Properties of Citrus Viroids: Symptom Expression and Dwarfing

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ABSTRACT. Single viroid sources were selected from the viroid collection maintained at Instituto Valenciano de Investigaciones Agrarias (IVIA) to evaluate the field performance of infected trees. The assays were conducted in Corsica and Spain using the same viroid sources. Trifoliate orange seedlings infected with *Citrus exocortis viroid* (CEVd) showed severe stunting, yellow blotching of the twigs and bark scaling symptoms characteristic of exocortis disease. Bark scaling and stunting were also observed when trifoliate orange was used as a rootstock. Trifoliate orange seedlings and rootstocks infected with three different variants of Citrus viroid II (CVd-II), IIa, IIb and IIc, showed characteristic bark cracks. Only CVd-IIb and CVd-IIc variants induced cachexia symptoms on Orlando tangelo seedlings and on the scion of Clementine trees grafted on trifoliate orange. Citrus viroid I (CVd-I) and especially *Citrus viroid III* (CVd-III) caused a considerable reduction in size of trifoliate orange seedlings also observed when this species was used as a rootstock. No bark symptoms were associated with CVd-I and CVd-III infections. *Citrus viroid IV* (CVd-IV) infection also caused bark cracking symptoms and size reduction on Clementine trees grafted on trifoliate orange but these effects were not apparent on trifoliate orange seedlings. The results of these studies confirm that only CEVd induces the exocortis symptoms as initially described. The bark cracking symptoms and the reduced size resulting from infection with other citrus viroids should not be equated with "exocortis".

Citrus trees are natural hosts of several viroids. The identification of citrus viroids other than *Citrus exocortis viroid* (CEVd) was initially performed on the basis of their electrophoretic migration of viroid-like RNA bands in 5% sPAGE, homology determined by molecular hybridization against specific cDNA probes, host range and specific symptoms on the Etrog citron indicator (4, 5, 12, 13, 16, 25, 26, 28, 29, 31). Sequencing information demonstrated that the viroid-like RNAs identified were variants of five different viroid species: CEVd, Citrus viroid I (CVd-I, also known as *Citrus bent leaf viroid*), Citrus viroid II (CVd-II) which includes the citrus variants of *Hop stunt viroid* (HSVd), *Citrus viroid III* (CVd-III) and *Citrus viroid IV* (CVd-IV). Recently another viroid, named Citrus viroid-Os, sharing only 68% homology with CVd-III has been described as a new viroid species (11). However, only two diseases have been clearly attributed to viroids, exocortis and cachexia. Citrus exocortis was described as a bark shelling disorder associated with a dwarfing of trees grafted on trifoliate orange and some of its hybrids (7). Cachexia is a gumming and wood pitting disease, first described on Orlando tangelo, which affects mandarins and mandarin hybrids, kumquats, Palestine sweet lime and alemow (3).

Since the symptoms induced by citrus viroids in a common indicator, Etrog citron, range from severe dwarfing and epinasty to mild vein necrosis and leaf tip browning, citrus viroids were initially all considered to be related to the exocortis disease. Since most field isolates have been found to occur as viroids mixtures in naturally infected trees, the contribution of each viroid to the characteristic symptoms of bark cracking, bark scaling and dwarfing needed to be further evaluated. Following the finding that citrus viroids were independently-transmissible RNA molecules, a collection of single viroids maintained in Etrog citron was available to undertake this type of study which was initiated in 1989.

Field isolates in naturally infected trees have been found to contain viroid mixtures which may produce the classical symptoms of

exocortis or cachexia in susceptible hosts. Additionally, some viroid combinations can influence the expression of exocortis and cachexia symptoms or can have independent effects on tree behavior. A dwarfing effect is well known for CEVd but has been also reported for CVd-III (17, 18, 23, 27). Inoculations with mixtures of viroids revealed a major effect of CEVd on dwarfing and yield performance which could be modified by additional viroids (10, 14, 17, 18). Intensity of bark scaling appeared to be dependent on the combination of viroids including CEVd (10). The effects on fruit yield associated with the presence of viroid mixtures including CEVd were also observed in trees grafted on rootstocks which did not express scaling, such as sour orange and Swingle citrumelo (1, 14). Some viroid combinations without CEVd were also found to induce a significant lower fruit yield both on susceptible rootstock (Troyer citrange) and on tolerant rootstock (sour orange) (24).

