Citrus Viroids: Symptom Expression and Performance of Washington Navel Sweet Orange Trees Grafted on Carrizo Citrange

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Abstract

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Citrus are natural hosts of several viroid species. *Citrus exocortis viroid* (CEVd) and *Hop stunt viroid* (HSVd) are the causal agents of two well-known diseases of citrus, exocortis and cachexia. Other viroids have been found to induce specific symptoms and different degrees of stunting in trees grafted on trifoliate orange and trifoliate orange hybrids. A field assay was initiated in 1989 to establish the effect of CEVd, HSVd, *Citrus bent leaf viroid* (CBLVd), *Citrus dwarfing viroid* (CDVd), and *Citrus bark cracking viroid* (CBCVd) on Washington navel sweet orange trees grafted on Carrizo citrange rootstock. Here we report the effect of viroid infection on symptom expression, tree size, fruit production and quality evaluated from 2004 to 2007. Vegeta-

tive growth was affected by viroid infection with height and canopy volume being reduced. No bark scaling symptoms were observed in CEVd-infected trees albeit they presented lesions and blisters in the roots. Bark cracking symptoms were consistently observed in CBCVd-infected trees that were smaller with enhanced productivity and fruit size. No major effects were found as a result of infection with CBLVd, HSVd, or CDVd. The quality of the fruits was not affected by viroid infection, except for the low diameter of the fruits harvested from HSVd-infected trees. An interesting effect was identified in terms of tree productivity increase (yield/canopy volume) as a result of infection with CEVd, CDVd, and especially CBCVd.

Citrus exocortis disease was described in 1948 as a bark shelling or scaling disorder affecting trees grown on the trifoliate orange (*Poncirus trifoliata* (L.) Raf.) rootstock (13). Once graft transmission was demonstrated (5,6), the disease was considered to be of viral etiology. With the discovery of viroids as a new class of plant pathogens (10), the exocortis disease was demonstrated to be associated with the *Citrus exocortis viroid* (CEVd) (44).

The use of Etrog citron (*Citrus medica* L.) for biological indexing purposes revealed a variety of symptoms which were erroneously considered, for many years, as evidence for the existence of CEVd strains (37). In 1985, Schlemmer et al. (39) provided the first evidence indicating that viroids other than CEVd were responsible for the mild and moderate symptoms observed on inoculated citrons. Subsequently, with the development of a double electrophoresis system (sequential polyacrylamide gel electrophoresis, sPAGE), a number of circular RNAs with faster migration than CEVd were consistently identified in field isolates, and their viroid nature established by infectivity assays on Etrog citron (11,12). Five viroids were initially characterized taking into consideration their electrophoretic migration in 5% sPAGE, sequence similarity determined by molecular hybridization against specific cDNA probes and specific symptoms on Etrog citron (12). Further se-

quencing and biological assays confirmed that their molecular and biological properties were consistent with the viroid species concept proposed by the International Committee on Taxonomy of Viruses (ICTV) (www.ictvonline.org/virusTaxonomy.asp). In addition to CEVd, *Citrus bent leaf viroid* (CBLVd), *Hop stunt viroid* (HSVd), *Citrus dwarfing viroid* (CDVd), and *Citrus bark cracking viroid* (CBCVd) have been recognized as true viroid species (1,33,34,38). More recently, two additional viroids, *Citrus viroid VI* (CVd-VI) (former *Citrus viroid OS*) and *Citrus viroid V* (CVd-V) have also been reported (23,45,46). These two viroids were not included in the present study that was initiated before they were described, and none of the viroid sources used contains CVd-V and/or CVd-VI.

The citrus cachexia disease, also known as xyloporosis, was first described in 1948 as discoloration, gumming, and browning of phloem tissues, wood pitting and bark cracking on Orlando tangelo (*C. paradisi* Macf. × *C. reticulata* Blanco) (9), and was suspected of being caused by a viroid (36). Characterization of citrus viroids other than CEVd led to the identification of specific variants of HSVd, in which a specific 5/6-nucleotide motif located in the Variable (V) domain ("cachexia expression motif") was responsible for its pathogenicity, as the causal agent of the cachexia disease (35,43,47).

Even though viroids had been shown to induce different degrees of dwarfing in certain rootstock/scion combinations (21,22,29,42), the effect of each viroid on field grown trees was not fully evaluated until 2004, when the results of a long-term field assay in which clementine trees grafted on trifoliate orange rootstock inoculated with several viroid sources, were evaluated (48). Furthermore, CDVd was shown to be a suitable choice to control tree size in the high-density plantings of Washington navel sweet orange trees (*Citrus sinensis* L.) grafted on trifoliate orange rootstock (50,51).

Here we report the results of an assay conducted to evaluate the performance of Washington navel sweet orange grafted on the exocortis sensitive Carrizo citrange (*P. trifoliata* × *C. sinensis*) rootstock in which we used the same viroid inoculum sources used by Vernière et al. (48).

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Materials and Methods

Viroid sources. Single viroid sources of CEVd, CBLVd, HSVd, CDVd, and CBCVd were selected from the viroid collection maintained at Instituto Valenciano de Investigaciones Agrarias (IVIA). Each source had been maintained in Etrog citron plants grafted on rough lemon that had been periodically tested to verify that they contained the expected viroid. When this assay was initiated in 1989, only limited information was available regarding the molecular and biological properties of these viroid sources. The information available now and the results of their agronomic characterization on clementine trees grafted on trifoliate orange (48) can be summarized as follows:

CEVd. Two isolates, CEVd-117 (EU872276) and CEVd-129 (AF298178), were chosen because they differed in the intensity of the symptoms induced in citron and *Gynura aurantiaca* (8). Further sequencing and biological characterization demonstrated that in the sequences of these isolates (8,20), presented in the Pathogenicity (P) and Variable (V) domains, the molecular characteristics of CEVd variants had been classified as class A and class B (severe and mild, respectively) based on their pathogenicity on tomato (52). Their further characterization revealed that hosts had a marked effect on the population of CEVd haplotypes as well as in their response to infection (7,26). In addition, clementine trees grafted on trifoliate orange showed only subtle differences in terms of the symptoms induced by these two CEVd isolates (48).

CBLVd. Two isolates, CVd-Ia (EU872278) and CVd-Ib (GQ260200), were initially selected because they differed in their electrophoretic mobility in sPAGE analysis. Isolate CVd-Ia had

been recovered from a field source from Spain, and it was highly homologous to the CBLVd (CVd-Ia) reported by Semancik et al. (42) but contained a set of nucleotide changes clustered in the left of the viroid secondary structure (16,19). Isolate CVd-Ib (kindly provided by J. S. Semancik, University of California, Riverside) was similar to the type strain of CBLVd (4).

HSVd. Isolate CVd-IIa (AF213503) had been biologically characterized as a non-cachexia-inducing variant, and sequencing demonstrated that it lacked the “cachexia expression motif” in the V domain of the viroid secondary structure (31,47). Isolates CVd-IIb (AF213488) and CVd-IIc (AF213492) had been characterized as cachexia inducing variants, and sequencing demonstrated that they contained the “cachexia expression motif” (31,47).

CDVd. Isolates CVd-IIIa (S76452) and CV-IIIb (AF184147) (kindly provided by J. S. Semancik, University of California, Riverside) were isolated from California and differed in 11 nucleotide changes (34). CVd-IIIC (AF184149) and CVd-IIId (EU934019) were selected from Spain and characterized as distinct variants of CDVd (16,27).

