Serological Differentiation of the Citrus Tristeza Virus Isolates Causing Stem Pitting in Sweet Orange

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ABSTRACT

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Citrus tristeza virus (CTV) complex comprises a number of isolates or strains producing several economically important disease syndromes in commercial *Citrus* spp. The stem pitting syndrome is the most important, and causes substantial losses in many citrus-producing regions of the world. In an attempt to develop a serological tool to rapidly differentiate stem pitting isolates of CTV, we evaluated many combinations of trapping and detecting antibodies in an indirect double-antibody sandwich (I-DAS) enzyme-linked immunosorbent assay (ELISA). Two combinations of trapping and detecting antibodies were found suitable for differentiating stem pitting isolates in extracts of infected sweet orange plants. One used a polyclonal serum raised against bacterially expressed CTV coat protein (CP) for trapping and a conformational monoclonal antibody 3E10 for detection, and the other used two polyclonal antisera generated against bacterially expressed CTV CP. Seventy-six CTV isolates from 20 countries, including 35 that cause stem pitting in sweet orange plants, were analyzed in I-DAS-ELISA using different combinations of polyclonal and monoclonal antibodies for trapping and as intermediate detecting antibodies. The ELISA format developed produces a strong positive signal for CTV isolates that cause stem pitting in sweet orange plants and a negative ELISA signal for CTV isolates that do not cause stem pitting. When combined with data on a universal ELISA format, i.e., reacting with a broad range of CTV isolates, these selective ELISA formats allowed reliable serological differentiation of CTV isolates that caused stem pitting in infected sweet orange plants.

The citrus tristeza virus (CTV) complex is one of the major graft-transmissible pathogens limiting profitable citrus production worldwide (2,3,9). It causes several types of disease syndromes in *Citrus* spp. From the disease management point of view, the two most important syndromes are decline and stem pitting. The decline

syndrome occurs when a sweet orange or another susceptible scion grafted onto a sour orange rootstock becomes infected with CTV. A virus-induced graft incompatibility forms, which results in decline and frequently rapid death of the tree. Most isolates of CTV are symptomless in sweet orange on tolerant rootstocks, but stem pitting occurs in decline-tolerant scion-rootstock combinations following infection with stem pitting isolates of CTV. It is caused by abnormalities in phloem and xylem development and results in tree stunting, reduced vigor, and losses in fruit size, quality, and yield; however, the tree does not die (7,17). In areas where either decline or stem pitting isolates are not wide spread, management of the CTV may depend on excluding or controlling specific disease-inducing isolates. Spread of the brown citrus aphid (*Toxoptera citricida*), the most efficient CTV vector, and an anticipated increase in incidence of the severe stem pitting isolates of CTV once this vector is established (17) have increased the need to find reliable, cost efficient, and simple methods to detect and differentiate severe stem pitting CTV isolates in the field from more benign isolates, which do not cause stem pitting.

Serological techniques have long been used for detecting CTV (1,6,10), and en-

zyme-linked immunosorbent assay (ELISA) is the most convenient, reliable, fast, and relatively inexpensive procedure. In 1990, Permar et al. (16) reported that the monoclonal antibody (MAb) MCA-13 could differentiate between Florida isolates causing decline and those that did not cause decline. It has been used extensively in Florida for indexing purposes and is currently the only serological tool available for large-scale differentiation of CTV isolates in the field. MCA-13 cannot, however, distinguish between decline and stem pitting isolates, and it may fail to react to some decline isolates from outside of Florida. Evaluation of other MAbs in many laboratories has failed to produce an MAb specific to stem pitting isolates.

Systematic screening of the 30 CTV-specific MAbs resulted in assigning them to five groups based on epitope specificity (14). MCA-13 binds a linear, continuous epitope located within 11 amino acid residues of the coat protein (CP) (14,15). However, 19 of the 30 MAbs were found to bind conformational epitopes that could not be defined by simple molecular genetic methods (14). At the same time, several potentially promising MAbs that sometimes gave indications of useful selectivity (11; S. M. Garnsey, *unpublished*; D. J. Gumpf, *personal communication*) all fell into the large group of MAbs directed toward conformational epitopes (14).

