Serological Detection of *Citrus tristeza virus* with Antibodies Developed to the Recombinant Coat Protein

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ABSTRACT

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Antibodies specific for the recombinant coat protein (rCP) of the p25 gene of *Citrus tristeza virus* (CTV) were developed in goats and rabbits and further evaluated as a complete kit for the detection of the virus using healthy and CTV-infected tissue. The combination of goat T1 used as primary (coating) and rabbit C3 as intermediate (detecting) rCP antibodies reacted efficiently, with optical density at 405 nm (OD₄₀₅) values between 0.250 and 2.000 with samples from an international collection of diverse CTV isolates. The CTV isolates tested cause a broad spectrum of disease syndromes in different citrus hosts. The OD₄₀₅ values for healthy tissue were less than 0.100. Likewise, the combination of goat T1 and rabbit C3 rCP antibodies gave consistent results for CTV-positive and -negative sample discrimination when directly compared with the Central California Tristeza Eradication Agency (CCTEA) antibodies used for large-scale CTV detection and a commercially available CTV serological detection kit. The combination of goat T1 and rabbit C3 rCP antibodies showed its suitability for large-scale indexing with samples collected in commercial groves as part of the CCTEA's regular monitoring program. The evaluation included 41,195 samples from 301 commercial groves from districts 1, 2, and 3. In total, 26 trees (0.063%) were found to be CTV positive using the T1/C3 rCP antibody combination. Results of this research provide evidence that rCP antibodies can be efficiently used for both capturing and detecting CTV antigens in double-antibody sandwich indirect enzyme-linked immunosorbent assay.

Citrus tristeza virus (CTV) is distributed worldwide and causes the most economically important virus disease of citrus (33). CTV infects nearly all *Citrus* spp., cultivars, and intergeneric hybrids and some citrus relatives (3,24); however, the most dramatic effect is the decline induced on plants grafted on sour orange (*Citrus aurantium* L.) rootstock. In addition, there are some strains of the virus that cause stem-pitting in the trunk and main branches on some oranges (*C. sinensis* (L.) Osb.), grapefruit (*C. paradisi* L.), and Tahiti lime (*C. latifolia* Tan.) regardless of the rootstocks, which frequently leads to severe tree deterioration and poor growth and yield (33). In the last 70 years, severe CTV epidemics

have occurred in different countries, with estimated tree losses of over 100 million citrus plants grafted onto sour orange rootstock (5,9,33). Countries where CTV has caused extensive tree losses include Argentina, Australia, Brazil, Dominican Republic, Israel, Jamaica, South Africa, Spain, the United States, and Venezuela, among others (5,9,14,22,33). Significant efforts are conducted every year in several countries to detect CTV-infected trees in order to prevent virus spread and future CTV epidemics, mostly in those citrus areas where sour orange still predominates as the main rootstock (5,7,9,18,22,28,33,37,38). Since the enzyme-linked immunosorbent assay (ELISA) test (10) was implemented for CTV (2), several ELISA-based protocols have been developed (4,8,15,34) and ELISA has become an indispensable tool for large-scale diagnosis of CTV worldwide for both research (6,9,17,18,35,36,42) and disease-management purposes (5,7,12,22,28,32,38). Antibody production for CTV diagnosis, either polyclonal (4,16) (R. F. Lee, *unpublished*) or monoclonal (6,27,40), has been accomplished in the past by using purified viri-

ons as immunogens. Although some of these antibodies were produced and used for specific research purposes, others have been available as serological detection kits by several commercial suppliers for large-scale diagnosis of CTV worldwide. In addition, several research groups have developed polyclonal antibodies to the recombinant p25 coat protein (rCP) of CTV (23,25,39). However, those rCP antibodies have shown inconsistent results as trapping antibodies under nondenaturing conditions (1,25,26); hence, their use at a large scale have been limited only as intermediate antibodies in double-antibody sandwich indirect (DASI)-ELISA (25,31) (M. L. Polek, *unpublished*). We have developed antibodies to the rCP of several CTV isolates from Mexico in goats and rabbits that have a remarkable suitability to perform as both primary (trapping) and intermediate (detecting) antibodies in DASI-ELISA tests (19). This work reports the evaluation of these rCP antibodies to detect the virus from a collection of CTV isolates from diverse geographic origins and compare their performance to a commercially available CTV detection kit and the established protocol from the Central California Tristeza Eradication Agency (CCTEA), and to determine their suitability for large-scale field indexing.

