

## Biological characterization of *Citrus tristeza virus* strains in lemon in Tucumán, Argentina

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### ABSTRACT

Tucumán province is the largest lemon producer in the world, with a production of 1.3 million tons. Unfortunally, tristeza disease has been present in Argentina since 1930 and is endemic. *Citrus tristeza virus* isolates from the main lemon varieties in Tucumán province were biologically characterized on the basis of vein clearing, vein corking, leaf cupping, stunting, stem pitting and other symptoms using standard indicator plants. The lemon varieties were: Frost Eureka, Frost Lisbon, Limoneira 8A and Genoa EEAT. The indicator plants used were Mexican lime, Eureka lemon, Duncan grapefruit, Pineapple sweet orange and sour orange seedlings, plus Ruby Blood sweet orange budded on sour orange rootstock. Symptoms on each of the indicator plants were rated from 0 (no reaction) to 5 (most severe). A total rating for each isolate was obtained by summing the ratings on each indicator plant. Differences among lemon isolates were observed. Genoa isolate induced least growth while Limoneira isolate induced the most severe stem pitting. Little difference was observed between Eureka and Lisbon isolate.

**Key words:** lemon varieties, CTV isolates, indicator plants, symptoms rating.

### RESUMEN

#### Caracterización biológica de aislamientos del virus de la tristeza de los cítricos de limoneros en Tucumán, Argentina

La Argentina es el primer país productor de limón en el mundo y la provincia de Tucumán lidera esta producción con 1,3 millones de toneladas. Desafortunadamente la tristeza de los cítricos es una enfermedad endémica en la Argentina desde 1930.

En el presente trabajo se informa sobre la caracterización biológica realizada a distintos aislamientos del virus de la tristeza de los cítricos de las principales variedades comerciales de limoneros. Las variedades de limoneros estudiadas fueron: Eureka Frost, Lisboa Frost, Lisboa Limoneira 8 A y Génova EEAT. La caracterización se realizó en las plantas indicadoras estándares para este tipo de pruebas y fueron: plantines de lima mexicana, limón Eureka, pomelo Duncan, naranjo dulce Pineapple y naranjo agrio; y plantas injertadas de naranjo dulce Ruby Blood en naranjo agrio. Los principales síntomas evaluados fueron clorosis y suberización de nervaduras, acopamiento de las hojas, enanismo y acanaladuras en la madera. Los síntomas en cada uno de los indicadores se clasificaron en una escala desde 0 (ausencia de síntomas) hasta 5 (síntomas muy severos). La valoración final para cada aislamiento se obtuvo de la suma de los valores obtenidos en cada indicador. Se encontraron diferencias entre los aislamientos. El aislamiento de limonero Génova EEAT resultó el más enanizante, mientras que el "stem pitting" más severo fue causado por el aislamiento del Lisboa Limonera 8 A. No hubo diferencias importantes entre los aislamientos de Eureka y Lisboa.

**Palabras clave:** variedades de limoneros, aislamientos de CTV, plantas indicadoras, severidad de síntomas.

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The *Citrus tristeza virus* (CTV) isolate which becomes permanently established in a citrus tree depends on its variety. All citrus species grown in the field naturally infected with tristeza can pick up the virus that caused stem pitting in the small fruited lime (Mexican), but only certain species pick up the virus which additionally causes the seedling yellow reaction in sour orange. CTV recovered from lemon only causes stem pitting in lime (Mc Clean, 1977).

Tucumán province, Argentina, is one of the leading lemon producing areas in the world and several lemon varieties are very well adapted to the region. The most efficient vector of tristeza, *Toxoptera citricida*, is present in the area and the disease is thus endemic.

The purpose of this study was to biologically characterize the CTV isolates collected from different commercial lemon varieties. Characterization was performed on the basis of differences in symptomatology in the different indicator plants.

CTV isolates from the main lemon varieties in Tucumán province were biologically characterized on the basis of vein clearing, vein corking, leaf cupping, stunting, stem pitting and other symptoms, using standard indicator plants (Garnsey *et al.*, 1987; Roistacher, 1991 and Moreno, 1991; Rocha Peña *et al.*, 1993; Polek *et al.*, 2005). The lemon varieties tested were Frost Eureka, Frost Lisbon, Limoneira 8A Lisbon and Genoa EEAT, from the citrus germplasm bank of the Estación Experimental Agroindustrial Obispo Colombres (EEAOC). The indicator plants used were Mexican lime, Eureka lemon, Duncan grapefruit, Pineapple sweet orange and sour orange seedlings, plus Ruby Blood sweet orange budded on sour orange rootstock (sweet/sour). Four plants of each indicator were graft-inoculated using three blind buds from the source plants. They were grown as a single stem and they were cut back after inoculation to force new growth. Greenhouse temperatures during the study were maintained between 18 and 27°C.

Symptoms in each of the indicator plants were evaluated four times during a 12-month period (third, fifth, ninth and twelfth months after inoculation) and symptoms were rated: 0: no reaction; 1: very mild; 2: mild; 3: moderate; 4: severe and 5: very severe. The individual symptom reaction scores for each host plant (n=4) were averaged. A total rating for each isolate was obtained by summing the ratings on each indicator plant (Table 1). One evaluator was involved in rating all tests and in making the final scoring. Stem pitting was determined at the end of the test. The main symptoms observed in indicator plants for all lemon isolates were the following: Mexican lime showed moderate to severe vein clearing (Figure 1), very mild to moderate leaf cupping (Figure 2), moderate to severe stunting (Figure 3) and moderate to very severe stem pitting, with areas of porous wood pitting along with

brownish gum-like deposits (Figure 4) as described by Garnsey *et al.* (2005). Vein corking was not observed.