CBCVd. The single isolate of CBCVd (GQ60216) (kindly provided by J. S. Semancik, University of California, Riverside) was from California, and it had been characterized to the type CBCVd strain (17).

Plant materials and inoculation. In June 1989, 1-year-old Washington navel trees grafted on Carrizo citrange were graft-inoculated with one of these 12 viroid isolates (six plants per viroid treatment and six noninoculated controls). The following year the trees were transplanted in a randomized block arrangement in a calcareous, alkaline (pH around 8.0), sandy-loam soil. The field

Fig. 1. Field plot of Washington navel trees grafted on Carrizo citrange graft-inoculated with 12 viroid isolates. **A**, Aspect of the trees when the experiment was terminated. **B**, Trunk in which the bark had been scraped to show the presence of pitting and gumming symptoms. **C**, Trees decapitated 50 cm above the bud-union. **D**, Trees in which the bark of the stumps had been partially removed for a final evaluation of symptoms on the bark and the wood above and below the bud union. **E**, Stumps being pulled away from the soil. **F**, Stumps from which the remaining trunk had been cut away. **G**, Measuring the fresh weight of the root system.

plot was located at the Instituto Valenciano de Investigaciones Agrarias (IVIA), Moncada (Valencia, Spain) on the east coast of Spain (Fig. 1A).

The trees were subjected to the standard pruning and harvesting operations of the region. In order to shape the canopy of the trees, two major prunings were performed in 1991 and 1993, and suckers were removed in all subsequent years. Tools were disinfested with a commercial sodium hypochlorite solution (4%) between trees. In 2006–2007, all the trees were indexed to verify that they were actually infected with the respective viroid isolates and that the noninoculated controls had remained viroid-free.

Viroid indexing. Bark (5 g) stripped from young shoots was powdered in liquid nitrogen and homogenized in 5 ml of extraction buffer (0.4 M Tris-HCl, pH 8.9; 1% (wt/vol) sodium dodecyl sulfate (SDS); 5 mM EDTA, pH 7.0; 4% (vol/vol) 2-mercaptoethanol); and 15 ml of water-saturated phenol. The total nucleic acids were partitioned in 2 M LiCl, and the soluble fraction was concentrated by ethanol precipitation and resuspended in 300 µl of TKM buffer (10 mM Tris-HCl; 10 mM KCl; 0.1 mM MgCl₂, pH 7.4) (41). Aliquots of these nucleic acid preparations were analyzed by northern blot hybridization as described by Murcia et al. (28). Briefly, the RNAs separated in 5% PAGE (60 mA, 2 h) were

Table 1. Symptoms observed on viroid-infected Washington navel orange trees grafted on citrange Carrizo

Treatment		Bark scaling	Bark cracking		Pegs	Gummy pits
Viroid	Isolate	No. trees ^a	Intensity	No. trees ^a	No. trees ^a	No. trees ^a
Control		0/6	Mild	6/6	0/6	0/6
CEVd	CEVd-117	0/6	–	0/6	1/6	0/6
	CEVd-129	0/6	Mild	6/6	1/6	1/6
CBLVd	CBLVd-Ia	0/6	–	0/6	0/6	3/6
	CBLVd-Ib	0/6	–	0/6	0/6	2/6
HSVd	HSVd-IIa	0/6	–	0/6	1/6	3/6
	HSVd-IIb	0/6	–	0/6	1/6	1/6
	HSVd-IIc	0/6	Mild	1/6	0/6	2/6
CDVd	CDVd-IIIa	0/6	Mild	2/6	1/6	2/6
	CDVd-IIIb	0/6	–	0/6	0/6	0/6
	CDVd-IIIC	0/6	–	0/6	0/6	1/6
	CDVd-IIId	0/6	–	0/6	1/6	1/6
CBCVd	CBCVd	0/6	Severe	6/6	0/6	0/6

^a Number of trees presenting symptoms over the total number of trees.

Fig. 2. A, Unspecific bark cracking extending from the rootstock to the scion. B, Green streaks observed in the wood after removing the bark. C, Small pits in the cambial site of the bark. D and E, Horizontal streaks of pits containing gum pockets. F, Unaffected wood.

Fig. 3. A, Lesions observed in the roots of trees infected with *Citrus exocortis* viroid (CEVd)-117. B, Roots of noninfected controls.

Table 2. Vegetative growth parameters of viroid (species and isolates) infected Washington navel sweet orange trees grafted on citrange Carrizo

Treatment	Tree height (m)		Rootstock circumference (cm)		Scion circumference (cm)		Canopy volume (m ³)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Viroid species								
Control	2.17	0.095	51.59	2.985	41.55	2.956	5.69	0.487
CEVd	1.89	0.076	49.72	2.516	39.46	2.244	4.44	0.349
CBLVd	2.21	0.076	54.18	2.482	42.02	2.192	5.81	0.349
HSVd	2.13	0.069	54.13	2.482	44.19	2.086	5.56	0.289
CDVd	1.90	0.064	50.13	2.370	38.69	1.964	4.70	0.259
CBCVd	1.64	0.095	46.76	3.234	40.67	3.316	3.60	0.486
Viroid isolates								
Control	2.16	0.096	51.57	2.993	41.55	2.956	5.69	0.487
CEVd-117	1.84	0.096	49.91	3.236	40.92	3.316	4.15	0.487
CEVd-129	1.94	0.096	49.50	2.788	38.00	2.669	4.72	0.487
CBLVd-Ia	2.14	0.096	54.35	2.969	42.88	2.942	5.73	0.487
CBLVd-Ib	2.28	0.096	54.75	2.993	41.15	2.956	5.90	0.487
HSVd-IIa	2.14	0.096	49.17	2.993	40.55	2.956	5.28	0.487
HSVd-IIb	2.28	0.096	58.12	3.596	47.56	3.818	5.25	0.487
HSVd-IIc	1.84	0.096	54.95	2.969	44.48	2.942	6.14	0.487
CDVd-IIIa	1.86	0.096	50.87	2.993	42.25	2.956	4.68	0.487
CDVd-IIIb	1.92	0.096	54.01	3.600	36.40	3.815	4.77	0.487
CDVd-IIIC	1.90	0.104	48.30	3.233	35.22	3.303	4.49	0.534
CDVd-IIId	1.90	0.096	46.90	3.236	40.92	3.316	4.87	0.487
CBCVd	1.63	0.096	46.65	3.236	40.67	3.316	3.60	0.487

P values obtained when comparing data of viroid-infected treatments with the noninoculated controls (*P* values lower than 0.05 are shown shaded).

