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Failure of 100 Mild Citrus Tristeza Virus Isolates from California to Cross Protect Against a Challenge by Severe Sweet Orange Stem Pitting Isolates

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Authors
Roistacher, C. N.
Dodds, J. A.

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Failure of 100 Mild Citrus Tristeza Virus Isolates from California to Cross Protect Against a Challenge by Severe Sweet Orange Stem Pitting Isolates

C. N. Roistacher and J. A. Dodds

ABSTRACT. The discovery of a severe sweet orange stem pitting isolate in field 12b of the University of California orchards at Riverside resulted in extensive search and destroy surveys for exotic isolates of the tristeza virus. These exotic isolates have the potential to destroy a citrus industry. Therefore, experiments were designed to test whether local mild isolates of CTV found throughout California would cross-protect against a challenge with the 12b and other sweet orange stem pitting isolates. This paper reports the results of three experiments where over 100 relatively mild CTV isolates collected from throughout California and inoculated into sweet orange were challenge inoculated with severe CTV stem pitting isolates. Results showed little to no cross-protection.

Between 1977-1980, severe isolates of seedling yellows tristeza virus (CTV-SY) and stem pitting tristeza virus (CTV-SP) were found spreading naturally in the citrus orchards at the Citrus Research Center (CRC) of the University of California at Riverside (UCR) (5, 22). Some isolates were found to stem pit sweet orange, grapefruit and tangelo and various hybrid seedlings or scions in the field and in greenhouse grown plants. One isolate, found spreading in field 12b at the CRC severely stem pitted and stunted inoculated plants of sweet orange and grapefruit. It was transmitted by *Aphis gossypii* at 100% efficiency (23). This isolate was similar in its destructive potential to the Capao Bonito CTV-SP reported from Brazil (14, 15), the navel CTV-SP reported from Peru (20) or the Ortanique tangor CTV-SP isolate reported from Australia (4). All of these severe and exotic CTV-SP isolates stem pit sweet orange, reduce fruit size, lower yields and stunt trees. They all have the potential to destroy a citrus industry within any country if introduced and effectively transmitted.

The discovery of this 12b isolate in the fields at UCR gave deep concern and resulted in an extensive program to search for and destroy all exotic CTV isolates at the CRC. Over 20,000 trees in the University orchards and in various locations in southern California were indexed for presence of CTV-SY and CTV-SP. Over 260 trees were found with severe CTV isolates and these trees were destroyed or replaced after elimination of the pathogens by shoot tip grafting and/or thermotherapy (9, 25). A number of CTV isolates found in the various fields were preserved by inoculation into sweet orange seedlings as holding plants and were held in the virus bank in the screenhouse at the Rubidoux indexing facility of the Department of Plant Pathology.

There is currently deep concern for the possible invasion of *Toxoptera citricida* into the United States (11, 25, 26). This aphid is the principal vector for most isolates of CTV throughout the world. It is probable that severe or exotic isolates of CTV, including ones similar to the 12b isolate, are present in various parts of California in a 'sleeping' state, perhaps in illegal imports or in backyard trees. If *T. citricida* enters California and becomes endemic, these exotic CTV isolates could be efficiently transmitted and would spread into surrounding citrus plantings with the potential for severe damage to the industry. Envisioning this possibility, experiments were begun in 1981 to test whether local and relatively mild isolates of CTV found throughout California would have any cross-protective ability against a challenge by severe CTV-SP isolates such as the 12b isolate. In a preliminary report, little to no protection by 19 CTV isolates was found when they were challenged with the 12b isolate (29). This paper reports on the results of three experiments where over 100 CTV isolates found in California citrus were inoculated into sweet orange and challenge inoculated with severe sweet orange CTV-SP isolates.

MATERIALS AND METHODS

All CTV isolates were collected from citrus trees which tested positive for CTV by graft transmission to appropriate indicator plants or by enzyme linked immunosorbent assay. Buds from each CTV positive source were graft-inoculated to a sweet orange seedling as a holding plant. The isolates were coded and held in quarantine in the virus bank at the Rubidoux screenhouse facility of the California Citrus Clonal Protection Program. All plants were grown under the UC system of plant growth (21).