Single viroid sources have been characterized by their reaction on Etrog citron indicators but limited data are available on their effect on commercial rootstocks and cultivars. Roistacher et al. (23) described distinct symptoms in trifoliate orange induced by CVd-I, CVd-II and CVd-III. Evaluation of the effects of single viroids on the classical hosts of exocortis and cachexia is then necessary to provide a better understanding of the cause-effect relationships between different CVds and symptom expression. Moreover, pure viroid sources will help to characterize dwarfing factors and to describe their effects on the performance of the trees in order to identify interesting viroid variants not associated with deleterious effects.

MATERIALS AND METHODS

Viroid sources. The following single viroid sources were selected from the viroid collection maintained at Instituto Valenciano de Investigaciones Agrarias (IVIA), Moncada, Spain, on inoculated citrons.

CEVd: Two sources (E-117 and E-129) differing in the severity of symptoms induced in citron and *Gynura* (6) were selected for that reason. Sequencing demonstrated that CEVd (E-117) (9) and CEVd (E-129) (2) are highly homologous to the CEVd sequences defined as class A and class B, respectively (30).

CVd-I: Two sources (Ia and Ib) were selected (8, 27).

CVd-II: Three sources (IIa-117, X-704 and X-707) were selected. The IIa-117 variant had been characterized as a non-cachexia variant (15), sharing the same molecular properties as CVd-IIa from California (21). X-704 and X-707 from the same original source as CVd-IIb and CVd-IIc (21), have been characterized as cachexia variants (15).

CVd-III: Variant IIIa was selected in California (20). Variants IIIb, IIIc and IIId were selected from Spanish sources, but their molecular characterization showed that their nucleotide sequence was virtually identical to CVd-IIIb from California (8, 20).

CVd-IV: The CVd-IV source was originally from California and has been characterized as highly homologous to CVd-IV from Israel (19).

Plant materials and inoculation. In Spring 1991, seedlings of an old-line of trifoliate orange, Orlando tangelo and sour orange were planted (density 5 m× 5 m) in three experimental field plots located at IVIA. In Spring 1992, the plants were graft-inoculated (four plants of trifoliate orange per treatment and five plants of Orlando tangelo and sour orange per treatment) with the viroid sources CEVd (E-117), CVd-I (Ia), CVd-II (IIa, IIb and IIc), CVd-III (IIId) and CVd-IV, and five plants of each species were left as non-inoculated controls. The seven treatments and the controls were randomly distributed. In 1999, the height (H) of the tree, the perimeter

of the trunk 10 cm above soil level and the diameter (D) of the canopy were measured. The canopy volume was calculated as $V = 0.7853HD^2$. For each species, the data were subjected to ANOVA. In those instances in which significant differences were found (trifoliate orange and Orlando tangelo), each treatment was compared to the control following the Dunnett method. Significant differences among treatments were determined following the Duncan's multiple range method.

Commune Clementine SRA85 from the Corsican budwood registration program was grafted in 1989 on Pomeroy trifoliate orange rootstocks which had been previously graft-inoculated with single source viroids. In Spring 1990, six replicates of each treatment were planted in a randomized block arrangement. Once the plantation was established in the field, all the trees were indexed at least twice during the 10-yr period. Two indexing methods were used: a) Biological indexing on Etrog citron followed by nucleic acid extraction and sPAGE analysis (5); b) Nucleic acid extraction of Clementine samples collected in July 1995 and sPAGE and slot-blot hybridization analysis using digoxygenin labeled probes (16, 25). Non-conforming trees (such as trees which did not fit to the viroid treatment due to the absence of detection of the viroid or addition of another variant) and those associated with problems affecting the parameters to be analyzed (such as snow damage which occurred in Winter 1996) were not taken into consideration for this study. Trunk circumferences of the scion and the rootstock were measured annually 10 cm above and below the bud line. The fruit yield per tree was also measured annually. Symptom expression was followed annually, and openings in the bark of the trunk have been made since 1998 to look for symptoms in the wood. The tree size and fruit yield data were submitted to ANOVA, and the significantly different means among the treatments for the different variables were compared following the Duncan method.