Biological characterization of the CTV isolates is currently performed on a standard set of five indicators, including sweet orange (5,8). This standardized virus testing is being used to study a collection of more than 300 CTV isolates from all major citrus-growing regions of the world established in a quarantine greenhouse facility in Beltsville, Maryland (8). This collection is ideally suited to search for new laboratory tools, including serology, that might demonstrate correlations of biochemical or antigenic characteristics with biological properties of the CTV isolates.

We initiated a serological survey of the CTV isolates using information on epitope specificity of a range of CTV-specific antibodies. This paper describes a serological system developed to distinguish among a wide range of isolates that cause stem pitting in sweet orange indicators from those that do not cause sweet orange stem pitting.

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MATERIALS AND METHODS

Source of isolates, maintenance, and sample collecting. CTV isolates were maintained in a quarantine greenhouse at Beltsville that houses a collection of exotic CTV isolates from all major citrus-growing regions of the world (5,8). Some Florida isolates were also maintained in Orlando and Lake Alfred under greenhouse conditions. Seventy-six isolates, the best-characterized based on symptoms incited in indicator plants, were selected for serological characterization (Table 1). Of these 76 isolates, 35 caused varying degrees of stem pitting in Madam Vinous sweet orange (OSP-positive isolates). Most of these isolates also caused other economically important syndromes, e.g., decline of sweet orange grafted onto sour orange rootstock and stem pitting in grapefruit (5,8). The remaining group of 41 isolates did not cause stem pitting of sweet orange (OSP-negative isolates) and consisted of 26 that caused decline of sweet orange grafted onto sour orange, 18 that caused stem pitting in grapefruit, 10 that caused seedling yellows, and 7 that caused only mild vein-clearing in Mexican lime (S. M. Garnsey, *personal communication*).

The isolates were maintained in Madam Vinous sweet orange plants (*Citrus sinensis* (L.) Osb.) under greenhouse conditions. Plant tissue for antigen preparation was collected in batches of 20 to 50 isolates for medium-scale experiments from 1994 to 1997. Samples were tested in all seasons of the year. Two experiments included all 76 isolates and were conducted in February and June 1997.

Preparation of plant extracts for ELISA. Because of restrictions on the movement of infected material from the Beltsville collection, three different methods were used for sample preparation. Fresh tissue was used for some Florida isolates maintained in Orlando or Lake Alfred. Freeze-dried and air-dried samples were prepared in the Beltsville greenhouse for exotic CTV isolates before shipping the samples to the Lake Alfred lab, or in Lake Alfred and Orlando for local Florida isolates.

To prepare a freeze-dried sample, approximately 0.5 g of bark tissue was peeled from young flush, chopped finely with a razor blade, and homogenized in 5 ml of 50 mM Tris-HCl, pH 8.0, then stored at 4°C for 16 h, and filtered through cheesecloth. Two-ml aliquots were lyophilized by a procedure described by Garnsey et al. (5). Freeze-dried extracts were rehydrated in 2 ml of distilled water just before use in ELISA.

To prepare dry tissue samples, 0.5 g of bark tissue was chopped with a razor blade and allowed to air-dry for 1 to 2 days at room temperature. These samples, termed "air-dried," were stored over silica gel desiccant at 4°C. Prior to use for ELISA, the air-dried samples were rehydrated

overnight in 5 ml of 0.02 M sodium carbonate buffer, pH 9.6, homogenized, and incubated overnight at 4°C. The samples were clarified by low-speed centrifugation, and the resulting supernatant was used in ELISA. Fresh tissue extracts were prepared similarly, excluding the drying step.

Sources of polyclonal antisera and MAbs. Three polyclonal antisera were used in these tests. Rabbit antiserum 1052 was generated against purified virions of the T36 isolate of CTV and described earlier (12–14). Antisera 109 and 110 were generated against a full-size bacterially expressed CTV CP (amino acids 2–223) fused to a maltose binding protein (MBP) tag in rabbit (109), as described (12,14), and chicken (110) (this paper). Antiserum 109 had antigenic specificity similar to the previously described anti-MBP-CP serum (12), but it had a higher titer. In some experiments, the IgG fraction was isolated from antisera using protein A-Sepharose affinity column chromatography or ammonium sulfate precipitation. CTV-specific globulin fraction also was isolated from the egg yolk produced by the chicken used to produce antiserum 110. Eggs were collected from the immunized bird for 7 weeks, and the yolks were separated and pooled. They were homogenized with 3 volumes of 1× PBS and stirred with an equal volume of chloroform for 1 h at room temperature. After centrifugation at 8,000 × g for 30 min, the supernatant was collected and used as a source of chicken antibodies 110. CTV-specific mouse MAb 3E10 ascites were kindly provided by M.-C. Tsai and H.-J. Su (National Taiwan University, Taipei).