Experiment 1. Nineteen California CTV isolates were used in this experiment. Eleven of the isolates with T-numbers as shown in Table 1 were routinely used as standard CTV positive controls and periodically indexed for CTV over a period of 28 yr. Results of their reaction in indicator plants were regularly recorded. The remaining eight isolates were collected from the CRC fields in Riverside. All 19 isolates were indexed to seedlings of grapefruit, sour orange, lemon and sweet orange to test their pathogenic characteristics. None were found to contain severe CTV-SY or CTV-SP components, and all were designated as citrus tristeza virus 'non-reactive' (CTV-NR). However, all were suspected to have the ability to cause quick decline on sour orange rootstock, hence the reluctance to designate them as mild isolates.

Two buds taken from each of the 19 sweet orange CTV-NR source plants were graft-inoculated to each of five seedlings of Madam Vinous sweet orange on May 29, 1981. All seedlings were cut back to approximately 20-25 cm from the soil surface, and new growth was trained to grow as a single shoot. Three months after inoculation, two of the five plants in each of the 19 pre-inoculated groups were challenge-inoculated with two buds of the severe 12b CTV-SP isolate.

The three remaining plants were vector challenged with *A. gossypii* by the method described and illustrated by Roistacher (19). Acquisition feeding was for 24 hr on their acquisition hosts and aphids were then transferred for a 24 hr challenge transmission feeding. The source plants used for aphid transmission feeding were sweet orange seedlings containing the 12b CTV-SP isolate. These plants were periodically cut back to force new growth for desirable aphid feeding. After transmission feeding the aphids were counted and killed with a 1% nicotine sulfate spray. The aphid count after the transmission feeding ranged from approximately 100 to 200 individuals per plant.

All plants were uniformly cut back on January 17, 1982 and the new growth was trained as a single shoot. On May 17, 1982, one year after the initial inoculation and 8 to 9 months after bud and vector challenge, all plants were measured, peeled and critically examined for symptoms of stem pitting to evaluate the effectiveness of cross protection. Ratings for stem pitting were: 0 = negative; 1-2 = mild; 3 = moderate: 4-5 = severe; 6 = very severe.

Experiment 2. Twenty CTV isolates were collected from fields 7b, 7c and 21d at the CRC. These isolates were tested for their ability to protect sweet orange seedlings against a bud-challenge from four severe sweet orange CTV-SP isolates. All 20 isolates were pre-indexed to seedlings of grapefruit, sour orange, lemon, Mexican lime and sweet orange and were defined as CTV-NR by their symptom response in these indicators. On May 29, 1981, two buds from each of these 20 CTV-NR isolates were graft-inoculated to five seedlings each of Madam Vinous sweet orange. All inoculated seedlings were cut back to approximately 20-25 cm from the soil surface, and new growth trained as single shoots. Four months after the initial inoculation, four plants were challenge-inoculated with two bud-grafts from each of four respective CTV-SP isolates. Plants were cut back again

TABLE 1
RESULTS OF CROSS PROTECTION TESTS IN MADAM VINOUS SWEET ORANGE OF 19 CTV-NR ISOLATES AFTER A CHALLENGE WITH THE SEVERE 12b CTV-SP ISOLATE BY BUD-GRAFT OR *APHIS GOSSYPII* INOCULATION

CTV-NR code[x]	Original location	Original source	Stem pitting after challenge[y]	
			Bud	Vector
T-500	Riverside, UCR	Valencia	6	6
T-502	Indio	Grapefruit	6	5
T-505	Tulare Co.	Valencia	6	6
T-508	Ventura Co.	Lemon	6	6
T-509	Orange Co.	Grapefruit	6	6
T-510	San Bernardino Co.	Grapefruit	6	6
T-511	Ventura Co.	Valencia	6	6
T-514	Tulare Co.	Valencia	5	6
T-516	Orange Co.	Sweet orange	5	–
T-519	Riverside, UCR	Meyer Lemon	6	6
T-521	Orange Co.	Sweet orange	6	5
19	Riverside, UCR	Sweet orange	6	4
150	Riverside, UCR	Mandarin	4	4
222	Orange Co.	Mandarin	6	6
267	Riverside, UCR	Valencia	6	6
608	Riverside, UCR	Sweet orange	6	6
1259	Riverside, UCR	Lemelo	6	6
1491	Riverside, UCR	Sweet Lime	6	6
1851	Riverside, UCR	Rough lemon	6	3
Average of 6 bud-challenged controls[z]			5.7	–
Average of 9 vector-challenged controls[z]			–	5.3
Average of 6 non-inoculated controls			0	0