	Tree height (m)	Rootstock circumference (cm)	Scion circumference (cm)	Canopy volume (m ³)
Viroid species				
Block	0.124	0.119	0.303	
Block*Viroid	0.357	0.327		0.265
Year	<0.0001	—	—	<0.0001
Viroid	<0.0001	0.105	0.309	0.001
Year*Viroid	0.846	—	—	0.062
Control vs. CEVd	0.037	0.919	0.934	0.126
Control vs. CBLVd	0.986	0.801	0.999	0.999
Control vs. HSVd	0.991	0.798	0.838	0.998
Control vs. CDVd	0.028	0.967	0.775	0.226
Control vs. CBCVd	0.0002	0.453	0.999	0.013
Viroid isolates				
Block	0.108	0.114	0.303	
Year	<0.0001	—	—	<0.0001
Isolate	<0.0001	0.105	0.519	0.019
Year*Isolate	0.206	—	—	0.291
Control vs. CEVd-117	0.069	0.999	1.000	0.203
Control vs. CEVd-129	0.358	0.998	0.965	0.722
Control vs. CBLVd-Ia	1.000	0.998	1.000	1.000
Control vs. CBLVd-Ib	0.962	0.995	1.000	1.000
Control vs. HSVd-IIa	0.867	0.995	1.000	0.999
Control vs. HSVd-IIb	0.999	0.530	0.798	0.998
Control vs. HSVd-IIc	0.992	0.954	0.995	0.998
Control vs. CDVd-IIIa	0.097	1.000	1.000	0.675
Control vs. CDVd-IIIb	0.291	0.998	0.902	0.775
Control vs. CDVd-IIIC	0.259	0.971	0.647	0.529
Control vs. CDVd-IIId	0.217	0.795	1.000	0.859
Control vs. CBCVd	0.0004	0.747	1.000	0.031

electroblotted (400 mA for 2 h) from the gel to Nylon membranes, immobilized by UV cross-linking, and hybridized with viroid-specific DIG-labeled DNA probes generated by polymerase chain reaction (PCR) using as templates plasmids containing full-length viroid DNA inserts (30). Prehybridization and hybridization were performed in 50% formamide, and the DIG-labeled hybrids were detected with an anti-DIG-alkaline phosphatase conjugate (Fab fragments) and visualized by autoradiography with the chemiluminescence substrate CSPD (Roche).

Symptom evaluation. Bark symptoms (scaling and cracking) were evaluated when the experiment was terminated in 2008 (Fig. 1A). The bark was scraped above and below the bud-union to verify the presence of pitting and gumming symptoms (Fig. 1B). The trees were decapitated 50 cm above the bud-union (Fig. 1C), and the bark of the stumps was removed for a final evaluation of symptoms on the bark and the wood above and below the bud-union (Fig. 1D). Finally, the stumps were pulled out of the soil (Fig. 1E) for a final evaluation of symptoms in the roots. After cutting away the remaining trunk (Fig. 1F), the fresh weight of the root system was measured after removing the soil that was attached to the roots (Fig. 1G).

Tree growth and fruit yield. Tree height, trunk circumferences of the scion and the rootstock (10 cm above and below the bud line) were measured in 2004 and 2007. The canopy volume was estimated as $V_c = 4/3\pi R^3$ (R being the radius of the canopy calculated from the canopy height and two perpendicular width measurements). Fruits were harvested manually, and fruit yield data were recorded in 2004, 2006, and 2007.

Fruit quality. From October 2005 to February 2006, the evolution of the color and size (length and diameter) of 10 randomly collected fruits from each tree was recorded at monthly intervals. Color index was determined using a colorimeter (Minolta CR-300) (24) taking into consideration the average of three independent measurements made at the equatorial zone of the fruit. During the same period, measurements of fruit length and diameter were also recorded from the same sampled fruits.

External fruit quality parameters were determined using fruit samples (10 fruits per tree) collected during the 2007 harvest and were recorded as follows: (i) fruit shape determined as the average of the diameter/length values; (ii) average of rind thickness measured at the equatorial zone using a digital caliper; (iii) bulk weight of the 10 fruits of each sample; (iv) bulk fruit size (volume) measured using the water displacement method; and (v) fruit density calculated as quotient between weight and fruit volume.

Internal fruit quality parameters were determined using fruit samples (10 fruits per tree) collected during the 2007 harvest and were recorded as follows: (i) volume of juice extracted from the 10 fruit samples using a squeezer (Zumonat C40) that operates at 14 kg/cm² and contains a series of filters retaining the large particles; (ii) juice density measured with a densitometer (Proton 1000-1100) calibrated at 15°C; (iii) soluble solids measured with a refractometer (Atago PR-101); (iv) total acidity measured by titration of 100 ml of juice with NaOH 0.1 N and correcting the volume of NaOH ($\times 1.28$) to obtain the corresponding percentage (%) of citric acid; and (v) maturity index calculated as the quotient of percentage of soluble solids and acidity percentage.

Statistical analysis. The effect of viroid infection and the effect of viroid isolates was performed by repeated measures ANOVA. Comparisons with the control were done with the Dunnett test. Analysis was performed using the Statgraphics Plus 5.1 software.

Results

Symptoms induced by viroid infection. No bark scaling symptoms were observed in any of the treatments (Table 1). Unspecific mild bark cracking symptoms were observed in several treatments, including the viroid-free controls. This mild bark cracking affected the rootstock as well as the scion (Fig. 2A). Severe bark cracking symptoms were consistently found in the rootstock of all the trees infected with CBCVd (Table 1). As reported earlier in the case of trees grafted in trifoliate orange (48),

severe cracking was more perceptible by observing the characteristic green streaks easily observable after scraping the bark (Fig. 2B).

After removing the bark, small pits were observed on the cambial side of the bark of Carrizo citrange rootstock (Fig. 2C) that were not associated with any of the treatments. Horizontal streaks of pits containing gum pockets (Fig. 2D and E) somewhat similar to the “wood pitting”, “gum pocket”, and “gummy pitting” disorders described in Argentina, South Africa, and Australia, respectively (14,15,18,25,40), were also observed in a few trees of several treatments. When the experiment was terminated and the trees were pulled out, small lesions were observed in the roots of CEVd-infected trees (Fig. 3).

Effect of viroid infection on tree size and fruit harvest.

Vegetative growth. As shown in Table 2, vegetative growth was affected by viroid infection with height and canopy volume being significantly reduced. Mean parameters (height, rootstock, and scion circumferences and canopy volume) of trees infected with

Table 3. Root weight of viroid (species and isolates) infected Washington navel sweet orange trees grafted on citrange Carrizo

Treatment	Rootstock weight (kg)	
	Mean	SE
Viroid species		
Control	15.67	3.640
CEVd	13.27	2.674
CBLVd	17.18	2.674
HSVd	19.53	2.261
CDVd	14.47	2.066
CBCVd	11.53	3.640
Viroid isolates		
Control	15.67	3.645
CEVd-117	12.37	3.645
CEVd-129	14.17	3.645
CBLVd-Ia	16.92	3.645
CBLVd-Ib	17.42	3.645
HSVd-IIa	25.84	3.645
HSVd-IIb	16.17	3.645
HSVd-IIc	16.59	3.645
CDVd-IIIa	13.67	3.645
CDVd-IIIb	15.50	3.645
CDVd-IIIC	15.30	3.992
CDVd-IIId	13.45	3.645
CBCVd	11.53	3.645

P values obtained when comparing data of viroid-infected treatments with the noninoculated controls.

