ISSN 0370-5404
NOTA TÉCNICA

Rev. Ind. y Agríc. de Tucumán
Tomo 87 (1): 45-48; 2010

Confirmation of the presence of citrus viroids in citrus orchards in Northwestern Argentina*

Julia Figueroa**, Ana Figueroa Castellanos**, Lucas Foguet**,
Cecilia Escobar** and Beatriz Stein**

ABSTRACT

Citrus viroids cause two well known diseases: exocortis and cachexia. In the citrus area of Northwestern Argentina, the presence of viroids was reported in the 1960's and diagnosis was based only on visual symptoms on susceptible rootstocks. From 2004 onwards, budwood samples from citrus trees showing viroid symptoms have been collected in Tucumán, Salta and Jujuy provinces. Biological indexing has been performed using Etrog citron Arizona 861-S1 grafted on rough lemon seedlings as the indicator plant. Since 2006, molecular diagnosis has also been performed using sequential polyacrylamide gel electrophoresis (sPAGE). The presence of viroids has been confirmed for the first time in northwestern region, using both techniques. Symptoms obtained in inoculated Etrog citron vary from mild to very severe. sPAGE utilization showed that most infections consisted of mixtures of two or more viroids. All viroid isolates are kept in the virus bank at the Citrus Sanitation Center of EEAOC in Tucumán. Currently, 22 isolates from Northwestern Argentina are stored and further characterization of these viroids will be done using PCR.

Key words: exocortis, cachexia, indexing.

RESUMEN

Confirmación de la presencia de viroides de citrus en quintas cítricas en el Noroeste Argentino

Los viroides de los citrus causan dos enfermedades bien conocidas en las regiones citrícolas del mundo: exocortis y caquexia. Las citas de viroides en fincas cítricas del Noroeste Argentino (NOA) se remontan a 1960 y el correspondiente diagnóstico se basaba sólo en observaciones de síntomas en portainjertos susceptibles. En 2004, se comenzó con la recolección de muestras de árboles con síntomas de viroides en las provincias de Tucumán, Salta y Jujuy. Se realizó el diagnóstico biológico, usando como planta indicadora el cidro Etrog Arizona 861- S1 inoculado sobre limón rugoso. En 2006 se comenzó también con el diagnóstico molecular usando la técnica de electroforesis secuencial en geles de poliacrilamida (sPAGE), siendo esta la primera vez que se confirmó la presencia de viroides en el NOA utilizando ambas técnicas. Los síntomas obtenidos en los cidros inoculados variaron desde leves a muy severos. Con el sPAGE se determinó que muchas de las infecciones eran mezclas de dos o más viroides. Todos los aislados se mantienen en el banco de virus del Centro de Saneamiento de Citrus, que hasta el momento posee 22 aislamientos correspondientes al NOA. Posteriormente se realizará la caracterización de estos utilizando la técnica de reacción en cadena de la polimerasa (PCR).

Palabras clave: exocortis, caquexia, diagnóstico.

** This technical note is based on a paper published in Proceedings of the 17th Conference of the International Organization of Citrus Virologists, 2007.*

*** Sección Fruticultura, EEAOC. saneamiento@eeaoc.org.ar*

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

Exocortis was first reported in Northwestern Argentina in 1959 (Wallace, 1959). Later, Foguet and Oste (1968) observed exocortis symptoms in 40% of 147 citrus trees of different species in some old rootstock trials. Field diagnoses of exocortis and cachexia were performed for the first time in 1965 and in 1984 (Foguet, 1966; Tan Jun *et al*., 1984).

In the 1970's, the Estación Experimental Agroindustrial Obispo Colombres (EEAOC) released budwood of nucellar clones for citrus growers and nurserymen (Foguet *et al*., 2000). The propagation of infected budwood from other sources and mechanical transmission with tools and knives spread the viroid diseases. The use of trifoliate Flying Dragon as a rootstock for lemon gave symptomatic evidence of the presence of viroids in some citrus groves. Although it is not an important disease, yet it may represent a potential risk.

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

During the period 2003-2006, samples were collected from symptomatic trees showing bark scaling on susceptible rootstocks (Figure 1) and from trees showing no symptoms, but with some degree of stunting, in Tucumán, Salta and Jujuy provinces. Samples were taken from grapefruit, sweet orange, lemon, Tahiti lime and Cleopatra mandarin field trees. All the isolates were kept in Pineapple sweet oranges 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 blind buds. Negative controls were non-inoculated plants. The inoculated plants were maintained in a greenhouse at 28-32ºC (Roistacher, 1991) for one year. All inoculated citrons developed symptoms with intensities ranging from severe to mild. Severe symptoms, associated with CEVd (Figure 2), appeared in four to ten weeks, whereas mild symptoms (Figure 3), associated with other viroids, took from three to six months to appear. Severe symptoms were very severe

Figure 1. Typical bark scaling in Valencia on Carrizo citrange.

Figure 2. Severe epinasty induced by pure CEVd.

Figure 3. Leaf symptoms induced in citron by a viroid infection.

stunting and severe leaf epinasty; and the underside of the leaves showed brown, necrotic and cracked midveins. Moderate symptoms were characterized by mild stunting and mild epinasty. Mild symptoms were a very mild leaf epinasty affecting only a few leaves.