RESULTS

Relationship between symptom expression and citrus viroids. Trifoliate orange seedlings infected with CEVd showed severe stunting, yellow blotching of the twigs and the bark scaling symptoms characteristic of the exocortis disease (Table 1). Bark scaling and stunting symptoms were also observed when trifoliate orange was used as a rootstock (Fig. 1A, Table 2). No bark scaling was observed on trifoliate orange seedlings nor on the rootstock of Clementine trees

TABLE 1
VIROID SYMPTOMS ON SEEDLINGS OF THREE CITRUS TYPES

Treatment		Species		
Viroid	Variant	Trifoliate orange	Orlando tangelo	Sour orange
Control		—[a]	—	—
CEVd	E-117	Bark cracking	—	—
CVd-I	I a	—	—	—
CVd-II	II a	Bark cracking	—	—
	II b	Bark cracking	Gum exudates	—
	II c	Bark cracking	Gum exudates	—
CVd-III	III d		—	—
CVd-IV		—	—	—

[a] = no reaction.

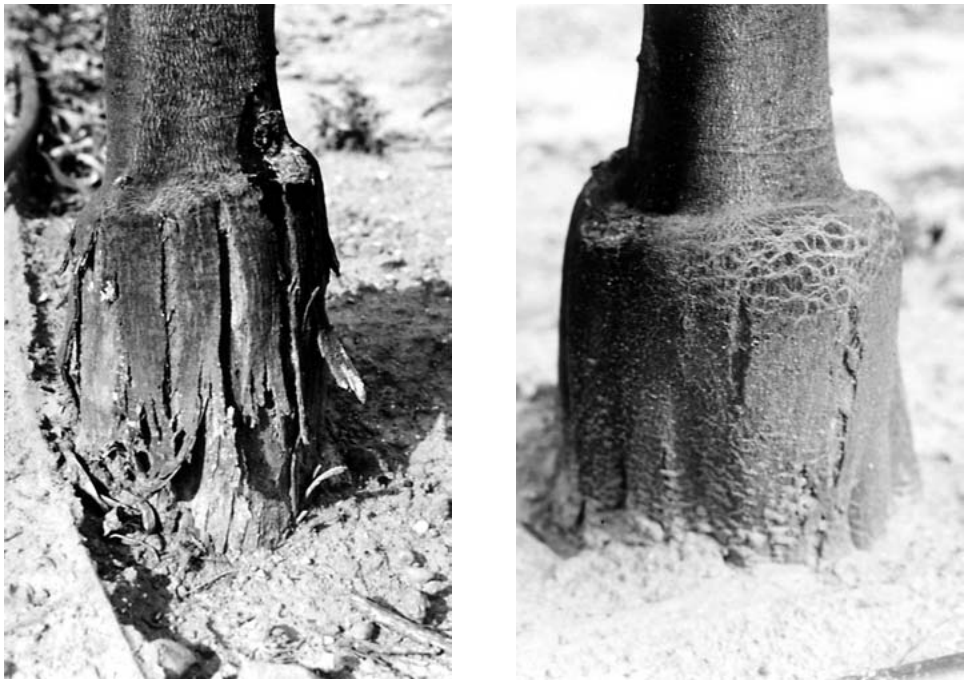

Fig. 1. Symptom expression on the rootstock Pomeroy trifoliate orange of 10-yr-old Clementine tree in Corsica. A. Severe bark scaling on CEVd-117 infected tree. B. Bark cracks on CVd-II infected tree.

grafted on trifoliate orange infected with CVd-I, CVd-II, CVd-III and CVd-IV. No scaling was observed on Orlando tangelo and sour orange seedings.

Trifoliate orange seedlings infected with three different sources of CVd-II (IIa, IIb and IIc) showed characteristic bark cracks, also observed when trifoliate orange was used as a rootstock (Fig. 1B). Cracking was also associated with CEVd, CVd-IV and to a less extent with CVd-I when trifoliate orange was used as a rootstock (Table 2). Only CVd-IIb and CVd-IIc variants induced cachexia symptoms on Orlando tangelo seedlings and on the scion of Clementine trees grafted on trifoliate orange (Fig. 2).