ELISA procedure. Indirect double-antibody sandwich (I-DAS) ELISAs were performed following the general protocol of Clark and Adams (4) with modifications described previously (13). Wells of Immulon 2 microtiter plates (Dynatech, Chantilly, VA) were coated with 200 µl of globulins at 1 µg/ml or with antiserum at 1:1000 dilution in 0.02 M sodium carbonate buffer (pH 9.6) and incubated for 4 h at 37°C or 16 to 24 h at 4°C. Plates were washed with 1× PBS buffer containing 0.1% Tween 20 (PBST) and rinsed 3 to 5 times with deionized water, and 200 µl of plant extract was loaded into each well. Plates were incubated with plant extracts for 16 to 20 h at 4°C, washed with PBST, and an intermediate detecting antibody at the appropriate concentration was applied to the wells in PBST buffer containing 0.2% bovine serum albumin (BSA) and 2% polyvinylpyrrolidone (PVP). After incubation for 4 h at 37°C (or, alternatively, for 16 to 20 h at 4°C), plates were washed extensively with PBST, and goat anti-rabbit (Sigma A-3687) or goat anti-mouse (Sigma A-3562) IgG-conjugates with alkaline phosphatase at 1:30,000 dilution in PBST with 0.2% BSA and 2% PVP were added, and the plates were incubated 4 h at

37°C (or 16 to 20 h at 4°C). The plates were washed with PBST, and 0.6 mg/ml of *p*-nitrophenyl phosphate (Sigma) in 0.1 M diethanolamine buffer, pH 9.8, was added as a substrate. The color reaction was monitored by measuring absorbance at 405 nm using an ELISA reader.

The ELISA experiments were optimized, in regard to the concentration of coating and detecting antibodies, for a maximal signal in both selective (with a positive control) and polyspecific (for all isolates) systems, i.e., OD₄₀₅ reading being not less than 1.0. Samples with no reaction in a polyspecific ELISA were excluded from the analysis as virus-negative. Samples with the OD₄₀₅ readings less than 1.0 in the universal ELISA format were also disregarded as producing inconclusive results. Several different combinations of coating and intermediate detecting antibodies were tested to differentiate CTV isolates. In each experiment, every isolate was analyzed in two replicates, and the resulting readings were averaged and cross-checked against corresponding two-replicate readings in a control experiment with a universal or polyspecific ELISA format, i.e., having no isolate specificity.

RESULTS AND DISCUSSION

Optimization of the I-DAS-ELISA format. For initial experiments, two Florida decline-inducing isolates were used: T36, a typical widespread isolate (18), and T68, originally isolated from illegally imported budwood (S. M. Garnsey, *unpublished*) and atypical for Florida. Both isolates cause decline in sweet orange grafted onto sour orange rootstocks; however, only T68 causes stem pitting in sweet orange (Table 1). Figure 1 illustrates the selective combination of the antiserum 109 used as a coating and the 3E10 MAb used for detection, which reacted to the OSP-positive isolate T68 but not to the OSP-negative T36. The opposite combination, MAb 3E10 for coating and the antiserum 109 for detection, was found to be nonselective toward T36 and T68 isolates (Fig. 1). Later, an additional 74 CTV isolates with previously defined symptomatology in a standardized set of indicator plants were used to verify the selectivity toward stem pitting symptoms in sweet orange.

To test the selective potential of other combinations of coating and detecting antibodies, we used several other available antisera against bacterially expressed CP fragments (12–14). Two combinations of coating and detecting antibodies were found to be selective for CTV isolates that caused stem pitting in sweet orange: (i) coating with the antiserum 109 and detecting with the MAb 3E10 at 20 ng of IgG per ml; and (ii) coating with the antiserum 110 and detecting with the antiserum 109 at 1:10,000 dilution. Two other combinations of coating and detecting antibodies detected all 74 isolates tested: (iii) coating

Table 1. Reaction of 76 citrus tristeza virus (CTV) isolates from 20 countries in indirect double-body sandwich enzyme-linked immunosorbent assay (I-DAS-ELISA) with combinations of trapping and detecting antibodies selective (italic) and nonselective for stem pitting reaction induced in Madam Vinous sweet orange seedlings

