Detection of a Tristeza-Seedling Yellows Strain in Spain

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ABSTRACT. In late 1984, some growers were found to be using budwood of a very early maturing satsuma mandarin of uncertain origin, for topworking citrus orchards near Valencia. Indexing of this material showed that it was infected with a very severe strain of citrus tristeza virus (CTV). It induced severe stunting, stem pitting, vein clearing and vein corking in Mexican lime and *C. macrophylla;* conspicuous stunting and seedling-yellows on Eureka lemon and Duncan grapefruit and stem pitting on sweet orange, grapefruit and rough lemon. None of the common CTV strains previously studied in Spain induces these severe symptoms. There is some evidence indicating that the original satsuma was illegally introduced from Japan. A program has been established for the eradication of this CTV strain.

Tristeza is the most serious citrus disease in Spain. It is present in all citrus areas at different levels (2, 4) and has killed more than ten million trees of sweet orange, mandarin and grapefruit on sour orange rootstock. However, the tristeza strains prevalent in Spain are relatively mild. They do not contain the seedling yellows component, they do not produce stem pitting in grapefruit or sweet orange, and they are easily controlled in the field by the use of tristeza-tolerant rootstocks (1, 4).

At the end of 1984, a routine test of a satsuma mandarin of unknown origin was done. It gave a tristeza-positive reaction in enzyme-linked immunosorbent assay (ELISA), and it induced severe tristeza symptoms on Etrog citron S-1 incubated in a greenhouse at 27-32 C that was being used for exocortis indexing. This symptomatology was not previously observed with any of the Spanish citrus tristeza virus (CTV) isolates, and thus this new strain was characterized biologically and the results reported here.

MATERIAL AND METHODS

An infected citron plant was kept and coded as CTV strain T-387 and used for aphid transmission experiments (3), and the original satsuma was coded as CTV strain T-388 and used for this study. Ten plants of each of the following indicator plants were graft inoculated with two bark patches of T-388:

— Mexican lime and *C. macrophylla* to determine vein clearing, vein corking, stem pitting and stunting symptoms

— Duncan grapefruit, Eureka lemon and sour orange to determine the seedling-yellows, stem pitting and stunting symptoms

— Pinapple sweet orange seedlings to determine stem pitting and stunting effects

— Salustiana, Navelina and Valencia sweet oranges grafted on rough lemon to study stem pitting symptoms

— Navelina, Salustiana and Valencia late sweet oranges, Marsh grapefruit and Clausellina satsuma grafted on sour orange to determine tristeza decline.

Ten plants of each of the same indicators were graft inoculated with the typical Spanish CTV isolate T-300 as positive controls and another ten plants of each indicator were used as negative controls.

All plants were incubated in a greenhouse at 18-26 C. Observations were made for 1 year after inoculation. At the end of this period, plants were measured to evaluate total growth and bark peeled to observe stem pitting. Vein clearing, seedling yellows and stem pitting symptoms were rated as: 0, no symptoms; 1, very mild; 2, mild; 3, moderate; 4, se-

vere; and 5, very severe. Stunting was evaluated by measuring the total length of all twigs produced by each plant.

Statistical analysis was done and means separated by Tukey's test.

RESULTS AND DISCUSSION

The new CTV strain T-388 induced severe vein clearing symptoms on Mexican lime and *C. macrophylla*, that were significantly different from those induced by the CTV strain T-300 (table 1). In addition, T-388 induced vein corking in all plants of both hosts (fig. 1).

The foliar symptoms induced by T-388 on Mexican lime are similar to those induced by the CTV strain T-308, that was found in a calamondin tree of unknown origin. This strain was previously evaluated and was the most severe CTV strain found in Spain (1). The only difference is that T-388 consistently induced vein corking, while T-308 induced this symptom only occasionally.

Strain T-388 induced clear moderate seedling yellows symptoms on Duncan grapefruit (fig. 2) and Eureka lemon, but did not affect sour orange. T-300 did not induce these reactions in the same experiment and none of the Spanish isolates previously evaluated produced seedling yellows (1).

