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Characterization of some psorosis and concave gum isolates from northwestern Argentina*

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ABSTRACT

The Citrus Sanitation Center of the Estación Experimental Agroindustrial Obispo Colombres in Tucumán, Argentina, has developed a virus bank of various graft-transmissible citrus pathogens found in northwestern Argentina. In this bank, several psorosis and concave gum isolates are maintained in Pineapple sweet orange seedlings. In order to characterize these pathogens, 11 isolates were indexed to seedlings of Pineapple sweet orange, Dweet tangor, Eureka lemon plus Etrog citron budded on rough lemon seedlings. Cross protection was applied for identifying psorosis-A. Symptoms obtained were variable and ranged from mild to very severe. A clear effect of temperature on symptom expression, and distinct differences in the reactions between psorosis-A and concave gum viruses were detected.

Key words: biological indexing, cross protection, TAS-ELISA.HP (horseradish peroxidase), symptom rating.

RESUMEN
Caracterización de aislamientos de psorosis y concave gum del noroeste argentino

El Centro de Saneamiento de Citrus de la Estación Experimental Agroindustrial Obispo Colombres, de Tucumán, Argentina, ha constituido un banco de virus de cítricos con material recolectado en la región noroeste del país. El mismo cuenta con varios aislamientos de psorosis y concave gum que se mantienen en plantas de naranjo dulce Pineapple. Con el objetivo de caracterizar biológicamente 11 de estos aislamientos, se inocularon plantines de naranjo dulce Pineapple, Dweet tangor, limonero Eureka y plantas injertadas de cidro Etrog en limoneros rugoso. Las pruebas con Pineapple se realizaron por duplicado bajo dos condiciones de temperatura: frías y calientes. La confirmación de psorosis A se realizó mediante prueba de protección cruzada con un aislamiento de psorosis B. Los resultados obtenidos muestran una amplia diversidad biológica entre los aislamientos, con expresión de síntomas que variaron desde suaves a muy severos. Se confirmó la presencia de psorosis A y se encontró que los aislamientos de “concave gum” del banco de virus no estaban en mezcla con psorosis. El efecto de la temperatura en la manifestación de los síntomas fue significativo y se observaron claras diferencias entre los síntomas de psorosis y concave gum.

Palabras clave: diagnóstico biológico, protección cruzada, TAS-ELISA.HP (horseradish peroxidase), severidad de síntomas.

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INTRODUCTION

Citrus psorosis is a damaging disease caused by *Citrus psorosis virus* (CPsV). It induces typical bark scaling lesions on sweet orange, mandarin and grapefruit trunks and limbs, and occasionally ringspot symptoms on their leaves and fruit. Wood staining often accompanies bark scaling on infected branches and trunks. Sour orange, lemon, pummelo and rough lemon usually show no external bark symptoms (Roistacher, 1991). Based on symptom expression, two types of psorosis were proposed by Fawcett and Klotz (1938): psorosis-A and psorosis-B. Later, Wallace (1957) showed that psorosis-A protects against a challenge from the more severe bark lesions produced by psorosis-B.

The causal agent of the disease is *Citrus psorosis virus* (CPsV), a type member of Ophiovirus genus with a genome of three single-stranded RNAs of negative polarity (Milne *et al*., 2000; Zanek *et al*., 2006).

Several methods are available for CPsV detection (Martín *et al*., 2004). Biological indexing is undertaken by graft-inoculating citrus indicator plants, and then testing for cross protection with a severe isolate (Roistacher and Calavan, 1965; Roistacher, 1991, 1993). DAS-ELISA (García *et al*., 1997), TAS-ELISA-AP (AP, alkaline phosphatase) (Alioto *et al*., 1999) and TAS-ELISA-HP (HP, horseradish peroxidase) (Zanek *et al*., 2006) are methods developed and applied for diagnosis in field trees. Several primers have been designed for CPsV detection by RT-PCR, thus providing alternative methods for diagnosis.

The disease is widespread in many parts of the world, including South America and the Mediterranean areas (Roistacher, 1993). In Argentina, psorosis is epidemic in the northeastern region, whereas in the northwestern region, although present it is not commonly observed in the orchards. It was introduced into Tucumán around 1910, when citrus growers imported sweet orange varieties from the United States. Since 1938, different authors have described its presence (Fawcett, 1938; Fawcett, 1939; Fernández Valiela, 1961; Foguet, 1966). Field diagnosis of the disease was practised and trees with bark lesion symptoms were eliminated. This probably contributed to the limited spread of the disease in Tucumán (Fawcett, 1938).

