

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. Tristeza disease has been present in Argentina since 1930 and it 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 indexed 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. Double Antibody Sandwich (DAS) ELISA diagnosis using a polyclonal antibody was also performed.

The *Citrus tristeza virus* (CTV) isolate that becomes permanently established in a citrus tree may depend on the variety of the citrus tree. All citrus species in the field naturally infected with CTV, can acquire the virus that caused stem pitting in the small fruited lime (Mexican), but only certain species acquire the virus which, in addition, causes the seedling yellows reaction in sour orange. CTV recovered from lemon only caused stem pitting in lime (3).

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

The objective of this study was to characterize the tristeza isolates in different commercial varieties of lemon.

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 (2, 4, 5, 6, 7). 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. 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 bark patches 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 to 27 °C.

Symptoms on each of the indicator plants were evaluated four times during a 12-mo period (third, fifth, ninth and twelfth months after inoculation) and symptoms were rated from 0 (no reaction).

TABLE 1
BIOLOGICAL REACTION OF LEMON *CITRUS TRISTEZA* ISOLATES IN DIFFERENT INDICATOR PLANTS

Mexican lime ¹		Lemon CTV isolates			
Symptoms	Negative control	Frost Eureka	Genoa EEAT	Frost Lisbon	Limoneira 8 A
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
Total ranking²	0.0	12.8	13.8	10.6	14.0
Sour orange ¹		Lemon CTV isolates			
Symptoms	Negative control	Frost Eureka	Genoa EEAT	Frost Lisbon	Limoneira 8 A
Height	176.0 cm	172.0 cm	123.0 cm	177.0cm	169.0cm
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
Total ranking²	0.0	1.0	2.3	0.0	2.5
Frost Eureka lemon ¹		Lemon CTV isolates			
Symptoms	Negative control	Frost Eureka	Genoa EEAT	Frost Lisbon	Limoneira 8 A
Height	176.0cm	149.5 cm	135.0 cm	158.0cm	150.5cm
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
Total ranking²	0.0	1.5	2.8	1.5	2.0
Ruby blood orange /sour orange ¹		Lemon CTV isolates			
Symptoms	Negative control	Frost Eureka	Genoa EEAT	Frost Lisbon	Limoneira 8 A
Height	135.8 cm	119.3 cm	101.0 cm	120.6cm	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
Total ranking²	0.0	1.0	2.0	1.0	2.3
Duncan grapefruit ¹		Lemon CTV isolates			
Symptoms	Negative control	Frost Eureka	Genoa EEAT	Frost Lisbon	Limoneira 8 A
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
Total ranking²	0.0	2.5	2.0	1.5	4.8
Pineapple sweet orange ¹		Lemon CTV isolates			
Symptoms	Negative control	Frost Eureka	Genoa EEAT	Frost Lisbon	Limoneira 8 A
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
Total ranking²	0.0	1.0	2.0	1.0	1.0

¹Indicator plant

²Rating scale of 0 to 5, with 0 being no symptoms observed



Fig. 1. Vein clearing caused by *Citrus tristeza virus* in Mexican lime



Fig. 2. Cupping on leaves of Mexican lime caused by *Citrus tritseza virus*.

to 5 (most 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 on indicator plants for all lemon isolates were: on Mexican lime: moderate to severe vein clearing (Fig. 1), leaf cupping (Fig. 2), severe stunting (Fig. 3) and stem pitting with areas of porous wood pitting along with brownish gum-like deposits (Fig. 4) as described by Garnsey et al. (1). Vein corking was not observed.



Fig. 3. Stunting in Mexican lime 6 mo after inoculation with *Citrus tristeza virus* (negative control is on the right).



Fig. 4. Stem pitting with areas of porous wood in Mexican lime with *Citrus tristeza virus* Limoneira 8 A isolate.



Fig. 5. Stem pitting in Duncan grapefruit: negative control (left), *Citrus tristeza virus* Lisbon isolate (middle) and CTV Limoneira isolate (right).

Sweet/sour, Pineapple sweet orange, grapefruit and lemon seedlings showed mild stunting. Stem pitting was mild to moderate with wood bristles and porous wood pitting on grapefruit (Fig. 5). Only the Eureka isolate showed mild stem pitting in Eureka lemon indicator plants.

None of the CTV isolates assayed induced foliar symptoms of vein clearing, vein corking or leaf yellowing on Duncan grapefruit, sour orange, Pineapple sweet orange, Eureka lemon or sweet/sour. Foliar symptoms were observed only on Mexican lime. The Genoa EEAT lemon isolate induced more severe stunting in all indicator plants. This reaction was more evident on Mexican lime seedlings with an average height of 47.9 cm compared with a height of 64.0 cm of plants inoculated with isolates from Limoneira isolates, 70.1 cm by isolates from Lisbon and 79.9 cm isolates from Eureka. Negative control seedlings averaged 208.8 cm.

The main differences between lemon isolates were observed in stem pitting and stunting reactions. The Genoa isolate induced the least growth whereas the 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 indicator plants. Results are summarized in Table 2.

TABLE 2
SUMMARY OF THE *CITRUS TRISTEZA VIRUS* BIOLOGICAL INDEXING RESULTS OF
LEMON CULTIVARS FROM TUCUMÁN, ARGENTINA

CTV Isolate	Total rating	Notable plant symptoms	Mexican lime	Pineapple Sw. or.	Indicator plant			
					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 indicators.	13.8	2.0	2.8	2.0	2.3	2.0
Limoneira 8A	26.6	Most severe stem pitting in all indicators – only one with 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

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