Tree size. CEVd induced a severe stunting and reduction of the diameter of the trunk and the canopy size on trifoliate orange seedlings and rootstocks (Tables 3 and 4). CVd-I and especially CVd-III caused a considerable reduction in size of trifoliate orange seedlings also observed when this species was used as a rootstock. The size reduction on the Clementine scion was more pronounced with the CVd-III viroids (Table 4).

The two cachexia variants of CVd-II (IIb and IIc) induced a significant reduction of size of Orlando tangelo seedlings (Table 3). A reduction of the trunk perimeter was also noticeable on the Clementine tree grafted on trifoliate orange when infected with CVd-IIc (Table 4). No effect was observed on sour orange.

No stunting effect was observed on the two susceptible rootstocks inoculated with CVd-IV (Table 3). Additionally, CVd-IV did not induce any stunting or reduction of the trunk perimeter of the Clementine grafted on trifoliate orange (Table 4).

Fruit yield. During the first 8 yr of harvesting, the mean cumulative fruit weight for the healthy Clementine was 376.7 kg/tree. A significant

TABLE 2
SYMPTOMS INDUCED BY VIROIDS ON CLEMENTINE TREES GRAFTED ON TRIFOLIATE ORANGE AFTER 10 YR

Treatment		Bark symptoms		Gumming	
Viroid	Variant	Scaling	Cracking	Scion	Rootstock
Control		0/5[1]	0/5	0/5	1/5
CEVd	E-117	6/6	*	0/6	0/6
	E-129	6/6	6/6	0/6	2/6
CVd-I	I a	0/5	1/5	0/5	1/5
	I b	0/5	0/5	0/5	4/5
CVd-II	II a	0/5	2/5	0/5	1/5
	II b	0/5	4/5	5/5	0/5
	II c	0/6	5/6	6/6	0/6
CVd-III	III a	0/6	0/6	0/6	2/6
	III b	0/6	0/6	0/6	1/6
	III c	0/6	0/6	0/6	4/6
	III d	0/6	0/6	0/6	4/6
CVd-IV		0/6	3/6	0/6	1/6

*Development of severe scaling masked the cracking symptoms observed in previous years.
[1]Number of affected trees/total number of trees in the treatment.

decrease of the cumulative weight was observed for the trees infected with CEVd variants yielding 201.6 and 195.1 kg per tree for CEVd-117 and CEVd-129 respectively (Table 5). The variants of CVd-III induced also a significant reduction in fruit production. The effects of the other viroids, CVd-I, CVd-II and CVd-IV were not significantly different from the control, except for the cachexia variant CVd-IIc.

Fig. 2. Symptom of gumming on the Commune Clementine scion of 9-yr-old CVd-IIc infected tree.

TABLE 3
EFFECT OF SINGLE VIROID INFECTION ON SIZE OF CITRUS SEEDLINGS[1]

	Species								
	Trifoliate orange			Orlando tangelo			Sour orange		
Viroids	Height (m)	Perimeter (cm)	Canopy (m^3)	Height (m)	Perimeter (cm)	Canopy (m^3)	Height (m)	Perimeter (cm)	Canopy (m^3)
Control	2.55	27.00	7.82	2.26	27.20	5.00	2.48	26.60	6.51
CEVd	1.44 c	18.75 c	2.58 d	1.98 a	24.70 a	4.27 a	2.66	26.20	7.44
CVd-I	2.35 ab	20.87 c	5.46 bc	2.18 a	27.00 a	4.78 a	2.74	26.40	7.79
CVd-II a	2.60 a	22.62 bc	6.60 abc	2.08 a	25.40 a	4.34 a	2.64	26.80	6.62
CVd-II b	2.77 a	25.62 ab	7.66 ab	1.72 b	21.40 b	2.45 b	2.68	26.80	7.23
CVd-II c	2.57 a	26.12 ab	8.84 a	1.64 b	21.50 b	2.33 b	2.58	26.30	6.52
CVd-III	1.95 bc	20.37 c	3.94 cd	2.20 a	27.70 a	5.35 a	2.60	26.50	7.59
CVd-IV	2.60 a	27.87 a	8.05 ab	2.14 a	26.00 a	5.24 a	2.76	28.70	8.93
CME	0.140**	7.286**	3.144**	0.047**	4.619**	0.959**	0.081	6.716	2.165

[1]Level of significance found on ANOVA.
**$P < 0.01$. In each column mean values followed by the same letter are not significantly different and those followed by different letters are significantly different.