[x]T-numbers are the standard CTV-NR isolates used as positive controls in the University of California budwood indexing program. They consistently gave little or no reaction on sweet orange.
[y]Ratings: 0 = negative; 1-2 = mild; 3 = moderate; 4-5 = severe; 6 = very severe (Fig. 1).
[z]Healthy plants were challenge-inoculated with the 12b CTV-SP isolate.

and new growth trained as single shoots. These challenge inoculum sources were pre-selected for their severe reaction on indicators, but especially for their ability to stem pit sweet orange. The isolates selected for challenge were:

SY-556 and SY-557 - Warner tristeza. These two sources were collected from declining navel orange trees at the Warner Ranch in Hawaii in 1971 by Dr. E. C. Calavan. Both isolates induced severe vein corking in Mexican lime leaves, and seedling yellows in both grapefruit and sour orange. In addition, both isolates induced stem pitting in grapefruit and sweet orange. The SY-556 isolate also severely stem pitted sour orange seedlings.

SY-558, the Kona or Waialua orange originated in Hawaii, probably of Japanese origin and was introduced in 1914 into the variety collection at UCR as CRC 451. It induced severe seedling yellows in grapefruit, sour orange and lemon indicators and severely stem pitted sweet orange.

SY-568 - the 12b isolate was found in a Minneola tangelo in field 12b at the CRC (5) and induced severe seedling yellows in grapefruit, sour orange, lemon and sweet orange seedlings. It severely stem pitted both grapefruit and sweet orange.

On January 11, 1982, approximately 4 months after challenge inoculation, all plants were again cut back and new growth trained to grow as single shoots. On May 17, 1982, one year after the initial inoculation and approximately 8 months after the challenge inoculation all plants were harvested and evaluated for appearance, growth and stem pitting. Stem pitting was rated as: 0 = negative; 1-2 = mild; 3 = moderate; 4-5 = severe; 6-7 = very severe.

Experiment 3. Sixty two CTV-NR isolates were collected from various fields in southern California. Most were from the orchards at the University of California. Some were from the experimental orchards at the South Coast Field Station in Orange County and others were from various orchards in Riverside, San Bernardino and Ventura Counties. These collections were made during the statewide survey for exotic isolates of CTV conducted in the early 1980's. All of these isolates indexed positive for CTV in Mexican lime and also had been inoculated into one Madam Vinous sweet orange seedling as part of the survey. None of them induced stem pitting or yellows in the Madam Vinous. Fifteen weeks after the initial inoculation, the Madam Vinous seedlings were challenge-inoculated with two bud-grafts of the 12b isolate and cut back to force a single shoot. All plants were harvested and evaluated for seedling yellows and stem pitting 4 to 6 months after the challenge inoculation. Stem pitting was rated as in Experiment 2.

RESULTS

Experiment 1. As shown in Table 1, there was no protection by any of the 19 CTV-NR isolates to either bud or vector challenge by the severe 12b CTV-SP isolate. There was also no difference between bud or vector challenge in the final stem pitting reaction induced by the severe 12b isolate. Stem pitting was equally intense in all Madam Vinous sweet orange indicator seedlings as shown in Fig. 1.

Experiment 2. As shown in Table 2, there was little or no protection by any of the 20 CTV-NR isolates in Madam Vinous sweet orange seedlings after a challenge with each of the four sweet orange CTV-SP isolates. Stem pitting found in the challenged plants was similar to that of the non-protected but challenged control plants (Fig. 1).