Rootstock weight (kg)	
Viroid species	
Block	
Block*Viroid	0.2599
Viroid	0.3624
Control vs. CEVd	0.9625
Control vs. CBLVd	0.9950
Control vs. HSVd	0.7768
Control vs. CDVd	0.9975
Control vs. CBCVd	0.8398
Viroid isolates	
Block	
Viroid isolate	0.5230
Control vs. CEVd-117	0.9983
Control vs. CEVd-129	1.0000
Control vs. CBLVd-Ia	1.0000
Control vs. CBLVd-Ib	1.0000
Control vs. HSVd-IIa	0.3280
Control vs. HSVd-IIb	1.0000
Control vs. HSVd-IIc	1.0000
Control vs. CDVd-IIIa	1.0000
Control vs. CDVd-IIIb	1.0000
Control vs. CDVd-IIIC	1.0000
Control vs. CDVd-IIId	1.0000
Control vs. CBCVd	0.9890

CEVd, CDVd, or CBCVd were lower than those of the noninoculated control with CBCVd having the strongest effect. Rootstock and scion circumferences were only slightly affected. There was no significant block effect, and the interaction year*viroid was not significant, indicating that the observed effects were due to viroid infection. Comparison of vegetative growth parameters of each viroid isolate (Table 2), although not statistically different, indicated the following: (i) CEVd-117 had a stronger effect than CEVd-129 on tree height, scion circumference, and canopy volume; (ii) CBLVd-Ia had a stronger effect than CBLVd-Ib on tree height and canopy volume; (iii) HSVd-IIc had a stronger effect on tree height, whereas HSVd-IIa had stronger effect on rootstock and scion circumferences; and (iv) CDVd showed variable effects.

When the experiment was terminated, the fresh weight of the roots was evaluated. As shown in Table 3, viroid infection did not affect significantly this parameter. The mean fresh weights of trees infected with CEVd or with CBCVd were smaller than those of the noninfected controls, but the differences were not statistically significant. There was no significant block effect, indicating that the observed effects were due to viroid infection. When data from each isolate were compared (Table 3), the major deviations from the noninoculated controls were found in the case of trees infected with CBCVd, with the severe CEVd isolate (CEVd-117) and with two of the four CDVd isolates tested, but the differences were not statistically significant. Unexpectedly, the mean value of one of the HSVd isolates (HSVd-IIa) was higher than that of

Table 4. Fruit yields of viroid (species and isolates) infected Washington navel sweet orange trees grafted on citrange Carrizo

Treatment	Yield /canopy volume ^a		Annual yield (kg) ^b		Cumulative yield (kg) ^c	
	Mean	SE	Mean	SE	Mean	SE
Viroid species						
Control	15.79	1.533	66.42	7.062	199.25	21.371
CEVd	18.10	1.122	62.40	6.235	187.21	18.916
CBLVd	14.69	1.122	68.61	6.235	205.83	18.916
HSVd	16.66	0.946	68.31	5.934	204.92	18.022
CDVd	18.14	0.863	63.06	5.788	190.93	17.647
CBCVd	20.28	1.532	51.00	7.062	153.00	21.371
Viroid isolates						
Control	15.79	1.524	66.42	7.208	199.25	21.864
CEVd-117	18.98	1.524	59.58	7.208	178.75	21.864
CEVd-129	17.22	1.524	65.22	7.208	195.67	21.864
CBLVd-Ia	14.75	1.524	66.83	7.208	200.50	21.864
CBLVd-Ib	14.63	1.524	70.39	7.208	211.17	21.864
HSVd-IIa	15.64	1.524	66.03	7.208	198.08	21.864
HSVd-IIb	20.65	1.524	71.33	7.208	214.00	21.864
HSVd-IIc	13.69	1.524	67.56	7.208	202.67	21.864
CDVd-IIIa	17.81	1.524	62.86	7.208	188.58	21.864
CDVd-IIb	18.49	1.524	64.06	7.208	192.17	21.864
CDVd-IIc	19.25	1.670	63.19	7.359	194.34	23.172
CDVd-IIId	17.07	1.524	61.56	7.208	184.67	21.864
CBCVd	20.28	1.524	51.00	7.208	153.00	21.864

P values obtained when comparing data of viroid-infected treatments with the noninoculated controls (*P* values lower than 0.05 are shown shaded).

	Yield /canopy volume	Annual yield (kg)	Cumulative yield (kg)
Viroid species			
Block	0.256	0.094	0.092
Block*Viroid	0.252	0.036	0.057
Year	<0.0001	<0.0001	—
Viroid	0.043	0.210	0.204
Year*Viroid	0.029	0.178	—
Control vs. CEVd	0.533	0.964	0.961
Control vs. CBLVd	0.943	0.997	0.997
Control vs. HSVd	0.971	0.998	0.998
Control vs. CDVd	0.449	0.978	0.989
Control vs. CBCVd	0.126	0.193	0.184
Viroid isolates			
Block	0.234	0.076	0.076
Year	<0.0001	<0.0001	—
Isolate	0.025	0.563	0.568
Year*Isolate	0.021	0.405	—
Control vs. CEVd-117	0.651	0.977	0.978
Control vs. CEVd-129	0.997	1.000	1.000
Control vs. CBLVd-Ia	0.999	1.000	1.000
Control vs. CBLVd-Ib	0.999	0.999	0.999
Control vs. HSVd-IIa	1.000	1.000	1.000
Control vs. HSVd-IIb	0.184	0.998	0.998
Control vs. HSVd-IIc	0.952	1.000	1.000
Control vs. CDVd-IIIa	0.962	0.999	0.999
Control vs. CDVd-IIb	0.815	1.000	1.000
Control vs. CDVd-IIc	0.615	1.000	1.000
Control vs. CDVd-IIId	0.999	0.998	0.998
Control vs. CBCVd	0.257	0.322	0.324

^a Average of data collected in years 2004 and 2007.

^b Average of data collected in 2004, 2006, and 2007 (data for 2005 was not available).

^c Cumulative yield of data collected in 2004, 2006, and 2007 (data for 2005 was not available).

the noninoculated controls, but the difference was not statistically significant.

Fruit harvest. As shown in Table 4, fruit harvest parameters were affected by annual conditions as indicated by the “year” significance ($P < 0.0001$). No major differences in annual and cumulative yields were found between viroid-infected trees and the noninoculated controls, but an interesting effect was identified in terms of tree productivity increase (yield/canopy volume) ($P = 0.043$), which appears to be modulated by annual conditions as shown by the interaction “year*viroid” ($P = 0.029$). It must be mentioned that CBCVd induced the highest productivity (yield/canopy volume) in spite of the relatively small annual and cumulative yields which were compensated by the small canopy (Table 2). There were no significant block effects, and the interaction year*viroid was not significant, indicating that the observed effects were due to viroid infection.

Fruit characteristics. Only slight differences were found in color index during the fruit-ripening period, but the values became es-

entially identical at harvest time (Table 5). No statistical differences were found when the values of viroid-infected trees were compared with those of the noninoculated control or when the values of the various isolates were compared (Table 5). Slight differences were observed in some of the fruit parameters measured, which became more obvious at harvest time (Table 6). The results suggest that the fruits of HSVd-infected trees were slightly smaller (height, $P = 0.032$; diameter, $P = 0.086$) than those of the noninfected controls. When the fruit length and diameter of trees infected with each isolate were compared, no significant differences were found (Table 6), but the results suggest that the fruits of trees infected with isolate HSVd-IIc were somewhat smaller.