MATERIALS AND METHODS

Source of antibodies. Goat (T1) and rabbit (C3) antibodies were obtained against the rCP of CTV isolates MX08 and MX14 from Mexico, respectively (20). Untagged rCP from *Escherichia coli* transformed cells was purified using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and extensively dialyzed against phosphate-buffered saline (PBS) buffer prior to its use as immunogen (19). Goat (T1) and rabbit (C3) rCP antibodies were purified by ammonium sulfate precipitation and adjusted to 1.0 mg/ml (optical density at 280 nm [OD₂₈₀] = 1.45) by spectrophotometry (11). The CCTEA detection protocol consists of whole-virus CTV polyclonal antibody provided by the University of California Citrus Clonal Protection Program (CCPP) (Department of Plant

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Table 1. *Citrus tristeza virus* (CTV) isolates from the international collection of the United States Department of Agriculture confined under quarantine conditions at Beltsville, MD (13) used to evaluate the reactivity of goat T1 and rabbit C3 recombinant coat protein antibodies

Sample no.	CTV isolate	Origin	Biological properties ^a
1	B-002	United States	Mild
2	B-006	United States	DC,SY,GSP,OSP
3	B-007	South Africa	GSP
4	B-007-1	South Africa	GSP
5	B-012	Brazil	DC,SY,OSP
6	B-012-1	Brazil	DC,SY
7	B-014	Brazil	DC,SY,OSP
8	B-014-4	Brazil	DC,SY
9	B-014-6	Brazil	DC,SY,GSP,OSP
10	B-016	Brazil	DC,SY
11	B-017	Brazil	DC,SY,GSP,OSP
12	B-017-3	Brazil	DC,SY,OSP
13	B-023	Israel	GSP
14	B-023-9	Israel	DC,GSP
15	B-028	United States	DC,SY,GSP,OSP
16	B-030 R1	Japan	DC,SY,OSP
17	B-030-1	Japan	DC
18	B-030-2	Japan	DC,GSP
19	B-030-2-1	Japan	DC
20	B-030-4	Japan	DC,SY,GSP,OSP
21	B-031	Japan	GSP,OSP
22	B-031-2	Japan	SY,GSP,OSP
23	B-035	Spain	Mild
24	B-046	South Africa	DC,GSP
25	B-063	China	DC,SY,GSP,OSP
26	B-065	China	DC,SY,GSP,OSP
27	B-065-1	China	DC,SY,GSP,OSP
28	B-065-2	China	DC,SY,GSP
29	B-077	Brazil	DC,SY,GSP,OSP
30	B-077-1	Brazil	DC
31	B-083	China	DC,GSP
32	B-128	Colombia	DC,SY,OSP
33	B-134	Peru	DC,SY,GSP
34	B-135	Peru	DC,SY,GSP,OSP
35	B-144	China	DC,SY,GSP
36	B-181	Costa Rica	Mild
37	B-185	Japan	DC,SY,GSP,OSP
38	B-187	Australia	DC,SY,GSP
39	B-188	Japan	GSP
40	B-190	Japan	Mild
41	B-192	France	Mild
42	B-194	India	DC,SY,OSP
43	B-195	India	DC,SY,GSP,OSP
44	B-198	Israel	DC,GSP
45	B-198-1	Israel	GSP
46	B-199	Israel	DC,SY,GSP,OSP
47	B-199-1	Israel	DC,SY,OSP
48	B-211	Taiwan	DC,SY,GSP
49	B-215	Japan	Mild
50	B-222	India	DC,SY
51	B-227	India	DC,SY,GSP,OSP
52	B-252	Taiwan	DC
53	B-256	Indonesia	DC,SY,GSP,OSP
54	B-270	China	DC,SY,GSP,OSP
55	B-276	Costa Rica	Mild
56	B-280	Taiwan	DC,GSP
57	B-296	Dominican Republic	DC
58	B-297	Dominican Republic	DC,GSP
59	B-300	Puerto Rico	DC,GSP
60	B-305	Cuba	Mild
61	B-310	Cuba	DC,GSP
62	B-316	Taiwan	DC,SY,GSP,OSP
63	B-318	Taiwan	Mild
64	B-335	United States	DC,GSP
65	B-352	United States	DC,SY,GSP
66	B-365	Spain	DC
67	B-370	Australia	DC,SY,GSP,OSP
68	B-372	Australia	DC,SY,GSP,OSP
69	B-373	Australia	DC,SY,GSP,OSP

^a Mild = mild in sweet orange on sour orange combination, DC = decline in sweet orange on sour orange combination, SY = seedling yellows on sour orange, GSP = stem pitting on Duncan grapefruit, and OSP = stem pitting on Madam vinous sweet orange.

Pathology, University of California, Riverside) and rabbit polyclonal antibody developed to a maltose-binding recombinant CP of California SY568 CTV isolate (25). The third source of CTV antibodies was that of Agdia SRA 78900/1000 (Elkhart, IN).

