Confirmation of the Presence of Citrus Viroids in Citrus Orchards in Northwestern Argentina

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ABSTRACT. Citrus viroids cause two well-known diseases: exocortis and cachexia. In the northwestern citrus area of Argentina, the presence of viroids was reported in the 1960s and diagnosis was made only by visual symptoms on susceptible rootstocks. Beginning in 2004, budwood samples of citrus trees showing viroid symptoms were collected in Tucumán, Salta, and Jujuy provinces. Biological indexing was performed using Etrog citron Arizona 861-S1 grafted on rough lemon seedlings as the indicator plant. Beginning in 2006, molecular diagnosis was also performed using sPAGE. For the first time in northwestern region, the presence of viroids was confirmed using both techniques. Symptoms obtained in inoculated Etrog citron were variable and ranged from mild to very severe. Using sPAGE, we determined that most of infections were mixtures of two or more viroids. All viroid isolates are kept in the viroid bank at the Citrus Sanitation Center at Tucumán. Currently, we have 22 isolates from northwestern Argentina and further characterization of these viroids will be done using PCR.

Exocortis and cachexia, caused by *Citrus exocortis viroid* (CEVd) and by a specific variant of *Citrus viroid II* (CVd-IIb) respectively, are present in almost all citrus-growing regions of the world.

Exocortis was first reported in the northwestern region of Argentina by Wallace in 1959 (7). Later, Foguet (4) observed exocortis symptoms on 40% of 147 citrus trees of different species in some old rootstock trials. Field diagnosis of CEVd and cachexia were performed for the first time in 1965 and in 1984 (2, 6).

In the 1970s, the Estación Experimental Agroindustrial Obispo Colombres (EEAOC) released budwood of nucellar clones to citrus growers and nurserymen (3). The propagation of infected budwood from other sources and mechanical transmission with tools and knives spread the viroid diseases. The use of Flying Dragon trifoliate as a rootstock for lemon showed symptomatic evidence of presence of viroids in some citrus groves. As yet it is not an important disease, but may represent a potential risk.

The purpose of the present study was to confirm the presence of citrus viroids in the northwestern region of Argentina by biological and molecular indexing.

From 2003 to 2006, samples were collected from symptomatic trees showing bark scaling on susceptible rootstocks (Fig. 1) and from trees showing no symptoms but with some degree of stunting in Tucumán, Salta and Jujuy provinces. Samples were taken from field trees of grapefruit, sweet orange, lemon, Tahiti lime, and Cleopatra mandarin. All the isolates were maintained in Pineapple sweet orange under greenhouse conditions.

Biological indexing was performed using Etrog citron Arizona 861-S1 grafted on rough lemon rootstock as indicator plants. For each cultivar tested, four indicator plants were inoculated by grafting three bark patches and non-inoculated plants were the negative controls. The inoculated plants were maintained in a greenhouse at 28-32ºC (5) for 1 yr. All inoculated citrons developed symptoms with intensities ranging from severe to mild. Severe symptoms associated with CEVd (Fig. 2) appeared in 4 to 10 weeks, whereas mild symptoms (Fig. 3), associated with other viroids, took from 3 to 6 mo. Severe symptoms were a severe stunting and leaf epinasty and cracking, browning, and necrosis on the underside of midveins of the leaves.

Moderate symptoms were characterized by mild stunting and mild epinasty. Mild symptoms were a very mild leaf epinasty affecting only a few leaves.

Fig. 1. Typical exocortis bark scaling in Valencia sweet orange on Carrizo citrange rootstock.

Fig 2. Severe epinasty induced by pure *Citrus exocortis viroid.*

Fig. 3. Mild viroid leaf symptoms induced in citron.

In addition, inoculated citrons were analyzed by sequential polyacrylamide gel electrophoresis (sPAGE). Nucleic acid extraction and sPAGE were done according to Duran-Vila et al. (1). The results showed that viroids were present and most of the infections were mixtures of two or more viroids (Fig. 4). A summary of a characterization of different isolates is given in Table 1. All 22 viroid isolates are being maintained at the Citrus Sanitation Center of the EEAOC at Tucumán, Argentina. Further characterization will be done using PCR.

