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 pathogens of citrus found in northwestern Argentina. In this bank there are several psorosis and concave gum isolates that are maintained in Pineapple sweet orange seedlings. In order to characterize these pathogens, an experiment was conducted with 11 isolates by indexing them 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. There was a clear effect of temperature on symptom expression and distinct differences in the reactions between psorosis-A and concave gum.

Citrus psorosis is a damaging disease caused by *Citrus psorosis virus* (CPsV), type member of the genus *Ophiovirus* with a genome of three single-stranded RNAs of negative polarity (14, 25). It induces typical bark scaling lesions in the trunks and limbs of sweet orange, mandarin and grapefruit, and occasionally ringspot symptoms on leaves and fruit. Wood staining often accompanies bark scaling in infected branches and trunks. Sour orange, lemon, pummelo and rough lemon usually show no external bark symptoms (18). Two types of psorosis were proposed by Fawcett and Klotz (8): psorosis-A and Psorosis-B, and Wallace (24) showed that psorosis-A would protect a sweet orange seedling against challenge from the more severe bark lesion forming psorosis-B (21).

Several methods are available for detection (13). Biological indexing is done by graft-inoculating citrus indicator plants and then testing for cross protection with a severe isolate (18, 19, 23). DAS-ELISA (11), TAS-ELISA-AP (using alkaline phosphatase) (1) and TAS-ELISA-HP (using horseradish peroxidase) (25) are methods developed and applied for diagnosis in field trees, and several primers have been designed for CPsV detection by RT-PCR, providing alternative methods for diagnosis.

The disease is widespread in many parts of the world, including South America and the Mediterranean areas (23). In Argentina, psorosis is epidemic in the north-east and although it is also present in the northwestern region (NW), it is not commonly observed in the orchards. It was introduced to Tucumán around 1910, when citrus growers imported sweet orange varieties from United States, and since 1938 different authors have described its presence (6, 7, 8, 10, 25). Field diagnosis of the disease was practiced and when a tree with bark lesion symptoms was found it was eliminated. This probably contributed to the limited dispersal of the disease in Tucumán (7).

Although concave gum, impietratura and cristacortis induce young leaf symptoms in the same indicator plants, these diseases cannot be grouped with psorosis-A (22). These diseases all produce oak leaf patterns in leaves of field trees as well as 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 (16). They do not produce scaly bark but induce other trunk or fruit symptoms distinct from those of psorosis. In addition, isolates of concave gum, impietratura, and cristacortis do not contain a 48 kd protein commonly associated with psorosis and ringspot isolates (3, 4, 23) and shown to be the viral coat protein (2); tissues of concave gum infected trees do not react with psorosis-A antiserum (5).

The Citrus Sanitation Center of the EEAOC, since its founding in October 2004 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 (18-27ºC) 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 from samples taken to the laboratory by nurserymen and found positive after biological diagnosis. Isolates from field orchard trees were collected between 2003 and 2006 from symptomatic citrus trees in Tucumán, Jujuy and Salta provinces (Fig 1, 2, 3 and 4).

In order to obtain information on the biological diversity among isolates, 11 of them were biologically indexed as described by Roistacher (18, 21). Characteristics of the psorosis and concave gum sources from northwestern Argentina used in this experiment are given 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; but for concave gum isolates, seedlings of Dweet tangor were used instead of Eureka lemon. For each isolate tested, four indicator plants were inoculated by grafting three blind buds and non-inoculated plants were the negative controls. Psorosis-A was confirmed by challenging the infected Pineapple seedlings with psorosis-B bark inoculum (cross protection) 5 mo after inoculation.

Fig 1. Psorosis A bark scaling on Navel orange in Tucumán (Isolate R-0152).

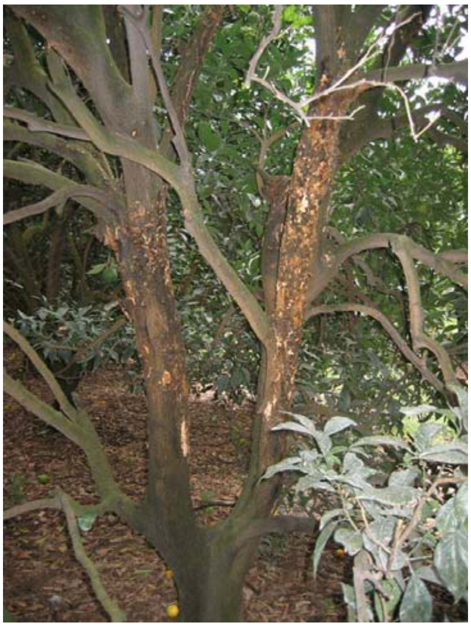

Fig 2. Psorosis B bark scaling on Westin orange in Tucumán (Isolate R-0052).