Experiment 3. Sixty out of the 62 CTV-NR isolates in Madam Vinous sweet orange gave no protection after a challenge with the severe 12b CTV-SP isolate. However, two isolates: Code 5427 from field 7C row 17 tree 10 at the CRC and Code 5754 from the Redlands area of San Bernardino County gave good protection as shown in Table 3. Madam Vinous sweet orange seedlings infected with isolate 7C-17-10 were also challenged by CTV-SP isolate SY-556. Only mild stem pitting developed, indicating some protection against a challenge by this isolate had occurred. Isolate 5754 from the Redlands area in grove 36, Row 7, Tree 2 came from a sweet orange budded on sweet orange rootstock and was in decline. Many navel orange trees in this grove were also in decline and showed various degrees of stem pitting. This grove was ultimately destroyed for a housing development.

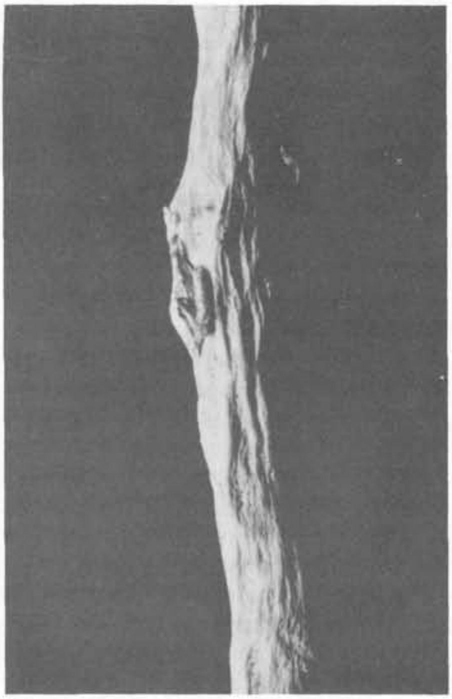

Fig. 1. Typical severe stem pitting induced in a Madam Vinous sweet orange seedling by the 12b CTV-SP isolate. Ninety-nine of 101 CTV-NR isolates found in California failed to protect against the stem pitting effects of this severe isolate.

TABLE 2
STEM PITTING IN SWEET ORANGE SEEDLINGS PRE-INOCULATED WITH 20 CTV-NR ISOLATES RESPECTIVELY AND CHALLENGED WITH FOUR SEVERE SWEET ORANGE STEM PITTING ISOLATES

Pre-inoculated CTV-NR source	Stem Pitting Ratings[w] Challenged with CTV-SP isolates: 556	557	568	558	Non-challenged controls[x]
336	2	5	4	3	0
354	5	4	6	4	2.5
392	4	5	6	3	1.5
412	4.5	5	4	3.5	2.5
415	4	4	5.5	1.5	1
523	5	4	5	2.5	1
525	4	4	6	3	2.5
562a	5.5	5	3.5	3	2.5
572	6	6	5	4	2
596e	3	4	3	3	1.5
602	3	7	5	4	0
606	2	4	5	3	3.5
614	5	5	6	5	1
1730	2	5	7	4	2
1736	5	6	6	4	0
1833	4	7	6	4.5	0
1828	4	4	4	5	1
1879	5	6	5	5	0
1873	4	6	5	5	3
1886	4	7	5	5	0
1888	5	6	6	5	1
Positive control[y]	3.7	6.7	6.6	4.2	–
Negative control[z]					0

[w]Stem pitting ratings: 0 = negative, 1-2 = mild, 3 = moderate, 4-5 = severe, 6-7 = very severe.
[x]Pre-inoculated Madam Vinous control seedlings containing the original CTV-NR source but not challenged.
[y]Each figure represents an average of 11 individual tests of non pre-inoculated Madam Vinous seedlings challenged respectively with each of the four severe CTV-SP isolates.
[z]Average of nine negative controls.

DISCUSSION

The two principal ways for exotic CTV isolates to be disseminated throughout a country are by distribution in propagative budwood or by vector transmission. Exotic CTV isolates may be present in early introductions such as in the Meyer lemon or illegally introduced satsuma or other symptomless hosts. They may be non-transmissible or poorly transmissible by local aphids. The CTV is initially spread by propagation and can remain in a dormant or 'sleeping' state in the tree. Without an active and well supported certification program, these exotic isolates can be distributed throughout a country via propagative budwood. Under some circumstances, transmissibility of the virus may change and local aphids can effectively spread the virus (3, 23). However, if *T. citricida* enters a country, it is highly probable that this aphid will spread these 'sleeping' CTV isolates as it did in Venezuela and in other countries (12, 18).