TABLE 4
EFFECT OF SINGLE VIROID INFECTION ON SIZE OF CLEMENTINE SRA85 ON POMEROY TRIFOLIATE ORANGE AFTER 10 YR

Treatment		Perimeter (cm)		
Viroid	Variant	Rootstock	Scion	Height (m)
Control		61.1 d[3]	45.8 e	2.96 c
CEVd	E-117	50.6 a	34.5 a	2.46 a
	E-129	51.8 ab	34.4 a	2.59 ab
CVd-I[1]	I	58.9 cd	41.5 cde	2.80 bc
CVd-II	II a	57.6 bcd	43.6 de	2.85 bc
	II b	54.9 abc	40.3 de	2.80 bc
	II c	52.7 ab	37.1 abc	2.65 ab
CVd-III	III a	52.6 ab	35.7 ab	2.69 abc
	III bcd[2]	54.6 abc	34.4 a	2.57 ab
CVd-IV		54.1 abc	40.5 bcd	2.80 bc
		$F = 3.22$, $p = 0.002$	$F = 6.79$, $p < 0.001$	$F = 3.08$, $p = 0.004$

[1]Mean of the variants CVd-Ia and Ib,
[2]Mean of the variants CVd-IIIb, IIIc and IIId.
[3]Means with the same letters are not significantly different (Duncan method).

DISCUSSION

The classical symptom of bark scaling initially associated with exocortis was only induced by CEVd. Cracks in the bark and stunting were also attributed to other single viroids. Thus, the bark cracking symptom and the reduced size resulting from infection with other citrus viroids should not be equated with "exocortis". Only the viroids CVd-IIb and IIc induced gumming in Orlando tangelo and in the Clementine scion, and are responsible of cachexia symptoms. The CVd-II variants, which share similar physical properties, appear to have different biological properties as previously shown (22). No additional symptom was specifically associated with a unique population of viroids as described elsewhere (23). Even though cracking was only consistently observed on trifoliate seedlings infected by CVd-II variants, cracks were associated with four different viroids on grafted trifoliate rootstock, but were not consistently observed, except for CEVd, 11 yr after inoculation. No deep pitting in the wood was observed with any single viroids when windows in the bark of trifoliate orange were opened. Presence of wood pitting will be further evaluated by peeling bark off the whole trunk when the assay is finished.

Dwarfing was strongly induced in CEVd-infected trifoliate orange seedlings or trees grafted on trifoliate

TABLE 5
EFFECT OF SINGLE VIROID INFECTION ON FRUIT YIELD OF CLEMENTINE SRA85 ON TRIFOLIATE ORANGE EXPRESSED BY CUMULATIVE WEIGHT 1992-1999

Treatment		Cumulative fruit weight 1992-99 kg/tree
Viroid	Variant	
Control		376.7 d[3]
CEVd	E-117	201.6 a
	E-129	195.1 a
CVd-I[1]	I	330.8 bcd
CVd-II	II a	333.3 cd
	II b	323.9 bcd
	II c	256.8 abc
CVd-III	II Ia	250.2 abc
	III bcd[2]	244.9 ab
CVd-IV		307.6 bcd
		$F = 5.73$, $p < 0.001$

[1]Mean of variants CVd-Ia and CVd-Ib.
[2]Mean of variants CVd-IIIb, CVd-IIIc and CVd-IIId.
[3]Means with the same letters are not significantly different (Duncan method).

