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Detection of Viroids in Five Commercial Citrus Cultivars in Texas

H. O. Miao, M. Skaria, and N. Solís-Gracia

ABSTRACT. Citrus exocortis viroid and other viroids were detected in four of the five major citrus cultivars tested from different South Texas locations. Inoculated Etrog citron indicator plants showed symptoms resembling reactions produced by CEVd, CVdII, CVdIII or CVdIV. The infection level was low or nil in two cultivars. The infection rates of the cultivars tested were 100% in Henderson grapefruit, 80% in Marrs orange, 75% in Navel orange, 27% in Rio Red grapefruit, and 0% in nucellar Ruby Red grapefruit.

Spread of the brown citrus aphid (BrCA) into the Caribbean Islands and Central American countries in the 1990s (4) prompted proactive strategies for citrus tristeza virus (CTV) management in Texas. That concern became stronger when the BrCA was detected in Florida in November, 1995. The major citrus crops in Texas are grapefruit and oranges grown on sour orange rootstock. The use of alternative rootstocks is one of the options the citrus industry would take as a CTV management strategy. However, viroid diseases can become a problem with the change of rootstock. Though the presence of viroids had been reported from Texas (3), the current CTV concern prompted additional viroid tests.

Inoculum source and viroid indexing. Budwood from three commercial grapefruit cultivars, Henderson, Rio Red, and Ruby Red and two sweet orange cultivars, Marrs early and N-33 navel was selected. In each orchard, 10 to 16 trees were chosen along the two principle diagonals. Four budsticks were collected from each tree and placed in a plastic bag and kept refrigerated until indexing. Indexing was done on rooted cuttings of Etrog citron 861-S-1 (5) by T-budding or bark chip grafting. One or two pieces of bark chips from each of four budsticks were grafted onto one or two citron cuttings per sample. A known citrus exocortis viroid (CEVd) positive, E9, and a healthy control were used for comparisons.

Inoculated plants were kept in a greenhouse at 26.5 to 36.5°C. Bud survival rate was recorded 2 to 3 wks after inoculation. Only plants with live inoculum were used in this test. The latent period and the types of reaction such as leaf epinasty, twisting, tip browning; midvein discoloration, necrosis, and cracking; petiole browning and wrinkle; stem necrosis and splits; and plant height were recorded once a week for the first 10 wks and twice a month thereafter, for a total of 9 mo.

Symptoms observed: Symptoms of viroid infection were visible as early as 7 wks after inoculation. Plants showed symptoms which included stunting, leaf epinasty; midvein necrosis and cracking; petiole necrosis, wrinkle, and twisting; as well as stem split and necrosis. Symptoms were detected in four of the five cultivars tested (Table 1). The incidence of viroid infection was 100% in Henderson grapefruit, 80% in Marrs orange, 75% in Navel orange, 27% in Rio Red, and 0% in Ruby Red grapefruits.

Inoculated plants showed symptoms resembling reactions produced by CEVd, CVdII, CVdIII or CVdIV (6). Most of the inoculated plants showed the severe stunting, severe leaf epinasty and necrosis in the midveins and petioles that are usu-

TABLE 1
DETECTION OF CEVd AND OTHER CITRUS VIROIDS IN FIVE COMMERCIAL CULTIVARS, BY INDEXING ON ETROG CITRON 861-S-1

Cultivars	Rootstock	Tree age years	Location	No. samples tested	Infection %
Henderson grapefruit	Swingle	5	Edinburg	10	100
Marrs orange	Sour orange	7 to 9	Mission	10	80
N-33 navel orange	Sour orange	2	La Feria	16	75
Rio Red grapefruit	Sour orange	2	Harlingen	15	27
Ruby Red grapefruit	Sour orange	30	Weslaco	10	0

ally associated with CEVd infection. Some plants showed milder symptoms like leaf drooping with petiole necrosis which are associated with citrus viroids III and IV. A few plants showed only petiole wrinkle or discoloration or tip browning of the leaves which may be associated with citrus viroid II.

Symptoms observed on Etrog citron suggest the presence of different citrus viroids in the indexed trees. An identification of the citrus viroids based only on the symptomatology in Etrog citron is difficult, since the symptom expression can be affected by environmental conditions. Also, mixed infections by different viroids may produce a synergistic effect (1). However, the severe symptoms and short incubation period observed in some of the indexed plants indicate the presence of CEVd. Mild reactions in some of the indicator plants may be due to the presence of citrus viroids other than CEVd.

The high incidence of viroid infection detected in Henderson grapefruit, Marrs orange, and N-33 navel orange suggests possible contamination of the source plants through infected budwood. The low or no incidence of viroids in Rio Red and Ruby Red grapefruit, respectively, are due to the unique origin of these cultivars. The commercial Rio Red was originally obtained by irradiation with thermal neutrons or X-rays to budsticks taken from nucellar Ruby Red trees grown from seed. The 27% infection detected in Rio Red may have been introduced through tools or regrafting of rootstocks where old-line buds failed to take. The Ruby Red block tested at the Citrus Center originated from nucellar plants where tool-borne contamination (2) was nil or very low.

Apart from this study, we have obtained several viroid isolates from various citrus trees. The tristeza virus and the BrCA threat is imminent in Texas. The use of pathogen-free budwood and alternative rootstocks are the two major strategies for managing CTV in the state.

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