Viroid infection affected external fruit quality parameters by reducing fruit length ($P = 0.033$), size ($P = 0.041$), and density with no effect on fruit shape (diameter/length), rind thickness, and fruit density (Table 7). It should be mentioned that, although the differences were not statistically significant, HSVd affected fruit sizes that are smaller than the controls, and CBCVd caused an

Table 5. Color evolution of fruits from viroid (species and isolates) infected Washington navel sweet orange trees grafted on Carrizo citrange

Treatment	Color 1 ^a		Color 2		Color 3		Color 4		Color 5		Color 6	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Viroid species												
Control	-11.03	0.719	0.81	1.165	7.99	0.676	10.02	0.406	12.04	0.317	14.57	0.504
CEVd	-10.88	0.560	0.31	1.016	7.30	0.602	10.36	0.316	11.95	0.241	14.49	0.367
CBLVd	-10.61	0.560	0.22	1.016	7.76	0.602	9.16	0.316	12.19	0.241	14.32	0.367
HSVd	-10.84	0.496	-0.17	0.962	7.38	0.574	9.77	0.280	12.04	0.209	14.47	0.308
CDVd	-11.39	0.467	0.38	0.938	7.12	0.563	9.70	0.264	11.94	0.195	14.71	0.280
CBCVd	-12.67	0.719	1.74	1.165	5.93	0.676	9.25	0.404	12.25	0.316	15.08	0.504
Viroid isolates												
Control	-11.03	0.713	0.81	1.162	7.99	0.629	10.03	0.405	12.04	0.317	14.57	0.504
CEVd-117	-11.02	0.713	0.19	1.162	7.22	0.629	10.27	0.405	12.01	0.317	13.84	0.504
CEVd-129	-10.74	0.713	0.43	1.162	7.38	0.629	10.44	0.405	11.89	0.317	15.15	0.504
CBLVd-Ia	-10.48	0.713	0.47	1.162	7.97	0.629	8.99	0.405	12.01	0.317	14.34	0.504
CBLVd-Ib	-10.74	0.713	-0.03	1.162	7.54	0.629	9.33	0.405	12.36	0.317	14.30	0.504
HSVd-IIa	-11.28	0.713	-0.94	1.162	6.79	0.629	9.57	0.405	12.32	0.317	14.57	0.504
HSVd-IIb	-10.39	0.713	0.44	1.162	8.23	0.629	9.97	0.405	11.91	0.317	15.06	0.504
HSVd-IIc	-10.83	0.713	-0.01	1.162	7.12	0.629	9.77	0.405	11.90	0.317	13.76	0.504
CDVd-IIIa	-11.56	0.713	-0.28	1.162	6.95	0.629	9.96	0.405	12.06	0.317	14.98	0.504
CDVd-IIIB	-11.87	0.713	0.30	1.162	6.90	0.629	9.62	0.405	11.80	0.317	14.13	0.504
CDVd-IIIC	-10.71	0.777	0.84	1.223	7.64	0.679	9.80	0.439	12.79	0.344	14.78	0.504
CDVd-IIID	-11.37	0.713	0.69	1.162	6.89	0.629	9.41	0.405	11.11	0.317	14.93	0.504
CBCVd	-12.67	0.713	1.74	1.162	5.93	0.629	9.25	0.405	12.25	0.317	15.08	0.504

P values obtained when comparing data of viroid infected treatments with the noninoculated controls.

	Color 1	Color 2	Color 3	Color 4	Color 5	Color 6
Viroid species						
Block	0.164	0.072	0.129	0.110	0.134	0.242
Block*Viroid	0.328	0.428	0.079			
Viroid	0.224	0.526	0.232	0.228	0.894	0.832
Control vs. CEVd	0.999	0.970	0.816	0.854	0.999	0.999
Control vs. CBLVd	0.968	0.944	0.998	0.166	0.984	0.983
Control vs. HSVd	0.999	0.684	0.865	0.940	1.000	0.999
Control vs. CDVd	0.978	0.979	0.628	0.840	0.997	0.999
Control vs. CBCVd	0.256	0.838	0.069	0.356	0.872	0.872
Viroid isolates						
Block	0.158	0.071	0.106	0.110	0.134	0.242
Block*Isolate	0.0001	<0.0001	<0.0001	0.436	0.023	0.0170
Isolate	0.568	0.831	0.274	0.221	0.101	0.515
Control vs. CEVd-117	1.000	1.000	0.951	1.000	1.000	0.922
Control vs. CEVd-129	1.000	1.000	0.992	0.985	1.000	0.985
Control vs. CBLVd-Ia	0.999	1.000	1.000	0.302	1.000	1.000
Control vs. CBLVd-Ib	1.000	0.995	1.000	0.755	0.990	1.000
Control vs. HSVd-IIa	1.000	0.646	0.633	0.980	0.997	1.000
Control vs. HSVd-IIb	0.997	1.000	1.000	1.000	1.000	0.996
Control vs. HSVd-IIc	1.000	0.996	0.905	1.000	1.000	0.872
Control vs. CDVd-IIIa	1.000	0.965	0.784	1.000	1.000	0.999
Control vs. CDVd-IIIB	0.977	1.000	0.738	0.991	1.000	0.998
Control vs. CDVd-IIIC	1.000	1.000	1.000	1.000	0.469	1.000
Control vs. CDVd-IIID	1.000	1.000	0.727	0.860	0.194	1.000
Control vs. CBCVd	0.480	0.989	0.086	0.643	1.000	0.995

^a Color was evaluated with a random sample of 10 fruits per tree. Data were collected monthly from October until harvesting time.

increase in fruit size and weight. When quality parameters of fruits from trees infected with each isolate were compared, no significant differences were found (Table 7). As indicated above, the results indicate that the fruits of trees infected with isolate HSVd-IIc were somewhat smaller.

Internal quality parameters of fruits harvested in 2007 are shown in Table 8. Fruits collected from CEVd- or CDVd-infected trees contained less soluble solids, but the differences were not highly significant ($P = 0.151$ and $P = 0.165$, respectively) with only minor effects on the maturity index. When quality parameters of fruits from trees infected with each isolate were compared, only relevant differences in the content of soluble solids were found in the case of fruits collected from trees infected with one of the CDVd isolates (CDVd-IIIc) ($P = 0.105$) (Table 8).

Discussion

The use of Etrog citron as the exocortis indicator for biological indexing purposes revealed a range of symptoms that were erroneously considered as evidence for the existence of different CEVd strains until viroids other than CEVd were identified and shown to induce specific symptoms in this indicator (11,12). Since naturally occurring field isolates contain more than a single viroid, the effect of each viroid on field grown trees was not established until 2004, when the results of a long-term field assay performed with clementine trees grafted on the trifoliate orange rootstock became available (48). Additional long-term field assays also revealed that viroid-induced effects might be attenuated or enhanced when trees were exposed to mixed viroid infections (49,51).

Table 6. Growth of fruits from viroid (species and isolates) infected Washington navel sweet orange trees grafted on Carrizo citrange^a