Although concave gum, impietratura and cristacortis induce young leaf symptoms in the same indicator plants, these diseases cannot be grouped with psorosis-A (Roistacher, 1981). All these diseases produce oak leaf patterns in leaves of both field trees and indicator plants, but they rarely induce shock symptoms in indicator plants. The concave gum pathogen will not protect against a challenge from psorosis –B lesion inoculum (Roistacher and Calavan, 1965) and does not produce scaly bark, but induces other trunk or fruit symptoms distinct from those of psorosis. In addition, concave gum, impietratura, and cristacortis isolates do not contain a 48 kd protein commonly associated with psorosis. Moreover, ringspot isolates (da Graça *et al*., 1991, 1993; Roistacher, 1993) and tissue of concave gum infected trees do not react with psorosis-A antiserum (D'Onghia *et al*., 1998).

Since its foundation in October 2004, the Citrus Sanitation Center of the EEAOC established a bank of graft-transmissible diseases of citrus which are maintained in holding plants of Pineapple sweet orange. Psorosis and concave gum isolates, kept in a cool greenhouse (18ºC-27ºC), were used together with other virus sources as positive controls in our indexing.

MATERIALS AND METHODS

Psorosis and concave gum sources were obtained from field trees and samples brought to the laboratory by nurserymen. They were found positive after biological diagnosis. Isolates were collected from symptomatic field trees of citrus orchards in the provinces of Tucumán, Jujuy and Salta, between 2003 and 2006 (Figures 1, 2, 3 and 4).

Figure 1. R-0152. Bark scaling produced by psorosis A.

Figure 2. R-0052. Bark scaling produced by psorosis B.

Figure 3. R-0083. Gum in a transversal trunk section.

Figure 4. R-0002. Atypical bark scaling.

In order to obtain information on the biological diversity among isolates, 11 were biologically indexed as described by Roistacher (Roistacher, 1991; Roistacher *et al*., 2000). Characteristics of the psorosis and concave gum sources from northwestern Argentina used in this experiment are displayed in Table 1. Seedlings of Pineapple sweet orange and Eureka lemon, plus Etrog citron budded on rough lemon, were used as indicator plants for characterization. In contrast, for concave gum isolates Dweet tangor seedlings were used instead of Eureka lemon. For each tested isolate, four indicator plants were inoculated by grafting three blind buds. Non-inoculated plants were the negative controls. Confirmation of psorosis-A was sought by challenging the infected Pineapple seedlings with psorosis-B bark inoculum (cross protection) five months after inoculation.

All indicator plants were grown in individual containers using an artificial substrate of peat moss and perlite. Fertilizers were applied using both micro and macronutrients in order to obtain uniform, healthy and vigorous plants. During the study, Etrog citron, Eureka lemon and Dweet tangor plants were kept under cool temperatures (24ºC to 27ºC maximum during the daytime and 18ºC to 21ºC minimum in the night). Pineapple sweet orange plants were divided into two groups: one held in a cool greenhouse and the other in a warm greenhouse, with temperatures of 28ºC to 33ºC maximum in the daytime and 25ºC to 27ºC minimum at night. In addition, supplementary

Table 1. Characteristics of the psorosis and concave gum sources from northwestern Argentina used in this experiment.

Isolate	Variety	Location	Notes
R-0052	Westin orange	Tucumán	Severe bark scaling. Gum in a transversal trunk section
R-0053	Westin orange	Tucumán	Severe bark scaling. Gum in a transversal trunk section
R-0083	Westin orange	Tucumán	Severe bark scaling. Gum in a transversal trunk section
R-0152	Navel orange	Tucumán	Bark scaling
R-0185	Marrs early orange	Tucumán	Bark scaling
R-0186	Marrs early orange	Tucumán	Bark scaling
R-0193	Westin orange	Tucumán	Bark scaling
R-0001	Cleopatra mandarin	Tucumán	Consistently induced OLP in indicator plants (Pineapple sweet orange)
R-0002	Valencia orange	Salta	Atypical bark scaling in field trees. Gum in a transversal trunk section
R-0106	Pineapple orange	Salta	Consistently induced OLP in indicator plants (Pineapple sweet orange)
R-0127	Carrizo citrange	Jujuy	Consistently induced OLP in indicator plants (Pineapple sweet orange)

lighting was given during winter months in order to enhance symptom expression (Roistacher, 1963).