Fig 3. Psorosis associated gum in a transversal section of a Westin orange trunk in Tucumán (Isolate R-0083).

Fig 4. Atypical bark scaling in a Valencia orange in Salta (Isolate R-0002).

TABLE 1
CHARACTERISTICS OF THE PSOROSIS AND CONCAVE GUM SOURCES FROM NORTHWESTERN ARGENTINA

Isolate	Variety	Location	Notes
R-0052	Westin orange	Tucumán	Severe bark scaling. Gum in tranversal trunk section
R-0053	Westin orange	Tucumán	Severe bark scaling. Gum in transversal trunk section
R-0083	Westin orange	Tucumán	Severe bark scaling. Gum in 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 Pineapple sweet orange
R-0002	Valencia orange	Salta	Atypical bark scaling in field tree. Gum in transversal trunk section
R-0106	Pineapple orange	Salta	Consistently induced OLP in Pineapple sweet orange
R-0127	Carrizo citrange	Jujuy	Consistently induced OLP in Pineapple sweet orange

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, citron, Eureka lemon and Dweet tangor plants were kept under cool temperatures (24º to 27ºC maximum day and 18º to 21º C minimum night). Pineapple sweet orange plants were divided into two groups; one held in the cool greenhouse and the other in a warm greenhouse with temperatures of 28ºC to 33ºC maximum day and 25º to 27ºC minimum night. In addition, supplemental lighting was given during winter months in order to enhance symptom expression (17).

Plants were inoculated in mid-August and symptoms were observed regularly beginning the fourth week after inoculation and ending 4½ mo later. Cross protection tests were then done using lesion inoculum Ps 243-1, from the University of California, Riverside virus bank. Symptoms observed were shock, leaf crinkle, mottle, oak leaf pattern (OLP) and flecking. Severity was ranked as follows: 0: no symptoms; +: mild symptoms; ++: moderate symptoms; +++: severe symptoms and ++++: very severe symptoms.

RESULTS AND DISCUSSION

A wide variation in symptom expression between isolates was found (Table 2) ranging from oak leaf pattern, shock, leaf crinkle, mottle, and flecking (Figs. 5, 6 and 7). Psorosis-A was confirmed with the cross protection test, and these results are also given in Table 2.

Fig 5. Concave gum induced oak leaf pattern in Dweet tangor (Isolate R-0001).

Fig 6. Shock reaction in Pineapple sweet orange.

Fig 7. R-0152. Psorosis mottle and leaf crinkle in Eureka lemon (Isolate R-0152)

TABLE 2
SYMPTOMS EXPRESSED BY PSOROSIS AND CONCAVE GUM ISOLATES IN INDICATOR PLANTS HELD IN COOL AND WARM TEMPERATURES

Isolate	Pineapple sweet orange				Etrog citron		Eureka lemon		Pineapple challenged
	Cool		Warm		Cool		Cool		
	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	(++)	(+)	(-)	(-)	(+)	(+)	(-)	(+)	(-)
							Dweet tangor		
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, PsB: psorosis B
Symptoms: (-) negative, (+): mild; (++): moderate; (+++):severe; (++++): very severe

Our results corroborate previous findings. The importance of temperature for symptom expression was evident, especially for the shock reaction. Cool temperatures favored appearance of shock symptoms in the young emerging shoots whereas 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 on Mexican lime plants (20).

Psorosis caused shock reaction in Pineapple sweet orange, citron and Eureka lemon under cool conditions whereas concave gum induced none of these symptoms. 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. The OLP symptoms, as well as those of psorosis, were more evident under cool conditions.

Isolate R-0002, showing unusual bark scaling (Fig. 4) and gum in a trunk section, induced OLP symptoms in indicator plants but gave no cross protection against psorosis-B challenge. TAS-ELISA-HP and RT-PCR were also performed (25) and gave negative results for CPsV. Citrus with psorosis-like bark lesions which index negative for psorosis-A have been described in Spain by Martin et al. (12) and for Bahía bark scaling in Brazil by Nickel et al. (15). Further investigation is underway to determine the character of this non-psorosis-A bark scaling.

This is the first time that this kind of assay was performed in our region and a large variability in symptom expression among the isolates was found. The data obtained provide information on the biological diversity among isolates from the northwestern citrus region of Argentina.

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