Stem pitting ranged from very mild to severe on grapefruit, with wood bristles and porous wood pitting (Figure 5). Only the Limoneira 8 A isolate showed a very mild stem pitting in sweet/sour orange indicator plants.

None of the CTV isolates assayed induced foliar symptoms of vein clearing, vein corking or leaf yellowing on grapefruit, sour orange, sweet orange, lemon or sweet/sour. Foliar symptoms were observed only in Mexican lime.

The Genoa EEAT lemon isolate induced more severe stunting in all the indicator plants studied. This reaction was more evident in Mexican lime seedlings of an average height of 47.9 cm, as compared with those of 64.0 cm induced by the Limoneira isolates, 70.1 cm for Lisbon isolates and 79.9 cm for Eureka isolates. Negative control seedlings averaged 208.75 cm. Sweet/sour, sweet orange, grapefruit and lemon seedlings showed very mild to mild stunting.

The main differences among lemon isolates were observed in terms of stem pitting and stunting reaction. The Genoa isolate induced the least growth while Limoneira isolate induced the most severe stem pitting. Little difference was observed between the Eureka and Lisbon isolates, but the latter showed the mildest symptoms in all the indicator plants. Results are summed up in Table 2.

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**Table 1. Biological reaction of lemon CTV isolates in different indicator plants.**

Indicator plant	Symptoms	Lemon CTV isolates				
		Negative control	Frost Eureka	Genoa EEAT	Frost Lisbon	Limoneira 8 A
Mexican lime	Height	208.8 cm	79.9 cm	47.9 cm	70.1 cm	64.0 cm
	Stunting or size reduction	0.0	3.0	4.0	3.0	3.0
	Vein clearing	0.0	3.5	4.0	2.5	3.0
	Leaf cupping	0.0	2.5	2.3	1.3	3.0
	Stem pitting	0.0	3.8	3.5	3.8	5.0
	<b>Total ranking</b>	<b>0.0</b>	<b>12.8</b>	<b>13.8</b>	<b>10.6</b>	<b>14.0</b>
Sour orange	Height	176.0 cm	172.0 cm	123.0 cm	177.0 cm	169.0 cm
	Stunting or size reduction	0.0	1.0	2.0	0.0	1.0
	Stem pitting	0.0	0.0	0.3	0.0	1.5
	<b>Total ranking</b>	<b>0.0</b>	<b>1.0</b>	<b>2.3</b>	<b>0.0</b>	<b>2.5</b>
Frost Eureka lemon	Height	176.0 cm	149.5 cm	135.0 cm	158.0 cm	150.5 cm
	Stunting or size reduction	0.0	1.0	2.0	1.0	1.0
	Stem pitting	0.0	0.5	0.8	0.5	1.0
	<b>Total ranking</b>	<b>0.0</b>	<b>1.5</b>	<b>2.8</b>	<b>1.5</b>	<b>2.0</b>
Ruby blood orange/sour orange	Height	135.8 cm	119.3 cm	101.0 cm	120.6 cm	121.8 cm
	Stunting or size reduction	0.0	1.0	2.0	1.0	1.0
	Stem pitting	0.0	0.0	0.0	0.0	1.3
	<b>Total ranking</b>	<b>0.0</b>	<b>1.0</b>	<b>2.0</b>	<b>1.0</b>	<b>2.3</b>
Duncan grapefruit	Height	115.8 cm	160.0 cm	114.3 cm	127.8 cm	97.5 cm
	Stunting or size reduction	0.0	0.0	1.0	0.0	1.0
	Stem pitting	0.0	2.5	1.0	1.5	3.8
	<b>Total ranking</b>	<b>0.0</b>	<b>2.5</b>	<b>2.0</b>	<b>1.5</b>	<b>4.8</b>
Pineapple sweet orange	Height	146.5 cm	121.5 cm	98.8 cm	122.0 cm	126.0 cm
	Stunting or size reduction	0.0	1.0	2.0	1.0	1.0
	<b>Total ranking</b>	<b>0.0</b>	<b>1.0</b>	<b>2.0</b>	<b>1.0</b>	<b>1.0</b>

Rating scale: 0: no reaction; 1: very mild; 2: mild; 3: moderate; 4 severe and 5: very severe.

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Figure 1. Vein clearing in Mexican lime.



Figure 2. Cupping on Mexican lime leaves.



Figure 3. Stunting in Mexican lime, six months after inoculation (negative control is on the right).



Figure 4. Stem pitting with areas of porous wood in Mexican lime with Limoneira 8 A isolate.



Figure 5. Stem pitting in Duncan grapefruit: negative control (left), Lisbon isolate (center) and Limoneira isolate (right).

**Table 2. Summary of CTV biological indexing results of lemon cultivars from Tucumán, Argentina.**

CTV Isolate	Total rating	Notable plant symptoms	Indicator plant					
			Mexican lime	Pineapple	Eureka lemon	Duncan grapefruit	Sour orange	Sweet/sour
Frost Eureka	19.8	No notable symptoms	12.8	1.0	1.5	2.5	1.0	1.0
Genoa EEAT	24.9	Most severe stunting in all indicator plants	13.8	2.0	2.8	2.0	2.3	2.0
Limoneira 8 A	26.6	Most severe stem pitting in all indicator plants the only one with stem pitting in sweet/sour	14.0	1.0	2.0	4.8	2.5	2.3
Frost Lisbon	15.6	Mildest symptoms in all indicator plants	10.6	1.0	1.5	1.5	0.0	1.0