathogen. The stunting effect of CEV in trees on sour orange is probably a new finding.

In general, the citrus viroids alone, or in combination, had little effect on yield, rind color, fruit size, or juice quality when present in navel orange trees on either sour orange or Troyer citrange stocks. There was some slight indication of tree size reduction by the citrus viroids but to date differences appear too small for commercial usage.

The use of CEV for stunting of citrus has been widely discussed and is somewhat controversial. There is a worldwide need for a dwarfed citrus tree primarily for economic reasons. The use of dwarfing rootstocks such as Flying Dragon trifoliate (18) appear promising where trifoliate can be successfully grown. The ethics of using or introducing any viroid that is highly transmissible by cutting tools into a grove, region, or country where it is not established are, in the judgment of these authors, questionable. The potential movement of the viroid to other existing or future scion-stock combinations might result in unforeseen problems; once introduced and present, the pathogen may be virtually impossible to eliminate from the area.

This is the first report, 8 yr after planting in the field, and further evaluations may in time substantiate

or modify these results. However, some results appear significant, i.e., the effect of sour orange rootstock on fruit production, rind color, and fruit size of navel oranges compared to trees on Troyer citrange. Also, the stunting effect of CEV on trees of navel orange on sour orange stock as well as trees on Troyer citrange stock appears to be significant.

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