Isolate ID ^a	Origin	OSP score ^b	ELISA reactions ^c			
			109/3E10	110/109	3E10/109	1052/3E10
B2	Florida	0	–	–	++++	++++
B3	Florida	0	–	–	++++	++++
B5	California	0	–	–	++++	++++
B6	California	3	++++	++++	++++	++++
B7	South Africa	0	–	–	++++	++++
B7-1	South Africa	0	–	–	++++	++++
B12	Brazil	2	++++	++++	++++	++++
B12-1	Brazil	0	–	–	++++	++++
B14	Brazil	3	++++	++++	++++	++++
B14-4	Brazil	0.5	++++	++	++++	++++
B14-6	Brazil	1	++	++	++++	++++
B16	Brazil	0	–	–	++++	++++
B17	Brazil	2	++++	++++	++++	++++
B17-3	Brazil	1	++++	++++	++++	++++
B23	Israel	0	–	–	++++	++++
B23-9	Israel	0	–	–	++++	++++
B28	Florida	1.5	++++	++++	++++	++++
B30	Japan	2	++++	++++	++++	++++
B30-1	Japan	0	–	–	++++	++++
B30-2	Japan	1	++++	++++	++++	++++
B30-2-1	Japan	0	–	–	++++	++++
B30-4	Japan	2	++++	++++	++++	++++
B31	Japan	1.5	++++	++++	++++	++++
B31-1	Japan	1	++++	++++	++++	++++
B31-2	Japan	1	++++	++++	++++	++++
B35	Spain	0	–	–	++++	++++
B46	South Africa	0.5	++++	++++	++++	++++
B46-1	South Africa	0	–	–	++++	++++
B63	China	2.8	++++	++++	++++	++++
B65	China	1	++++	++	++++	++++
B65-1	China	0.5	–	–	++++	++++
B65-2	China	0	–	–	++++	++++
B77	Brazil	1	++++	++	++++	++++
B77-1	Brazil	0	–	–	++++	++++
B77-2	Brazil	0	–	–	++++	++++
B83	China	0	–	–	++++	++++
B128	Colombia	0.5	–	–	++++	++++
B134	Peru	2	++++	++++	++++	++++
B135	Peru	1.5	++++	++++	++++	++++
B144	China	2	++++	++++	++++	++++
B181	Costa Rica	0	–	–	++++	++++
B185	Japan	1.5	++++	++++	++++	++++
B187	Australia	0	–	–	++++	++++
B188	Japan	0	–	–	++++	++++
B190	Japan	0	–	–	++++	++++
B192	France	0	–	–	++++	++++
B194	India	1.5	++++	++++	++++	++++
B195	India	2.5	++++	++++	++++	++++
B198	Israel	0	–	–	++++	++++
B198-1	Israel	0	–	–	++++	++++
B199	Israel	0.5	++++	++++	++++	++++
B199-1	Israel	0.5	++++	++++	++++	++++
B211	Taiwan	0	–	–	++++	++++
B215	Japan	0	–	–	++++	++++
B222	India	0	–	–	++++	++++
B227	India	3	++++	++++	++++	++++
B235	Taiwan	2	++++	++	++++	++++
B249	Venezuela	1	++++	++++	++++	++++
B252	Taiwan	0	–	–	++++	++++
B256	Indonesia	2.5	++++	++++	++++	++++
B270	China	1	++++	++++	++++	++++
B276	Costa Rica	0	–	–	++++	++++
B280	Taiwan	0	–	–	++++	++++
B296	Dominican Republic	0	–	–	++++	++++
B297	Dominican Republic	0	–	–	++++	++++
B300	Puerto Rico	0	–	–	++++	++++
B305	Cuba	0	–	–	++++	++++
B310	Cuba	0	–	–	++++	++++
B316	Taiwan	2	++++	++++	++++	++++
B318	Taiwan	0	–	–	++++	++++
B335	California	0	–	–	++++	++++
B352	California	0	–	–	++++	++++
B365	Spain	ND	–	–	++++	++++
B370	Australia	3	++++	++++	++++	++++
B372	Australia	2.5	++++	++++	++++	++++
B373	Australia	2	++++	++++	++++	++++

^a ID letter B means Beltsville collection. Dash means subpropagation by aphid transmission, with number after dash indicating number of aphid transmissions (5,8).