Treatment	Length 1 (mm)		Length 2 (mm)		Length 3 (mm)		Length 4 (mm)		Length 5 (mm)		Length 6 (mm)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Viroid species												
Control	71.86	0.853	75.42	1.164	77.25	1.17	79.05	1.254	77.71	1.178	84.49	1.237
CEVd	71.62	0.608	75.28	0.967	77.58	0.95	76.46	1.019	78.32	0.914	82.47	0.945
CBLVd	72.06	0.608	75.09	0.967	76.57	0.95	76.24	1.019	77.79	0.914	82.33	0.945
HSVd	71.47	0.501	74.78	0.892	76.95	0.86	76.87	0.927	78.21	0.806	80.97	0.825
CDVd	71.87	0.449	75.66	0.859	77.04	0.82	76.80	0.886	78.13	0.758	83.14	0.769
CBCVd	72.38	0.853	76.25	1.164	79.90	1.17	79.25	1.256	77.30	1.178	84.56	1.237
Viroid isolates												
Control	71.86	0.853	75.42	1.166	77.25	1.159	79.05	1.256	77.71	1.181	84.49	1.238
CEVd-117	72.06	0.853	75.06	1.166	77.44	1.159	76.44	1.256	76.96	1.181	82.35	1.238
CEVd-129	71.17	0.853	75.50	1.166	77.72	1.159	76.49	1.256	79.68	1.181	82.59	1.238
CBLVd-Ia	72.53	0.853	75.68	1.166	77.23	1.159	76.43	1.256	77.55	1.181	83.15	1.238
CBLVd-Ib	71.58	0.853	74.50	1.166	75.91	1.159	76.05	1.256	78.02	1.181	81.51	1.238
HSVd-IIa	71.72	0.853	74.88	1.166	77.31	1.159	77.20	1.256	79.28	1.181	81.13	1.238
HSVd-IIb	71.54	0.853	74.95	1.166	76.43	1.159	76.77	1.256	76.88	1.181	81.31	1.238
HSVd-IIc	71.16	0.853	74.50	1.166	77.11	1.159	76.64	1.256	78.47	1.181	80.47	1.238
CDVd-IIIa	71.12	0.853	75.02	1.166	76.24	1.159	76.83	1.256	78.22	1.181	82.47	1.238
CDVd-IIIb	72.01	0.853	76.26	1.166	76.81	1.159	77.11	1.256	77.89	1.181	82.37	1.238
CDVd-IIIc	72.22	0.934	75.48	1.264	77.66	1.269	76.55	1.348	79.07	1.294	85.05	1.344
CDVd-IIId	72.13	0.853	75.95	1.166	77.46	1.159	76.71	1.256	77.42	1.181	82.67	1.238
CBCVd	72.38	0.853	76.25	1.166	79.90	1.159	79.24	1.256	77.29	1.181	84.56	1.238

P values obtained when comparing data of viroid infected treatments with the noninoculated controls (*P* values lower than 0.05 are shown shaded).

	Length 1	Length 2	Length 3	Length 4	Length 5	Length 6
Viroid species						
Block	.	0.155	0.249	0.096		0.131
Block*Viroid	0.461	0.072	0.123	0.399	0.101	
Block*Isolate	0.006	0.087	0.014	0.011	0.005	0.0003
Viroid	0.944	0.899	0.313	0.121	0.982	0.033
Isolate	0.925	0.946	0.915	0.999	0.425	0.696
Control vs. CEVd	0.999	1.000	0.999	0.163	0.989	0.388
Control vs. CBLVd	0.999	0.999	0.979	0.116	1.000	0.330
Control vs. HSVd	0.988	0.977	0.999	0.245	0.994	0.032
Control vs. CDVd	1.000	0.999	0.999	0.204	0.997	0.653
Control vs. CBCVd	0.983	0.962	0.303	0.999	0.999	1.000
Viroid isolates						
Block	.	0.120	0.226	0.093		0.131
Block*Isolate	0.0002	0.005	0.000	0.002	<.0001	0.0003
Isolate	0.987	0.984	0.703	0.593	0.875	0.166
Control vs. CEVd-117	1.000	1.000	1.000	0.474	1.000	0.772
Control vs. CEVd-129	0.999	1.000	1.000	0.497	0.870	0.865
Control vs. CBLVd-Ia	0.999	1.000	1.000	0.470	1.000	0.984
Control vs. CBLVd-Ib	1.000	0.999	0.983	0.313	1.000	0.393
Control vs. HSVd-IIa	1.000	1.000	1.000	0.839	0.965	0.261
Control vs. HSVd-IIb	1.000	1.000	1.000	0.639	1.000	0.319
Control vs. HSVd-IIc	0.999	0.999	1.000	0.572	1.000	0.112
Control vs. CDVd-IIIa	0.999	1.000	0.998	0.667	1.000	0.822
Control vs. CDVd-IIIb	1.000	1.000	1.000	0.802	1.000	0.778
Control vs. CDVd-IIIc	1.000	1.000	1.000	0.593	0.992	1.000
Control vs. CDVd-IIId	1.000	1.000	1.000	0.609	1.000	0.892
Control vs. CBCVd	1.000	1.000	0.523	1.000	1.000	1.000

(continued on next page)

^a Fruit height was measured in a random sample of 10 fruits per tree. Data were collected monthly from October until harvesting time.

Field observations indicated that trees grafted on Troyer and Carrizo citranges were also viroid sensitive and developed mild exocortis symptoms, but only the effects of field sources containing several viroids had been reported (3,32). The present study reports the results of an attempt to establish the effect of single viroid sources on the performance of Washington navel sweet orange trees grafted on Carrizo citrange.

Unexpectedly, no bark scaling symptoms characteristic of the exocortis disease were observed as a result of viroid infection. However, CEVd-infected trees presented small lesions in the roots, a mild form of the symptoms recently described in trees grafted on Carrizo citrange that were infected with a field isolate containing CEVd, HSVd, CBLVd, and CDVd (3). Several trees presented bark-cracking symptoms only consistently observed in trees infected with CBCVd or with the mild CEVd isolate (CEVd-129) as

well as in the noninfected controls. In most instances, the bark cracking symptoms were mild and extended above the bud-union, suggesting that they might be the result of sunburn and not associated with viroid infection. However, in the case of CBCVd-infected trees, bark cracking was intense and mainly restricted to the rootstock, suggesting that it was a viroid induced effect as demonstrated earlier in trees grafted on trifoliate orange (48). As also reported earlier, the presence of pegs and gummy pits was not associated with viroid infection (48).

Infection with CEVd or CDVd also caused a slight reduction of tree growth and yield that was not as significant as reported in the case of trees grafted on trifoliate orange (48,51). Unexpectedly, whereas CBCVd did not affect the growth of trees grafted on trifoliate orange (48,51), the results of the present study showed that CBCVd infection caused the most significant reduction of tree

Table 6. (continued from previous page)^b

Treatment	Diameter 1 (mm)		Diameter 2 (mm)		Diameter 3 (mm)		Diameter 4 (mm)		Diameter 5 (mm)		Diameter 6 (mm)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Viroid species												
Control	72.49	0.731	75.96	0.808	78.24	0.952	77.99	0.978	78.35	0.999	83.85	1.493
CEVd	71.27	0.521	75.30	0.656	77.81	0.782	77.03	0.781	78.53	0.835	81.88	1.100
CBLVd	71.18	0.429	75.91	0.656	77.37	0.782	77.34	0.780	78.34	0.835	80.12	1.100
HSVd	71.44	0.521	75.32	0.597	77.33	0.716	77.25	0.703	78.29	0.773	80.10	0.933
CDVd	71.91	0.384	76.23	0.571	77.72	0.688	77.69	0.668	78.33	0.746	81.48	0.853
CBCVd	71.56	0.731	76.31	0.808	79.54	0.952	79.34	0.978	78.99	0.999	83.71	1.492
Viroid isolates												
Control	72.49	0.731	75.96	0.813	78.25	0.946	78.25	0.946	78.25	0.946	83.85	1.493
CEVd-117	71.12	0.731	75.32	0.813	77.29	0.946	77.29	0.946	77.29	0.946	81.80	1.493
CEVd-129	71.43	0.731	75.28	0.813	78.33	0.946	78.33	0.946	78.33	0.946	81.99	1.493
CBLVd-Ia	71.53	0.731	76.21	0.813	77.83	0.946	77.83	0.946	77.83	0.946	80.57	1.493
CBLVd-Ib	71.35	0.731	75.61	0.813	76.92	0.946	76.92	0.946	76.92	0.946	79.66	1.493
HSVd-IIa	71.45	0.731	75.53	0.813	77.30	0.946	77.30	0.946	77.30	0.946	79.47	1.493
HSVd-IIb	70.91	0.731	74.93	0.813	76.92	0.946	76.92	0.946	76.92	0.946	81.07	1.493
HSVd-IIc	71.18	0.731	75.50	0.813	77.78	0.946	77.78	0.946	77.78	0.946	79.76	1.493
CDVd-IIIa	72.07	0.731	76.37	0.813	77.46	0.946	77.46	0.946	77.46	0.946	80.72	1.493
CDVd-IIIb	71.10	0.731	75.84	0.813	76.92	0.946	76.92	0.946	76.92	0.946	81.42	1.493
CDVd-IIIc	72.54	0.805	76.35	0.883	78.94	1.025	78.94	1.025	78.94	1.025	82.65	1.633
CDVd-IIId	71.95	0.731	76.38	0.813	77.56	0.946	77.56	0.946	77.56	0.946	81.11	1.493
CBCVd	71.56	0.731	76.31	0.813	79.54	0.946	79.54	0.946	79.54	0.946	83.71	1.493