The CTV strain T-388 produced very mild stem pitting in Eureka lemon, mild to moderate stem pitting in Pineapple, Valencia, Navelina and Salustiana sweet oranges and rough

TABLE 1
VEIN CLEARING SYMPTOMS INDUCED BY CITRUS TRISTEZA VIRUS (CTV) STRAINS[z]

Host	CTV Strain		
	T-388	T-300	Control
Mexican lime	4.6	3.3	0
C. macrophylla	4.5	1.6	0

[z]Symptoms rated on a scale of: 0 = none; 1 = very mild; 2 = mild; 3 = moderate; 4 = severe; 5 = very severe. Rating is the average for ten plants of each replicate.

Fig. 1. Vein corking induced by citrus tristeza virus strain T-388 on Mexican Lime one year after inoculation.

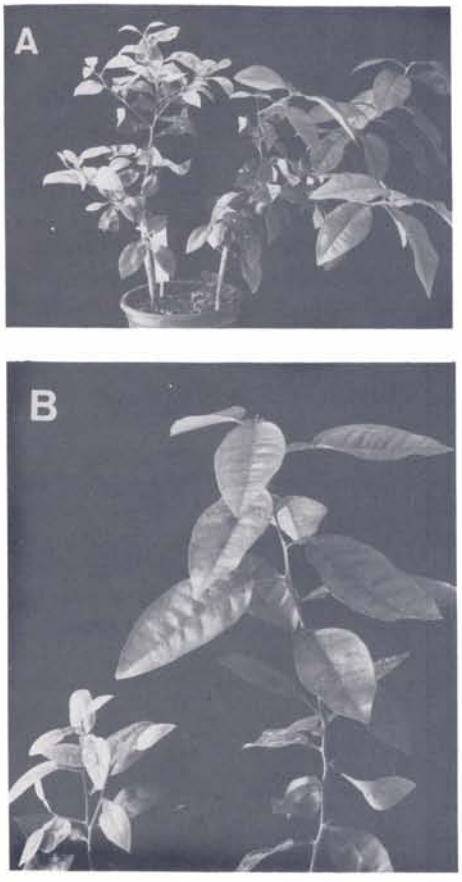

Fig. 2. Seedling-yellows reaction induced by citrus tristeza virus strain T-388 on Duncan grapefruit 4 months after inoculation. A, entire plants; B, detail. Left, infected plants; right, healthy controls.

TABLE 2
STEM PITTING SYMPTOMS INDUCED BY CITRUS TRISTEZA VIRUS (CTV) STRAINS[z]

Host	CTV Strains		
	T-388	T-300	Control
Mexican lime	4.6	2.0	0
Citrus macrophylla	4.0	2.3	0
Duncan grapefruit	3.5	0	0
Eureka lemon	0.6	0	0
Pineapple sweet orange	2.0	0	0
Salustiana sweet orange	2.7	0	0
Navelina sweet orange	2.6	0	0
Valencia sweet orange	2.3	0	0
Rough lemon	2.7	0	0

[z]Symptom rated on a scale of: 0 = none; 1 = very mild; 2 = mild; 3 = moderate; 4 = severe; 5 = very severe. Rating is average for the 10 plants of each replicate.

lemon, moderate to severe pitting in Duncan grapefruit and *C. macrophylla* and very severe pitting in Mexican lime (table 2, figs. 3 and 4). T-300 only induced mild stem pitting in Mexican lime and *C. macrophylla*. In previous experiments (1), none of the Spanish CTV isolates induced stem pitting in Duncan grapefruit or Pineapple sweet orange.

Table 3 shows the influence of CTV strains T-388 and T-300 on total growth of different hosts. T-300 did not significantly reduce the growth of any host. T-388 did not affect the growth of Pineapple sweet orange or sour orange, but reduced the growth of Mexican lime, *C. macrophylla*, Etrog citron, Duncan grapefruit and Eureka lemon 70 to 90% in relation to each control. None of the Spanish CTV isolates reduced the growth of Duncan grapefruit (1).

