

## Characterization and Indexing for Citrus Viroids in Cuba

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**ABSTRACT.** Biological studies on citrus viroids were performed in Cuba, and included the characterization of 15 viroid isolates on indicator plants, the determination of their effects on commercial scion/stock combinations, and the introduction of alternative methods of biological diagnosis of viroid infection. Biological characterization showed that all isolates contained a mixture of two or more viroids which induced mild to severe symptoms on Etrog citron Arizona 861 S-1 and Parson's Special mandarin. Inoculation with isolates containing *Citrus exocortis viroid* (CEVd), Citrus viroid II-b (CVD-IIb), *Citrus viroid III* (CVD-III) and *Citrus viroid IV* (CVD-IV) affected tree growth and production of Valencia orange and Marsh grapefruit on Troyer and Carrizo citrange rootstocks, Marsh grapefruit on alemow rootstock and Persian lime SRA-58' on alemow. In Star Ruby grapefruit on Swingle citrumelo only tree growth was affected. A new methodology for the simultaneous biological detection of citrus viroids was validated.

Success in the management of viroid-caused diseases is based on the knowledge of factors that favor viroid multiplication and symptom expression, the determination of viroid effects on citrus scion and rootstock combinations of horticultural interest, and the knowledge of characteristics of existing isolates, as well as the availability of effective diagnostic methods (2).

Biological studies were conducted on citrus viroid complexes in Cuba, including characterization of 15 isolates by inoculation on the indicator plants, Etrog citron Arizona 861 S-1 and Parson's Special mandarin. Viroid effects on six commercial scion/rootstock combinations under field conditions were also evaluated. New approaches for simultaneous biological detection of multiple viroids were developed and validated according to Peralta and Villoch (1).

Ten of 15 isolates analyzed induced severe symptoms on Etrog citron, while three were moderate and one was mild. On Parson's Special mandarin, six isolates caused severe symptoms and three caused

moderate and one mild symptoms. The reactions of these indicator plants suggested the presence of two or more viroid species in these isolates, and this was confirmed by Velázquez et al. (3).

Viroid infection of six citrus scion/rootstock combinations produced negative effects on tree growth and fruit production, although fruit quality was not affected. Reductions of canopy growth and fruit volume (percentage reduction relative to uninfected control) of infected trees are summarized in Table 1.

The new methodology for simultaneous biological detection of citrus viroids consisted of grafting indicator buds of Clemelin 11-20 on Cuban shaddock rootstocks and simultaneously inoculating with bark samples containing the suspected viroid isolates. Three months later, Etrog citron buds were grafted 5 to 10 cm above the bud union. Observations of citron sprouts were made for symptoms of exocortis and other viroids, and observations for symptoms on Clemelin 11-20 were made by removing bark to detect cachexia symptoms in the wood. Observa-

TABLE 1  
PERCENTAGE REDUCTION (RELATIVE TO UNINFECTED CONTROLS) IN CANOPY  
VOLUME AND FRUIT PRODUCTION OF VIROID INFECTED TREES

Scion/rootstock combination	Viroid species	Canopy volume (%)	Fruit production (%)
Olinda Valencia/Troyer citrange	CEVd	34-71	44-67
Olinda Valencia/Carrizo citrange	CEVd	63	59
Persian lime/Alemow	CEVd, HSVd, CVd III	43-44	40-88
Marsh grapefruit/Troyer citrange	CEVd	67	48
Marsh grapefruit/Alemow	HSVd	52	25
Star Ruby grapefruit/Swingle citrumelo	CEVd, HSVd, CVd III	32-47	0

tions were carried out over 6 mo. This methodology can be used as an alternative viroid diagnostic assay.

### LITERATURE CITED

1. Peralta, E. L. and A. Villoch  
1999. Metodología para la validación de ensayos inmunoquímicos y moleculares utilizados en el diagnóstico de fitopatógenos. *Rev. Fitopatol.* 34: 211.
2. Pérez, R.  
2004. Bases para el manejo de viroides de los cítricos. Tesis presentada en opción al grado de Doctor en Ciencias Agrícolas, Villa Clara, Cuba, 101 pp.
3. Velázquez, K., J. M. Pérez, R. Pérez, D. Rodríguez, I. Peña, and N. Duran-Vila  
2003. Caracterización biológica y molecular de aislamientos de viroides de cítricos de Cuba. *CitriFrut* 20(1): 11-17.