P values obtained when comparing data of viroid infected treatments with the noninoculated controls.

	Diameter 1 (mm)	Diameter 2 (mm)	Diameter 3 (mm)	Diameter 4 (mm)	Diameter 5 (mm)	Diameter 6 (mm)
Viroid species						
Block	0.398	0.170	0.139	0.107	0.264	0.198
Block*Viroid		0.105	0.140	0.400	0.042	
Block* Isolate	0.0003	0.074	0.019	0.029	0.042	0.0002
Viroid	0.596	0.716	0.393	0.344	0.994	0.095
Isolate	0.936	0.985	0.609	0.973	0.206	0.967
Control vs. CEVd	0.446	0.908	0.990	0.757	1.000	0.609
Control vs. CBLVd	0.569	1.000	0.851	0.927	1.000	0.115
Control vs. HSVd	0.333	0.904	0.807	0.869	1.000	0.086
Control vs. CDVd	0.890	0.997	0.971	0.996	1.000	0.376
Control vs. CBCVd	0.769	0.996	0.672	0.615	0.981	1.000
Viroid isolates						
Block	0.397	0.130	0.119	0.102	0.148	0.198
Block*Viroid	0.0003	0.007	0.001	0.007	0.001	0.0002
Block* Isolate	0.912	0.948	0.579	0.797	0.807	0.481
Control vs. CEVd-117	0.777	0.999	0.990	0.999	1.000	0.944
Control vs. CEVd-129	0.937	0.998	1.000	0.939	0.984	0.968
Control vs. CBLVd-Ia	0.968	1.000	1.000	1.000	1.000	0.556
Control vs. CBLVd-Ib	0.904	1.000	0.909	0.986	1.000	0.276
Control vs. HSVd-IIa	0.946	1.000	0.990	1.000	0.988	0.230
Control vs. HSVd-IIb	0.626	0.954	0.910	0.956	0.905	0.745
Control vs. HSVd-IIc	0.815	1.000	1.000	1.000	1.000	0.299
Control vs. CDVd-IIIa	1.000	1.000	0.998	1.000	1.000	0.615
Control vs. CDVd-IIIb	0.760	1.000	0.910	0.999	1.000	0.858
Control vs. CDVd-IIIc	1.000	1.000	1.000	1.000	1.000	1.000
Control vs. CDVd-IIId	1.000	1.000	1.000	1.000	1.000	0.757
Control vs. CBCVd	0.975	1.000	0.917	0.897	1.000	1.000

^b Fruit diameter was measured in a random sample of 10 fruits per tree. Data were collected monthly from October until harvesting time.

Table 7. External quality parameters of fruits from viroid (species and isolates) infected Washington navel sweet orange trees grafted on citrange Carrizo

Treatment	Diameter/length ^a		Rind thickness (mm) ^a		Fruit weight (g) ^b		Fruit size (ml) ^b		Fruit density (g/ml)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Viroid species										
Control	1.01	0.009	5.10	0.120	2933.3	131.14	3278.7	147.88	0.90	0.006
CEVd	1.01	0.007	5.08	0.091	2747.5	98.21	3098.7	111.73	0.89	0.005
CBLVd	1.03	0.007	5.16	0.091	2667.5	98.21	3008.5	111.73	0.89	0.006
HSVd	1.01	0.005	4.99	0.079	2623.3	84.43	2910.7	96.72	0.90	0.004
CDVd	1.02	0.005	4.93	0.074	2825.6	77.93	3129.4	89.68	0.90	0.004
CBCVd	1.01	0.009	5.09	0.120	3041.3	131.14	3378.5	147.88	0.90	0.006
Viroid isolates										
Control	1.01	0.009	5.10	0.120	2933.3	131.14	3278.7	147.88	0.90	0.006
CEVd-117	1.01	0.009	5.02	0.120	2742.0	131.14	3116.7	147.88	0.88	0.006
CEVd-129	1.01	0.009	5.13	0.120	2753.0	131.14	3080.7	147.88	0.90	0.006
CBLVd-Ia	1.03	0.009	5.13	0.120	2753.7	131.14	3107.7	147.88	0.89	0.006
CBLVd-Ib	1.02	0.009	5.19	0.120	2581.3	131.14	2909.3	147.88	0.89	0.006
HSVd-IIa	1.02	0.009	4.84	0.120	2657.0	131.14	2931.0	147.88	0.91	0.006
HSVd-IIb	1.00	0.009	5.35	0.120	2645.0	131.14	2981.3	147.88	0.89	0.006
HSVd-IIc	1.01	0.009	4.79	0.120	2568.0	131.14	2819.7	147.88	0.91	0.006
CDVd-IIIa	1.02	0.009	4.87	0.120	2789.2	131.14	3089.0	147.88	0.90	0.006
CDVd-IIIb	1.01	0.009	4.96	0.120	2790.3	131.14	3097.2	147.88	0.90	0.006
CDVd-IIIc	1.03	0.010	4.99	0.132	2990.8	143.02	3310.6	161.00	0.90	0.007
CDVd-IIId	1.02	0.009	4.89	0.120	2732.0	131.14	3020.7	147.88	0.91	0.006
CBCVd	1.01	0.009	5.09	0.120	3041.3	131.14	3378.5	147.88	0.90	0.006

P values obtained when comparing data of viroid infected treatments with the noninoculated controls (*P* values lower than 0.05 are shown shaded).