Plants of Navelina, Salustiana, and Valencia sweet orange, Marsh grapefruit and Clausellina satsuma grafted on sour orange and inoculated with T-388 showed pale, dull green leaves and chlorosis along the central vein of leaves. Plants inoculated with T-300 and controls did not show these symptoms. None of the Spanish CTV

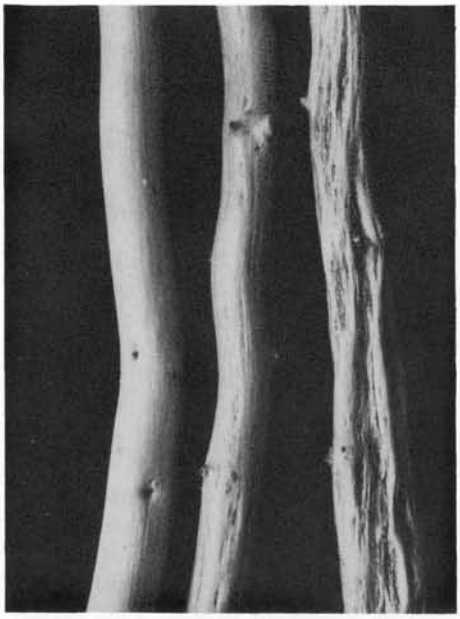

Fig. 3. Stem pitting induced by citrus tristeza virus strains on Mexican lime one year after inoculation. Left, healthy control; center, inoculated with strain T-300; right, inoculated with strain T-388.

isolates previously studied produced any symptom on Washington navel grafted on sour orange (1).

All these data clearly indicate that T-388 is a different and much more severe CTV strain than those previously existing in Spain.

Additional indexing of the original satsuma showed that, in addition to T-388, it was infected with exocortis, psorosis and vein enation, but it was free of infectious variegation, cachexia and tatter leaf.

Field trees topworked with the infected satsuma variety did not show stem pitting, except one Navelina sweet orange tree that had mild pitting. The lack of stem pitting may be due to the short period of time since topworking, that apparently started in some scattered trees in 1971-1972. However, many trees of sweet oranges and mandarins grafted on sour orange topworked with the infected satsuma had quick decline and died.

The new CTV strain T-388 was transmitted under experimental con-

Fig. 4. Stem pitting induced by citrus tristeza virus strain T-388 on rough lemon (left), Duncan grapefruit (center) and Pineapple sweet orange (right), 1 yr after inoculation.

ditions by *Aphis gossipii* with an efficiency of 60% (2). So far no natural field transmission has been observed.

TABLE 3
INFLUENCE OF CITRUS TRISTEZA VIRUS (CTV) STRAINS ON TOTAL GROWTH OF DIFFERENT HOSTS[z]

Host	CTV Strains[y]		
	T-388	T-300	Control
Mexican lime	110.4[b]	389.7[a]	537.2[a]
C. macrophylla	59.7[b]	594.0[a]	642.0[a]
Duncan grapefruit	82.4[b]	229.6[a]	267.5[a]
Eureka lemon	69.5[b]	252.7[a]	231.7[a]
Sour orange	248.4[a]	—	284.0[a]
Pineapple sweet orange	462.7[a]	438.0[a]	474.7[a]

[z]Data is the average of the total growth of branches expressed in centimeters of ten plants of each host inoculated with each CTV strain.
[y]Figures in rows followed by distinct letters are significantly different at the 5% level.

Present evidence indicates that the infected satsuma is an Okitsu type, illegally introduced from Japan. This explains the severity of the new strain, since severe CTV strains that induce the seedling yellows reaction and stem pitting in sweet oranges and other varieties are prevalent in Japan (5).

The new severe CTV strain poses a serious threat to the Spanish citrus industry, because it may produce stem pitting on sweet oranges and grapefruit grafted on tristeza-tolerant rootstocks. An eradication program has been established to eliminate trees topworked with the infected satsuma variety and surrounding trees that could have become naturally infected. This program is presently in operation and hopefully infected trees will be eradicated.

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