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## Citrus Exocortis and Citrus Cachexia Viroids in Commercial Groves of Tahiti Lime in México

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**ABSTRACT.** A survey for citrus viroids was carried out on Tahiti lime in several citrus regions of México. Detection of citrus viroids was conducted by reverse transcription-polymerase chain reaction (RT-PCR) and sequential polyacrylamide gel electrophoresis (sPAGE). Both citrus exocortis (CEVd) and citrus cachexia (CCaVd) viroids were consistently detected in 33 out of 35 samples analyzed from Tamaulipas, Veracruz, Tabasco, and Yucatán citrus groves. There was no evidence of infection with other citrus viroids in the samples analyzed. Disease symptoms present in the groves included in the survey were severe bark cracking on main branches and trunk. These symptoms were frequently associated with differing degrees of tree deterioration and poor growth. In addition, infected trees which were grafted on Alemow rootstock had reduced growth in trunk diameter below the bud union along with brownish lesions on the wood. The consistent presence of both CEVd and CCaVd in Tahiti lime groves from diverse geographical origins in México indicates the continuing use of infected propagative budwood sources.

Tahiti (Persian) lime has become a profitable citrus crop in México, mostly for its easy suitability for export and consumption as fresh fruit in local markets. In the early 1980's, there were only a few thousand ha of Tahiti lime, mostly in the region of Martínez de la Torre, Veracruz in the Gulf of México. However, in the last 15 yr, the crop has expanded to other parts of the country, and currently there are more than 18,500 ha distributed in the states of Veracruz and Tabasco in the Gulf of Mexico, and Oaxaca on the Pacific coast. Other states with increasing areas of Tahiti lime are Yucatan, Chiapas, Jalisco, and Colima (2, 5). These plantings of Tahiti lime plus an additional 80,000 ha of Mexican lime place México as the principal producer of acid limes worldwide (2, 5).

Tahiti lime has traditionally been grafted on sour orange rootstock; however, in recent years a significant number of new groves have been planted on either Alemow, Volkamer lemon, or Troyer and Carrizo citranges (Rocha-Peña, M. A. personal observations).

In the course of several field trips by the second author (MARP) throughout the main citrus areas of México, several viroid-like symptoms were observed in Tahiti lime groves. The symptoms observed were severe bark cracking on the trunk and main branches and reduced size of the trees. This work reports the association of citrus exocortis (CEVd) and citrus cachexia (CCaVd) viroids with these symptoms in most Tahiti lime-producing areas of México.

### MATERIALS AND METHODS

**Plant material.** Plant tissue sampling was addressed to trees showing poor growth and evident bark cracking in twigs and main branches. Dates of sample collection and temperature ranges are shown in Table 1. Budwood and tender tissue was collected from selected trees in Tahiti lime groves in four different citrus regions of the country: States of Tamaulipas (northeast); Veracruz (mid-Gulf of Mexico); Tabasco (southeast Gulf of Mexico); and Yucatán Peninsula.

TABLE 1  
DETECTION OF CITRUS EXOCORTIS (CEVd) AND CITRUS CACHEXIA (CCaVd) VIROIDS BY REVERSE TRANSCRIPTASE (RT)-POLYMERASE CHAIN REACTION (PCR) AND SEQUENTIAL POLYACRYLAMIDE GEL ELECTROPHORESIS (sPAGE) IN TAHITI LIME TREES FROM FOUR DIFFERENT GEOGRAPHICAL AREAS FROM MEXICO

Area	Date of sample collection	Temperature range °C	RT-PCR		sPAGE	
			CEVd	CCaVd	CEVd	CCaVd
Tamaulipas	07/17/96	29-35	7/7	7/7	7/7	0/7
Veracruz	10/02/96	27-33	7/7	7/7	7/7	0/7
Tabasco	01/29/96	20-26	8/8	8/8	8/8	0/8
Yucatán	11/10/97	28-32	11/13	11/13	11/13	0/13
TOTAL			33/35	33/35	33/35	0/35

Samples were shipped to the Research Unit of INIFAP in Monterrey where they were assayed.

**Reverse transcription-polymerase chain reaction (RT-PCR).** RT-PCR assays were run for the two viroids known to cause citrus diseases: CEVd and CCaVd. Primer sequences for both citrus viroids was as described by Yang et al. (17) and synthesized by Bio-Synthesis, Inc. (Lewisville, TX). RNA was extracted from the plant tissue and viroid-cDNA synthesized as follows: 5 µg of the final RNA extract from field or greenhouse indicator plants was mixed separately with 20 pmoles of the appropriate primers for CEVd or CCaVd and 4 µl of 5X RT buffer (250 mM Tris buffer, pH 8.3, 375 mM KCl and 15 mM MgCl<sub>2</sub>). The mixture was heated at 94°C for 3 min and sequentially transferred to ice for 2 min then to room temperature for 30 min. Then the reaction mixture was added: 2 µl of 0.1 M DTT, 5 U of AMV reverse transcriptase, and 500 µM of dNTP's, and the mixture incubated at 37°C for 1 h. Two µl of this reaction was subjected to DNA amplification with 40 pmoles of each set of primers, CEVd or CCaVd as appropriate, in the presence of PCR buffer (20 mM Tris buffer, pH 8.4, 50 mM KCl, 2 mM MgCl<sub>2</sub>, plus 200 µM dNTP's and 2.5 U Taq-DNA polymerase [Gibco]). PCR reactions were run in a Perkin Elmer thermocycler Model 9700, with thermal programs