	Diameter/length	Rind thickness (mm)	Fruit weight (g)	Fruit size (ml)	Fruit density (g/ml)
Viroid species					
Block		0.460	0.159	0.145	0.250
Block*Viroid		0.160			
Block*Isolate	0.003	0.115	-	-	-
Viroid	0.154	0.432	0.033	0.041	0.014
Isolate	0.811	0.032	0.829	0.775	0.189
Control vs. CEVd	1.000	1.000	0.530	0.643	0.785
Control vs. CBLVd	0.188	0.985	0.234	0.306	0.554
Control vs. HSVd	0.926	0.897	0.104	0.077	0.694
Control vs. CDVd	0.377	0.574	0.845	0.719	0.623
Control vs. CBCVd	1.000	1.000	0.927	0.964	0.990
Viroid isolates					
Block		0.420	0.159	0.145	0.250
Block*Isolate	0.003	0.020			
Isolate	0.467	0.072	0.206	0.210	0.024
Control vs. CEVd-117	1.000	1.000	0.909	0.984	0.643
Control vs. CEVd-129	1.000	1.000	0.935	0.940	1.000
Control vs. CBLVd-Ia	0.379	1.000	0.937	0.978	0.893
Control vs. CBLVd-Ib	0.743	1.000	0.300	0.368	0.964
Control vs. HSVd-IIa	0.805	0.649	0.585	0.440	0.780
Control vs. HSVd-IIb	1.000	0.636	0.535	0.627	0.993
Control vs. HSVd-IIc	1.000	0.421	0.261	0.155	0.377
Control vs. CDVd-IIIa	0.893	0.767	0.986	0.955	0.964
Control vs. CDVd-IIIb	0.999	0.989	0.986	0.966	1.000
Control vs. CDVd-IIIc	0.511	0.999	1.000	1.000	0.988
Control vs. CDVd-IIId	0.867	0.839	0.881	0.775	0.893
Control vs. CBCVd	1.000	1.000	0.999	1.000	1.000

^a Diameter, length, and rind thickness are the average measurements of the 10 fruits sampled from each tree.

^b Fruit weight and size were measured as the overall weight and volume of samples of 10 fruits collected from each tree.

size (height and canopy volume). As reported by Bani Hashemian et al. (3), reduction of tree size was associated with a small root system.

CBCVd-infected trees, in spite of producing smaller yields than the noninfected controls, resulted in a good yield efficiency (yield/canopy volume). The *P* values, however, were not small enough to infer if the differences were statistically significant. Further assays with larger numbers of trees would be necessary to confirm the sensitivity of Carrizo citrange to CBCVd infection and to postulate its usefulness to control tree size in high-density plantations. With the exception of fruits harvested from HSVd-infected trees that presented a smaller caliber, viroid infection did not affect any of the fruit quality parameters evaluated.

In summary, the results of this study indicate that under the growing conditions in which the assay was performed, viroid

infection reduced height and canopy volume and affected fruit size without reducing yield. CBCVd produced interesting effects because in spite of reducing tree size, productivity and fruit size were enhanced. However, these results must be taken with caution because field isolates usually contain several viroids and the effect of mixed infections has been shown to affect the performance of the trees, as has been recently shown in a field assay conducted with 'Nules' clementine and 'Navelina' sweet orange trees grafted on Carrizo citrange (3), as well as in low performing commercial orchards of 'Nules' clementine and 'Ortanique' tanger (*C. reticulata* × *C. sinensis*), also grafted on Carrizo citrange (2). If the sensitivity of Carrizo citrange to CBCVd is confirmed, this viroid would be the best candidate to control the size of trees grafted on Carrizo citrange in high density plantings, as suggested in the case of CDVd for trees grafted on trifoliate orange (50).

Table 8. Internal quality parameters of fruits from viroid (species and isolates) infected Washington navel sweet orange trees grafted on citrange Carrizo

Treatment	Juice volume (ml) ^a		Juice density (g/ml)		Soluble solids (%)		Total acidity (%)		Maturity index	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Viroid species										
Control	1156.7	53.10	1.05	0.004	14.00	0.215	1.48	0.109	9.64	0.749
CEVd	1079.7	41.27	1.05	0.003	13.50	0.160	1.30	0.082	10.77	0.561
CBLVd	1079.7	41.27	1.06	0.003	13.89	0.160	1.37	0.082	10.39	0.561
HSVd	1041.1	36.49	1.05	0.003	13.89	0.136	1.42	0.071	9.94	0.483
CDVd	1109.3	34.34	1.05	0.003	13.55	0.125	1.30	0.065	10.90	0.446
CBCVd	1185.8	53.10	1.05	0.004	13.60	0.215	1.29	0.109	10.83	0.749
Viroid isolates										
Control	1156.7	53.32	1.05	0.003	14.00	0.215	1.483	0.109	9.64	0.749
CEVd-117	1088.3	53.32	1.05	0.003	13.50	0.215	1.387	0.109	10.42	0.749
CEVd-129	1070.0	53.32	1.05	0.003	13.50	0.215	1.222	0.109	11.12	0.749
CBLVd-Ia	1123.3	53.32	1.06	0.003	13.92	0.215	1.350	0.109	10.35	0.749
CBLVd-Ib	1035.0	53.32	1.06	0.003	13.87	0.215	1.383	0.109	10.42	0.749
HSVd-IIa	981.7	53.32	1.06	0.003	13.85	0.215	1.465	0.109	9.58	0.749
HSVd-IIb	1090.0	53.32	1.05	0.003	13.73	0.215	1.382	0.109	10.03	0.749
HSVd-IIc	1051.7	53.32	1.06	0.003	14.10	0.215	1.418	0.109	10.20	0.749
CDVd-IIIa	1103.3	53.32	1.05	0.003	13.57	0.215	1.233	0.109	11.42	0.749
CDVd-IIIb	1080.0	53.32	1.05	0.003	13.45	0.215	1.280	0.109	11.04	0.749
CDVd-IIIc	1194.9	58.06	1.05	0.003	13.23	0.235	1.235	0.119	10.94	0.817
CDVd-IIId	1058.3	53.32	1.05	0.003	13.97	0.215	1.445	0.109	10.20	0.749
CBCVd	1185.8	53.32	1.05	0.003	13.60	0.215	1.285	0.109	10.83	0.749

P values obtained when comparing data of viroid infected treatments with the noninoculated controls.

	Juice volume	Juice density	Soluble solids	Total acidity	Maturity index
Viroid species					
Block	0.191	0.410	0.181	0.1542	0.158
Block*Viroid	0.274				
Viroid	0.185	0.698	0.072	0.4011	0.401
Isolate	0.302	0.666	0.334	0.9226	0.923
Control vs. CEVd	0.539	0.475	0.151	0.4715	0.472
Control vs. CBLVd	0.539	0.909	0.982	0.7862	0.786
Control vs. HSVd	0.177	0.976	0.979	0.9907	0.991
Control vs. CDVd	0.833	0.638	0.165	0.3100	0.310
Control vs. CBCVd	0.987	0.596	0.429	0.5555	0.556
Viroid isolates					
Block	0.145	0.410	0.181	0.154	0.158
Isolate	0.184	0.781	0.135	0.660	0.797
Control vs. CEVd-117	0.955	0.890	0.483	0.998	0.990
Control vs. CEVd-129	0.835	0.890	0.479	0.425	0.655
Control vs. CBLVd-Ia	1.000	0.890	1.000	0.969	0.995
Control vs. CBLVd-Ib	0.477	1.000	1.000	0.997	0.990
Control vs. HSVd-IIa	0.116	0.890	1.000	1.000	1.000
Control vs. HSVd-IIb	0.962	1.000	0.969	0.996	1.000
Control vs. HSVd-IIc	0.652	1.000	1.000	1.000	1.000
Control vs. CDVd-IIIa	0.993	0.890	0.649	0.480	0.439
Control vs. CDVd-IIIb	0.911	1.000	0.366	0.718	0.717
Control vs. CDVd-IIIc	1.000	0.911	0.105	0.550	0.828
Control vs. CDVd-IIId	0.723	0.890	1.000	1.000	1.000
Control vs. CBCVd	1.000	0.890	0.734	0.743	0.859

^a Juice extracted from samples of 10 randomly collected fruits.

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