^b Orange stem pitting (OSP) score is an arbitrary scale, where 0 = no visible stem pitting and 3.0 = very severe stem pitting (5,8) based on reactions observed on Madam Vinous seedlings under greenhouse conditions in Beltsville; ND = not determined.

^c ELISA reactions represent OD₄₀₅ values obtained with two selective antibody combinations, 109/3E10 and 110/109, and two nonselective or universal combinations, 3E10/109 and 1052/3E10, expressed as follows: –, not exceeding double the healthy controls; +, 0.5 to 1.0; ++, 1.0 to 1.5; +++, 1.5 to 2.0; +++++, more than 2.0.

with the MAb 3E10 at 0.1 to 1 µg of IgG per ml and detecting with the antiserum 109 at 1:30,000 dilution; and (iv) coating with the antiserum 1052 at 1:1,000 dilution and detecting with the MAb 3E10 at 20 ng of IgG per ml. The latter two combinations were also suitable for a universal detection of approximately 300 other isolates present in the collection for which characterization of their biological properties is not yet concluded (data not shown).

Differentiation of the orange stem pitting CTV isolates. Data on screening of 76 CTV isolates with the universal and selective I-DAS-ELISA formats are summarized in Table 1. Initially, the format utilizing rabbit polyclonal serum 109 against the bacterially expressed, full-size CTV CP for trapping and an MAb 3E10 as detecting antibody was found selective. Later, chicken polyclonal antiserum 110 against bacterially expressed, full-size CTV CP was used for coating, with the rabbit antiserum 109 used as detecting antibody, with similar results (Table 1).

The two selective combinations of trapping and detecting antibodies (Table 1, fourth and fifth columns) demonstrated remarkable correlation of the ELISA reaction and the OSP score determined on sweet orange Madam Vinous seedlings grown in the Beltsville greenhouse. ELISA readings for all OSP-positive isolates tested (OSP score 0.5 or more) were usually 10 to 20 times over the healthy controls, while reading for OSP-negative isolates never exceeded double the readings for healthy controls. Only in two cases, CTV isolates B128 and B65-1 with the OSP score 0.5, did this correlation not seem to hold. B128 is a field isolate from Colombia causing stem pitting in grape-

fruit. However, this only deviation may be attributed to imperfections of the arbitrary scoring system, which sometimes experiences difficulties when applied to the CTV isolates causing mild or very mild stem pitting reactions in sweet orange (S. M. Garnsey, *unpublished observations*). Both selective ELISA formats tested are of a direct type, i.e., stem pitting isolates reacted strongly in DAS-ELISA with 109 coating/3E10 detection, or 110 coating/109 detection, whereas non-stem pitting isolates did not (Table 1).

Possible mechanisms of the OSP differentiation. The data obtained suggested that the selectivity of the DAS-ELISA system analyzed was not due to the better replication of the stem pitting isolates of the virus, as the universal ELISA format indicated high levels of the CTV antigen in all samples tested. The 3E10 epitope is apparently present in stem pitting as well as in non-stem pitting isolates, as the 3E10 MAb captured all the isolates tested when used as the trapping antibody in the universal ELISA format (Fig. 1; Table 1, sixth column). Thus, the absence of the 3E10 epitope in OSP-negative isolates cannot be a reason for the selectivity observed.

The MAb 3E10 was found specific to a conformational epitope present in the intact, full-size CTV CP (14). Screening of the panel of 76 CTV isolates by I-DAS-ELISA in the universal format revealed conservation of the 3E10 epitope in all the isolates tested (Table 1, sixth column). This conservation of the 3E10 epitope in all the CTV isolates under study seems to contradict the selectivity of the I-DAS-ELISA format, which utilized 3E10 as the detecting antibody. However, careful analysis of the data presented in Table 1

and available information on the structure of the CTV antigen might indicate that there is no contradiction.

MAb-mediated serological selectivity usually assumes presence or absence of a certain epitope in the antigen. An example of this type of differentiation for CTV is represented by MCA-13, an MAb specific to a linear, continuous epitope (16). When used as a detecting antibody, this MAb reacts selectively to the decline-inducing isolates from Florida, both in DAS-ELISA and in Western blot, and fails to react to a number of mild isolates (16). The reactivity of MCA-13 has been associated with the presence or absence of a specific amino acid sequence in the CTV CP (14,15). This type of differentiation may be termed "qualitative," since it is based on the presence or absence of a certain epitope. The selectivity described here is apparently of a different type.