of 94°, 55°, and 72°C for 30 sec each, and 94°, 50°, and 72°C for 30 sec each, for CEVd and CCaVd, respectively.

**Nucleic acid extraction and sequential polyacrylamide gel electrophoresis (sPAGE).** Nucleic acids from tender tissue from each tree/sample were phenol extracted and purified by the CF-11 cellulose chromatography method described by Baksh et al. (1) and followed by sPAGE as described by Duran-Vila et al. (4).

For RT-PCR and sPAGE analysis, proper healthy, CEVd- and CCaVd-infected citron or Parson's Special Mandarin plants, respectively, were included in every test.

## RESULTS AND DISCUSSION

The results of the survey for citrus viroids on Tahiti lime groves in México are summarized in Table 1. Both CEVd and CCaVd viroids were detected in 33 out of 35 field samples analyzed from Tamaulipas, Veracruz, Tabasco and Yucatán. When the same samples were analyzed by sPAGE, only CEVd was detected; no CCaVd nor any other citrus viroid was found (Table 1). This may have been due to a low titer of the viroids in the plants sampled since most of the samples were collected in fall-winter months of 1996-97. The lack of detection of CCaVd by sPAGE and its relatively easy detection by RT-PCR (Table 1) supports this possibility.



**Fig. 1. Disease symptoms associated to viroid infection in Tahiti lime groves in México. (A) Severe bark cracking on branches. (B) Brownish lesions on the wood noticeable when a portion of the bark is removed at the bud union on plants grafted on Alemow rootstock.**

The RT-PCR assay has become a highly sensitive and powerful tool for the study and detection of many

plant pathogens (6), and has also been adapted for detection of citrus viroids (7, 8, 16,17). In our study, we

used the RT-PCR assays for two well-defined CEVd and CCaVd viroid diseases of citrus (12). Other citrus viroid groups, such as CVd I, CVd II, CVd IIa, CVd III, CVd IV and CVd V, which are frequently associated with CEVd infections (3, 4, 15), may also have been present, but they were not tested for by the PCR method. Most of these other citrus viroids generally occur at lower concentrations than CEVd and sometimes can escape detection by sPAGE when the infected plants are not exposed to warm conditions (3, 4 15).

Most trees included in this study showed symptoms of bark cracking on trunk and main branches to different degrees (Fig. 1A). These symptoms were commonly associated with a reduction of tree size, and sometimes with a marked deteriorated condition of the canopy and poor growth. All trees sampled having bark cracking symptoms were shown to be infected by both CEVd and CCaVd (Table 1). Previous reports of bark cracking symptoms on Tahiti lime in Brazil were associated with CEVd on the basis on the reaction on inoculated citron indicator plants (13). Likewise, in a survey conducted in Venezuela for citrus viroids in Tahiti lime trees showing bark cracking of branches, CEVd and other citrus viroids were detected by RT-PCR and sPAGE (8). Both CEVd and CCaVd viroids were present as mixed infections in most of the samples analyzed in México (Table 1). It is not known if the bark cracking in trunk and branches is caused by either CEVd or CCaVd, or the combination of both because

they were always found as mixed infections (Table 1). The effect of CCaVd was evident on some trees grafted on Alemow rootstock. These trees showed a remarkable stunting and reduced trunk diameter below the bud union, along with brownish lesions on the wood which was noticeable when a portion of bark was removed at the bud union (Fig. 1B).

CEVd has been reported to commonly occur in sweet orange, grapefruit and mandarin groves in the northeastern states of Tamaulipas and Nuevo Leon (9, 14), and is presumed to be widespread in most old citrus groves grafted on sour orange rootstock throughout the country (10, 11). The occurrence of both CEVd and CCaVd in most Tahiti lime samples collected from diverse origins provides new information on the phytosanitary condition of this crop and indicates the continued use of infected budwood sources for the establishment of new Tahiti lime plantations. There were, however, a few samples that did not have CEVd and CCaVd, particularly in groves in the Yucatán Peninsula (Table 1). Inquiry as to the origin of those particular groves revealed that clean Tahiti lime budwood for propagation was brought from outside the country.

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