Plants were inoculated in mid-August and symptoms were observed regularly, beginning at the fourth week after inoculation and ending four and a half months later. Then, cross protection tests were carried out using lesion inoculum Ps 243-1, from the virus bank of University of California, Riverside. Symptoms observed were shock, leaf crinkle, mottle, oak leaf pattern (OLP) and flecking. Severity was ranked as follows: 0: negative, no symptoms; +: mild symptoms; ++: moderate symptoms; +++: severe symptoms and ++++: very severe symptoms.

RESULTS AND DISCUSSION

A wide variation in symptom expression among isolates was found (Table 2 and Table 3), including oak leaf pattern, shock, leaf crinkle, mottle and flecking (Figures 5, 6 and 7). Psorosis A was confirmed with the cross protection test and results are also given in Table 2 and Table 3.

Results obtained corroborate previous findings. The importance of temperature for symptom expression was evident, especially for shock reaction. Cool temperatures favored the appearance of shock symptoms in young emerging shoots. By contrast, under warm conditions symptoms were less clear and in some cases, shock and other symptoms did not appear, as had been reported with tristeza reaction in Mexican lime plants (Roistacher *et al*., 1974).

Psorosis induced shock reaction in Pineapple sweet orange, citron and Eureka lemon under cool conditions, whereas concave gum induced none of these symptoms. Only two isolates, R-0152 and R-0186, induced very severe shock symptoms in Eureka lemon and citron, respectively, under cool conditions.

There was much variability in symptom expression and very few symptoms were the same for the different isolates on all indicator plants.

Cross protection results for concave gum isolates were negative, so they were not in mixtures with psorosis. OLP symptoms, as well as those of psorosis, were more evident under cool conditions.

Isolate R-0002, showing an unusual bark scaling (Figure 4) and gum in a trunk section, induced OLP symptoms in indicator plants and gave no cross protection against psorosis-B challenge. TAS-ELISA-HP and RT-PCR tests were also performed (Zanek *et al*., 2006) and they were also found CPsV negative. Citrus plants with psorosis-like bark lesions which indexed negatively for psorosis-A have been reported by Martin *et al*. (2002) in Spain, as well as plants with Bahía bark scaling by Laranjeira *et al*. (2006), in Brazil. Further research is underway to determine the character of this non-psorosis-A bark scaling.

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Table 2. Symptoms expressed by psorosis isolates in indicator plants held under cool and warm temperatures.

Isolate	Pineapple sweet orange				Etrog citron		Eureka lemon		Pineapple
	Cool		Warm		Cool		Cool		challenged
	Shock	Young leaf	Shock	Young leaf	Shock	Young leaf	Shock	Young leaf	with Ps B
R-0052	(+)	(++)	(-)	(+)	(-)	(+++)	(-)	mot(+)	(-)
R-0053	(+++)	(+++)	(-)	(++)	(-)	mot, lc(+)	(+)	mot(+)	(-)
R-0083	(++++)	(+)	(-)	(+)	(+)	mot (++)	(-)	(-)	(-)
R-0152	(++++)	(++)	(-)	spot(+)	(++)	(+)	(++++)	lc, mot(++)	(-)
R-0185	(+++)	(+)	(-)	spot(++)	(-)	(+)	(-)	mot(+++)	(-)
R-0186	(++++)	(+)	(-)	(+)	(++++)	(-)	(-)	(-)	(-)
R-0193	(++)	(+)	(-)	(-)	(+)	(+)	(-)	(+)	(-)

Table 3. Symptoms expressed by concave gum isolates in indicator plants held under cool and warm temperatures.

Isolate	Pineapple sweet orange				Etrog citron		Eureka lemon		Pineapple
	Cool		Warm		Cool		Cool		challenged
	Shock	Young leaf	Shock	Young leaf	Shock	Young leaf	Shock	Young leaf	with Ps B
R-0001	(-)	OLP(+)	(-)	(-)	(-)	(-)	(-)	OLP(+)	(+)
R-0002	(-)	OLP(++)	(-)	(-)	(-)	(-)	(-)	OLP(+)	(+)
R-0106	(-)	OLP(++)	(-)	(-)	(-)	(-)	(-)	OLP(++)	(+)
R-0127	(-)	OLP(+++)	(-)	(-)	(-)	(-)	(-)	OLP(+++)	(+)

Cross protection: a negative reaction indicates protection. **Abbreviations:** lc: leaf crinckle; mot: mottle; OLP: oak leaf pattern; spot: spotting and PsB: psorosis B. **Symptoms:** (-) negative; (+): mild; (++): moderate; (+++): severe and (++++): very severe.

Figure 5. R-0001. Oak leaf pattern in Dweet tangor.

Figure 6. Shock reaction in Pineapple.

Figure 7. R-0152. Mottle and leaf crinkle in Eureka lemon.

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