orange. But dwarfing was also observed for CVd-III infected plants and to a lesser extent in CVd-I infected trifoliate orange seedlings. The two cachexia viroids induced dwarfing of Orlando tangelo, and only one significantly reduced the size of the Clementine. Dwarfing cannot be associated only with exocortis and cannot be considered as a specific symptom of the exocortis syndrome. In addition to the dwarfing effect, fruit yield was markedly reduced in CEVd-infected Clementine. A significant reduction was also observed for two of the four CVd-III variants, but other viroid variants inducing stunting did not reduce fruit yield. CVd-I and CVd-III, which do not induce any symptoms on trifoliate orange and mandarins, could represent useful dwarfing factors without any deleterious effect. This confirms the report of Semancik et al. (27) on the effects of these viroids on tree size and performance of Valencia orange grafted on Rubidoux trifoliate orange. They also showed that CVd-IIa has a dwarfing effect with a ratio yield/canopy volume which was actually superior. In our study, when infected with CVd-IIa, the Clementine grafted onto trifoliate orange displayed a slight reduction of size and yield compared to the healthy control but no clear-cut differences were noticeable. As the observations were made on trees of approximately the same age, this could be attributed to a slight different response on the combination Clementine/Pomeroy trifoliate orange. Additional observations should confirm the behaviour of CVd-IIa infected trees.

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LITERATURE CITED

1. Castle, W. S., R. R. Pelosi, and R. F. Lee
   1991. Growth and yield of young sweet orange trees on Swingle citrumelo roostock inoculated with citrus viroids. In: *Proc. 11th Conf. IOCV*, 214-219. IOCV, Riverside, CA.
2. Chaffai, M.
   2000. Mecanismos de defensa asociados a infecciones por viroids. Tesis doctoral. Universidad de Valencia.
3. Childs, J. F. L.
   1950. The cachexia disease of Orlando tangelo. Plant Dis. Rept. 34: 295-298.
4. Duran-Vila, N., J. A. Pina, J. F. Ballester, J. Juarez, R. Rivera-Bustamante, and J. S. Semancik
   1988. The citrus exocortis disease: a complex of viroid-RNAs. In: *Proc. 10th Conf. IOCV*, 152-164. IOCV, Riverside, CA.
5. Duran-Vila, N., C. N. Roistacher, R. Rivera-Bustamante, and J. S. Semancik
   1988. A definition of citrus viroid group and their relationship to the exocortis disease. J. Gen. Virol. 69: 3069-3080.
6. Duran-Vila, N. and J. S. Semancik
   1990. Variations in the cross protection effect between two strains of citrus exocortis viroid. Ann. Appl. Biol. 117: 367-377.
7. Fawcett, H. S. and L. J. Klotz
   1948. Exocortis of trifoliate orange. Citrus Leaves 28: 8-9.
8. Foissac, X. and N. Duran-Vila
   2000. Characterization of two citrus apscaviroids isolated in Spain. Arch. Virol. 145: 1075-1983.
9. Gandía, M., A. Palacio, and N. Duran-Vila
   2000. Variability of citrus exocortis viroid (CEVd). In: *Proc. 14th Conf. IOCV*, 265-272. IOCV, Riverside.
10. Hadas, R., and M. Bar-Joseph
    1991. Variation in tree size and rootstocks scaling of grapefruit trees inoculated with a complex of citrus viroids. In: *Proc. 11th Conf. IOCV*, 240-243. IOCV, Riverside, CA.