Antisera 109 and 110 were raised against the CP antigen of non-stem pitting isolate T36 (12), a well-known decline-inducing CTV isolate from Florida (18). This fact alone implies that a unique, hypothetical "stem pitting" epitope could not have been in the antigen used for the production of antisera 109 and 110. Selectivity of the described I-DAS-ELISA system for the orange stem pitting isolates of CTV reported here may be interpreted in two different ways. First, CTV antigen in non-stem pitting isolates may have a limited number of the 3E10 epitopes, which may be bound and saturated by the coating antibodies. This would prevent binding of the MAb 3E10 used as an intermediate detecting antibody. The second possibility is that the stability of the conformational 3E10 epitope may differ in stem pitting

Fig. 1. A drawing illustrating the orange stem pitting (OSP)-selective and universal enzyme-linked immunosorbent assay (ELISA)-based detection systems with the two Florida citrus tristeza virus (CTV) isolates, T-36 (Beltsville ID, B3) and T-68 (B28), as examples. Antibody designations are: 109, a polyclonal antiserum, and 3E10, a monoclonal antibody. The rightmost column shows the relative OD₄₀₅ signal in ELISA.

and non-stem pitting isolates. In this case, the 3E10 epitope in stem pitting isolates would retain its integrity after binding of the antigen to the trapping antibody, while it would be disrupted in the non-stem pitting isolates.

Recently, we reported an example of a substantial modulation of the antigenic reactivity of the CTV coat protein upon binding of the virus to the first, coating antibody (13). Specifically, binding of the conformational MAb caused marked changes in the antigenic reactivity of the CTV antigen, exposing cryptic linear epitopes (13). The data presented here also suggest that the differentiation observed is probably due to changes in antigenic reactivity of the CTV antigen mediated by the binding to the first, trapping antibody. The selectivity based on reactivity of CTV isolates toward a conformational, rather than a linear, epitope which we have described provides the first direct evidence that serology can be successfully used to differentiate CTV isolates causing stem pitting in sweet orange. Irrespective of the underlying mechanism(s), this technique may be of great help in differentiation of undesirable orange stem pitting isolates from other more benign isolates.

Interestingly, the I-DAS-ELISA format tested could not distinguish CTV isolates causing stem pitting in grapefruit (data not shown). This observation is in agreement with the biological testing data, which suggested that syndromes of stem pitting in grapefruit and in sweet orange are caused by two independent genetic determinants of CTV (5,8). The search for the ELISA-based differentiation of grapefruit stem pitting needs to be continued.

Serological differentiation of the orange stem pitting isolates of CTV: Applications. Differentiation of the stem pitting isolates from non-stem pitting isolates is becoming a major issue in management of the CTV infection in Florida, California, and other citrus-growing areas. CTV has long been observed to have a diversity of disease severity in different indicators. Testing of isolates in a collection of more than 300 accessions at Beltsville using a standardized set of five indicator plants has confirmed this diversity (5,8). Currently, isolates that cause severe stem pitting in sweet orange and grapefruit are rare in commercial citrus in the United States, but they are common in a number of other citrus-producing countries. With the brown citrus aphid spreading in North America and the Caribbean, stem pitting isolates of CTV are expected

to increase in incidence unless steps are taken to prevent their introduction and spread (7,17). Biological characterization is the most reliable procedure available today for the CTV isolate characterization and differentiation; however, like any biological technique, it is laborious, slow, and expensive. Lack of cost-efficient methods to distinguish stem pitting isolates from decline-inducing or symptomless isolates has been a major constraint in the management of CTV. The data presented here demonstrate that serological differentiation of the CTV isolates causing stem pitting in sweet orange is possible, and it can be accomplished even with polyclonal antisera raised against bacterially expressed virus antigens.

We believe that the ELISA-based technique utilizing inexpensive antisera against bacterially expressed CTV CP antigens will provide a suitable supplement and may be an alternative to biological indexing. The differentiating system described here is based on results with a limited number of isolates and needs to be tested on a larger scale and with field material. If successful, this system may be used to limit the spread of harmful orange stem pitting CTV isolates in commercial citrus groves.

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