In addition, inoculated citrons were analyzed by sequential polyacrylamide gel electrophoresis (sPAGE). Nucleic acid extraction and sPAGE were carried out according to Duran-Vila *et al*. (1993). The results showed that viroids were present and most of the infections were caused by mixtures of two or more viroids (Figure 4). A summary of a characterization of different isolates is given in Table 1.

All viroid isolates are kept in the viroid bank at the Citrus Sanitation Center of the EEAOC in Tucumán,

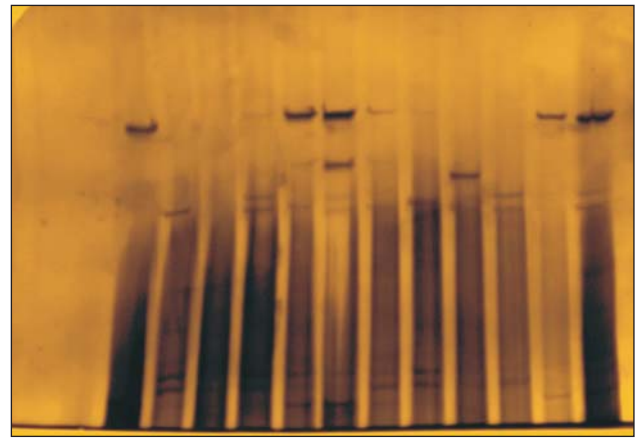

Figure 4. sPAGE nucleic acid analysis of different field isolates.

Table 1. Biological indexing and s-PAGE analysis of citrus viroid infected trees from Tucumán, Salta and Jujuy provinces, Argentina.

Province	Citrus type	Variety	Field symptoms	Symptoms in citron	Presence of viroids s-PAGE		
					CEVd	CVdII	other viroids
Tucumán	Mandarin	Cleopatra	No symptoms	moderate	(-)	(+)	(+)
Tucumán	Lemon	Limoneira 8 A Lisbon	Bark scaling	severe	(+)	(-)	(-)
Tucumán	Lemon	Limoneira 8 A Lisbon	No symptoms	moderate	(-)	(+)	(-)
Tucumán	Lemon	Limoneira 8 A Lisbon	Bark scaling	severe	(+)	(-)	(-)
Tucumán	Lemon	Frost Eureka	Bark scaling	mild	(-)	(+)	(-)
Tucumán	Orange	Ruby Blood	Bark scaling	severe	(+)	(+)	(-)
Tucumán	Orange	Ruby Blood	Bark scaling	severe	(+)	(-)	(-)
Tucumán	Orange	Ruby Blood	Bark scaling	severe	(+)	(+)	(-)
Tucumán	Lime	Tahiti	No symptoms	severe	(+)	(-)	(-)
Tucumán	Orange	Cape Nartge	No symptoms	mild	(-)	(+)	(-)
Salta	Orange	Pineapple	Bark scaling	severe	(+)	(+)	(+)
Salta	Orange	Jaffa	Stunt tree	severe	(+)	(+)	(+)
Salta	Orange	Jaffa	Tall plant in a block of stunted trees	moderate	(-)	(+)	(+)
Salta	Orange	Valencia	Stunting and bark scaling	moderate	(-)	(+)	(+)
Salta	Orange	Valencia	Bark scaling	severe	(+)	(-)	(-)
Salta	Grapefruit	Rouge la Toma	Bark scaling	moderate	(-)	(+)	(+)
Salta	Grapefruit	Rouge la Toma	Bark scaling	severe	(+)	(-)	(-)
Salta	Grapefruit	Rouge la Toma	No symptoms	severe	(-)	(+)	(+)
Salta	Grapefruit	Rouge la Toma	Bark scaling	severe	(+)	(-)	(-)
Salta	Grapefruit	Rouge la Toma	Stunting and bark scaling	severe	(+)	(-)	(-)
Salta	Grapefruit	Rouge la Toma	No symptoms	severe	(-)	(+)	(+)
Jujuy	Lemon	Frost Eureka	Bark scaling	severe	(+)	(+)	(+)

Argentina. Currently, We have 22 isolates from Northwestern Argentina and further characterization of these viroids will be done using PCR.

CITED REFERENCES

Duran-Vila, N.; J. A. Pina and L. Navarro. 1993. Improved indexing of citrus viroids. In: Proc. Conf. IOCV, 12, Riverside, USA, pp. 202-211.

Foguet, J. L. 1966. Enfermedades de los citrus reconocidas en Tucumán. Bol. Inf. Estac. Exp. Agríc. Tucumán (2): 24-28.

Foguet, J. L.; A. Blanco; H. Vinciguerra and J. L. González. 2000. El mejoramiento citrícola en la Estación Experimental Agroindustrial Obispo Colombres. Avance Agroind. 21 (2): 6-8.

Foguet, J. L. and C. A. Oste. 1968. Disorders of trifoliate orange rootstock in Tucumán, Argentina. In: Proc. Conf. IOCV, 4, Gainesville, USA, pp. 183-189.

Roistacher, C. N. 1991. Graft-transmissible diseases of citrus. Handbook for detection and diagnosis. FAO, Rome.

Tan Jun, R. J.; J. Palacios; M. A. García and G. Torres Leal. 1984. Virus disease influence in citrus plantations in Tucumán and Salta-Argentina. In: Proc. Conf. IOCV, 9, California, USA, pp. 362-364.

Wallace, J. M. 1959. Un estudio de las enfermedades de los citrus en algunos países de la América del Sur en 1959. (Informes Técnicos) INTA. Bs. As., Argentina.