11. Ito, T., H. Ieki, K. Ozaki, and T. Ito
2001. Characterization of a new citrus viroid species tentatively termed citrus viroid Os. Arch. Virol. 146: 975-982.
12. La Rosa, R., M. Tessitori, G. Albanese, A. Catara, and M. Davino
1993. Diagnosis of citrus exocortis and hop stunt-homologous citrus viroids by oligonucleotides probes. In: *Proc. 12th Conf. IOCV*, 435-437. IOCV, Riverside, CA.
13. Levy, L., A. Hadidi, and S. M. Garnsey
1992. Reverse-transcription-polymerase chain reaction assays for the rapid detection of citrus viroids using multiplex primers sets. Proc. Int. Soc. Citricult. 2: 800-803.
14. Nauer, E. M., C. N. Roistacher, E. C. Calavan, and T. L. Carson
1988. The effect of citrus exocortis viroid (CEV) and related mild citrus viroids (CV) on field performance of Washington navel orange on two rootstocks. In: *Proc. 10th Conf. IOCV*, 204-211. IOCV, Riverside, CA.
15. Palacio, A. and N. Duran-Vila
2000. Citrus cachexia disease: Molecular characterization of its viroid agent. In: *Proc 14th Conf. IOCV*, 273-281. IOCV, Riverside, CA.
16. Palacio-Bielsa, A., X. Foissac and N. Duran-Vila
1999. Indexing of citrus viroids by imprint hybridisation. Eur. J. Plant Pathol. 105: 897-903.
17. Polizzi, G., G. Albanese, A. Azzaro, M. Davino, and A. Catara
1991. Field evaluation of dwarfing effect of two combinations of citrus viroids on different citrus species. In: *Proc. 11th Conf. IOCV*, 230-233. IOCV, Riverside, CA.
18. Polizzi, G., A. Azzaro, and A. Catara
1992. Effects of citrus viroids on different rootstocks. Proc. Int. Soc. Citricult. 2: 797-799
19. Puchta, H., K. Ramm, R. Luckinger, R. Hadas, M. Bar-Joseph, and H. L. Sänger
1991. Primary and secondary structure of citrus viroid IV (CVd IV), a new chimeric viroid present in dwarfed grapefruit in Israel. Nucleic Acids Res. 19: 6640.
20. Rakowski, A. G., J. A. Szychowski, Z. S. Avena, and J. S. Semancik
1994. Nucleotide sequence and structural features of the Group III citrus viroids. J. Gen. Virol. 75: 3581-3584.
21. Reanwarakorn, K. and J. S. Semancik
1998. Regulation of pathogenicity in hop stunt viroid-related group II. J. Gen. Virol. 79: 3163-3171.
22. Reanwarakorn, K. and J. S. Semancik
1999. Correlation of hop stunt viroid variants to cachexia and xyloporosis diseases of citrus. Phytopathology 89: 568-574.
23. Roistacher, C. N., J. A. Bash, and J. S. Semancik
1993. Distinct disease symptoms in *Poncirus trifoliata* induced by three citrus viroids from three specific groups. In: *Proc. 12th Conf. IOCV*, 173-179. IOCV, Riverside, CA.
24. Roistacher, C. N., J. E. Pehrson, and J. S. Semancik
1991. Effect of citrus viroids and the influence of rootstocks on field performance of navel orange. In: *Proc. 11th Conf. IOCV*, 234-238. IOCV, Riverside, CA.
25. Romero-Durban, J., M. Cambra, and N. Duran-Vila
1995. A simple imprint-hybridization method for detection of viroids. J. Virol. Methods 55: 37-47.
26. Semancik, J. S. and N. Duran-Vila
1991. The grouping of citrus viroids: additional physical and biological determinants and relationships with disease of citrus. In: *Proc. 11th Conf. IOCV*, 178-188. IOCV, Riverside, CA.
27. Semancik, J. S., A. G. Rakowski, J. A. Bash, and D. J. Gumpf
1997. Application of selected viroids for dwarfing and enhancement of production of "Valencia" orange. J. Hort. Sci. 72: 563-570.
28. Semancik, J. S., C. N. Roistacher, R. Rivera-Bustamante, and N. Duran-Vila
1988. Citrus cachexia viroid, a new viroid of citrus: Relationship to viroids of the exocortis disease complex. J. Gen. Virol. 69: 3059-3068.
29. Tessitori, M., R. La Rosa, G. Albanese and A. Catara
1996. PCR diagnosis of citrus viroids in field samples. In: *Proc. 13th Conf. IOCV*, 230-235. IOCV, Riverside, CA.
30. Visvader, J. and R. H. Symons
1986. Replication of *in vitro* constructed viroid mutants: Location of the pathogenicity modulating domain of citrus exocortis viroid. EMBO J. 5: 2051-2055.
31. Yang, X., A. Hadidi, and S. M. Garnsey
1992. Enzymatic cDNA amplification of citrus exocortis and cachexia viroids from infected citrus hosts. Phytopathology 82: 279-285.