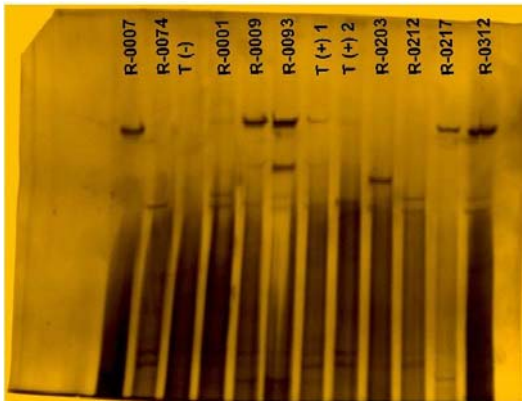

Fig 4. sPAGE nucleic acid analysis of different field isolates.

TABLE 1
BIOLOGICAL INDEXING AND S-PAGE ANALYSIS OF CITRUS VIROID-INFECTED TREES FROM TUCUMÁN, SALTA AND JUJUY PROVINCES, ARGENTINA

Isolate no. (Province)	**Citrus variety**	**Field symptoms**	**Symptoms on citron**	**Presence of viroids s-PAGE**		
				CEVd	**CVd-II**	**Other viroids**
R-0001 (Tucumán)	Cleopatra mandarin	No symptoms	moderate	**(-)**	**(+)**	**(+)**
R-0007 (Tucumán)	Limoneira 8 A Lisbon lemon	Bark scaling	severe	**(+)**	**(-)**	**(-)**
R-0079 (Tucumán)	Limoneira 8 A Lisbon lemon	No symptoms	moderate	**(-)**	**(+)**	**(-)**
R-0184 (Tucumán)	Limoneira 8 A Lisbon lemon	Bark scaling	severe	**(+)**	**(-)**	**(-)**
R-0150 (Tucumán)	Frost Eureka lemon	Bark scaling	mild	**(-)**	**(+)**	**(-)**
R-0009 (Tucumán)	Ruby Blood orange	Bark scaling	severe	**(+)**	**(+)**	**(-)**
R-0010 (Tucumán)	Ruby Blood orange	Bark scaling	severe	**(+)**	**(-)**	**(-)**
R-0011 (Tucumán)	Ruby Blood orange	Bark scaling	severe	**(+)**	**(+)**	**(-)**
R-0069 (Tucumán)	Tahiti lime	No symptoms	severe	**(+)**	**(-)**	**(-)**
R-0074 (Tucumán)	Cape Nartge orange	No symptoms	mild	**(-)**	**(+)**	**(-)**
R-0093 (Salta)	Pineapple orange	Bark scaling	severe	**(+)**	**(+)**	**(+)**
R-0095 (Salta)	Jaffa orange	Stunted tree	severe	**(+)**	**(+)**	**(+)**
R-0096 (Salta)	Jaffa orange	Tall plant in a block of stunted trees	moderate	**(-)**	**(+)**	**(+)**
R-0212 (Salta)	Valencia orange	Stunting and bark scaling	moderate	**(-)**	**(+)**	**(+)**
R-0217 (Salta)	Valencia orange	Bark scaling	severe	**(+)**	**(-)**	**(-)**
R-0202 (Salta)	Rouge la Toma grapefruit	Bark scaling	moderate	**(-)**	**(+)**	**(+)**
R-0204 (Salta)	Rouge la Toma grapefruit	Bark scaling	severe	**(+)**	**(-)**	**(-)**
R-0203 (Salta)	Rouge la Toma grapefruit	No symptoms	severe	**(-)**	**(+)**	**(+)**
R-0178 (Salta)	Rouge la Toma grapefruit	Bark scaling	severe	**(+)**	**(-)**	**(-)**
R-0205 (Salta)	Rouge la Toma grapefruit	Stunting and bark scaling	severe	**(+)**	**(-)**	**(-)**
R-0260 (Salta)	Rouge la Toma grapefruit	No symptoms	severe	**(-)**	**(+)**	**(+)**
R-0312 (Jujuy)	Frost Eureka lemon	Bark scaling	severe	**(+)**	**(+)**	**(+)**

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