Once introduced into a region or country, these exotic CTV isolates may be transmitted by either of the two primary aphid vectors *A. gossypii* and *T. citricida* superimposing on existing CTV-NR isolates and may eventually become dominant in an area (4, 5, 8, 14, 16, 20). In some cases, new CTV-SP isolates can effectively destroy or cripple all or part of an industry such as occurred in Brazil (15, 27), Peru (20),

TABLE 3
CROSS PROTECTION EVALUATION OF 62 CTV-NR ISOLATES IN SWEET ORANGE SEEDLINGS WHEN CHALLENGE-INOCULATED WITH THE SEVERE 12b SWEET ORANGE STEM PITTING ISOLATE

CTV sources and locations	Stem pitting ratings[x] after challenge with the severe 12b CTV-SP isolate	
	Range	Mean
60 sources from various southern California locations	4-7	4.9
Code 5426 from field 7c, Row 17 tree 10 at the CRC	0	0
Code 5754 from 36-7-2, Redlands, San Bernardino Co.	0	0
Non-preinoculated controls[y]	5-7	5.1
Healthy controls[z]	0	0

[x]Stem pitting ratings: 0 = negative; 1-2 = mild; 3 = moderate; 4-5 = severe; 6-7 = very severe.
[y]Seventeen healthy plants were challenge-inoculated with the 12b isolate.
[z]Six plants non-preinoculated and non-challenged.

Australia (16), the Reunion Islands (10) and recently in Florida (17).

Once an exotic CTV isolate is introduced and begins to spread, protective isolates will have to be developed. The two earlier in depth studies for finding protective isolates against CTV-SP were those by Costa and Müller (7) in Brazil for protection against the stem pitting problem in Pera sweet orange and Mexican lime, and by Balaraman and Ramakrishnan (1, 2) in India for protection against the stem pitting problem in their small fruited lime. Other protective isolates which have been reported were the 'Hassaku' HM-55 isolate in Japan (28); the experimental isolate LMS-6 in South Africa (6, 30); a number of mild protective isolates for grapefruit stem pitting in Australia (8) and experimental isolates T-49, T-50a and T-56 for sweet on sour protection in Florida (31). Protective isolates Z-5, 37, 37a and 40 were also developed by passage of CTV-SP through *Passiflora* sp. (24).

In California, a new severe CTV isolate which severely stem pitted sweet orange, grapefruit and tangelo was found spreading from field 12b in the orchards of the CRC (5). It was found to be readily transmitted by *A. gossypii* and if unchecked could have spread throughout the State. It had the ability to severely damage or even destroy the sweet orange and grapefruit industries in California. These studies show that the local isolates of tristeza present in California would not have protected against the spread of this 12b isolate; or of three other severe sweet orange stem pitting isolates of tristeza.

Ninety nine out of 101 CTV-NR isolates found in California gave no protection when challenged with the severe 12b CTV-SP isolate. The implications are that if a protective CTV isolate is to be found effective against an exotic such as the 12b isolate, it may be difficult to find it in existing tristeza sources within the State, but should be developed from the isolates which are currently destructive or spreading. There is evidence which supports the hypothesis that some CTV isolates may carry their own internal protective strains. This was shown in the passage of six CTV-SP isolates by vector through two *Passiflora* sp. (*P. caerulea* and *P. gracilis*). All of the six isolates obtained after passage showed exceptional protective ability against the strain from which they were derived (24). Also, Moreno *et al.* (13) presented evidence that severe strains of CTV may exist within citrus infected with apparently mild CTV isolates. This suggests that natural internal cross protection exists within the CTV-infected tree or plant.

The results of these experiments shows a general lack of protection by many existing isolates of CTV in California. This points up the serious problem which may be faced by any country which does not have strict quarantine regulations to prevent import of exotic or destructive pathogens, or which does not have a comprehensive certification program for citrus which could detect and perhaps limit the spread of severe CTV-SP in propagative budwood.

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