Tissue samples. Citrus tissue analyzed in this study were from three sources. (i) Citrus samples from the international collection of CTV isolates of the United States Department of Agriculture (USDA) located at Beltsville, MD (13). These CTV isolates represent the main groups of biologically characterized CTV strains from diverse geographic origins (Table 1). (ii) Citrus tissue from a collection of CTV isolates from California maintained under greenhouse conditions at the CCTEA at Tulare, CA (<http://www.cctea.org/web%20page%20averaged%20rev.pdf>). These CTV isolates represent all the groups of biologically characterized CTV strains from California. (iii) Citrus samples collected in commercial groves as part of the CCTEA's regular monitoring program in the San Joaquin Central Valley.

Tissue collection and sample preparation. Young, tender bark tissue was collected from the in planta CTV cultures maintained in quarantine in the USDA international collection of CTV isolates. The chopped bark tissue was homogenized using a Kleco tissue homogenizer (Garcia Machines, Visalia, CA) in extraction buffer (PBS; 8 mM Na₂HPO₄, 14 mM KH₂PO₄, 15 mM NaCl, pH 7.4 containing 0.05% Tween 20, 2% polyvinylpyrrolidone [Sigma PVP 40; Sigma-Aldrich, St. Louis], and 0.2% bovine serum albumin) at a ratio of 1/10 (grams fresh weight/volume). Following homogenization, the samples were aliquoted into glass vials and lyophilized. The lyophilized samples were stored at -20°C except while being shipped under quarantine permit. To prepare the samples for assay, distilled water was added to the lyophilized sample to bring the volume to the original measure. The California citrus tissue was collected under a standard methodology developed by the CCTEA (31). In all, 8 to 10 tender flushes with fully expanded leaves were collected from each citrus plant, placed in plastic bags, labeled, and brought to the laboratory in ice chests. Antigen samples were prepared by grinding 0.5 g of petiole tissue in 5 ml of extraction buffer using a Kleco tissue homogenizer (Garcia Machines).

Serological tests. All serological tests were performed according to the standard DAS-ELISA (11). Unless otherwise stated, 100 µl was used per well and three washings with PBS + 0.05% Tween 20 (PBS-Tween) were performed between steps. Polystyrene flat bottom Nunc Maxisorb microtiter plates (Rochester, NY) were coated with the corresponding trapping antibodies diluted in carbonate buffer (0.015 M NaHCO₃, 0.03 M NaCO₃, pH 9.6) and incubated for 6 h at 37°C. Antigen samples were incubated for

16 to 18 h at 5°C; each of the intermediate antibodies were diluted in conjugate buffer (PBS-Tween-PVP + 0.2% bovine serum albumin [wt/vol]) and incubated for 4 h at 37°C. After washing, the corresponding alkaline phosphatase enzyme conjugates were diluted in conjugate buffer and incubated for 2 h at 37°C. At the end of the incubations, the reaction with p-nitrophenyl phosphate (Sigma N 2765) at 1.0 mg/ml in 10% diethanolamine, pH 9.8, was quantified after 1 h at 405 nm (OD_{405}) using an ELISA plate reader. Goat T1 trapping and rabbit C3 intermediate rCP antibodies were used at 3 and 0.1 µg/ml, respectively; goat anti-rabbit immunoglobulin G alkaline phosphatase conjugate (Sigma A3812) was used at 1:30,000 dilution. CCTEA antibodies were used according to a preestablished protocol

(31) and Agdia's detection kit was used as recommended by the supplier. In each experiment, CTV antigens included two replications of each citrus sample. Standards of CTV-infected citrus tissue and extracts of sweet orange, grapefruit, and mandarin (*C. reticulata* Blanco) of healthy greenhouse-grown plants were included as positive and the negative controls, respectively. When OD_{405} readings were greater than two times the mean of the healthy controls, reactions were considered positive (+). Reactions with OD_{405} values less than 0.100 or two times the mean of the healthy controls were considered negative (-). The threshold for negative and positive reactions was only an estimated value and has been routinely used at the CCTEA in the annual regular monitoring program.

RESULTS

Reactivity of goat T1 and C3 rCP antibodies to a collection of CTV isolates from diverse geographic origins. This part of the work was conducted at the USDA National Clonal Germplasm Repository for Citrus and Dates located at Riverside, CA. Using goat T1 and rabbit C3 rCP antibodies to test all the CTV isolates, OD_{405} values between 0.250 and 2.200 were observed (Fig. 1). In all, 6 (8.69%) CTV isolates gave OD_{405} readings between 0.200 and 0.500, 9 (13.04%) between 0.500 and 1.000, 30 (43.47%) between 1.000 and 1.500, 20 (28.98%) between 1.500 and 2.000, and 4 (5.79%) higher than 2.000. Conversely, OD_{405} values for healthy tissue samples were lower than 0.100 (Fig. 1).

Fig. 1. Detection of *Citrus tristeza virus* (CTV) with T1 and C3 recombinant coat protein antibodies from the international collection of the United States Department of Agriculture confined under quarantine conditions at Beltsville, MD from diverse geographical origins. The upper bar of every optical density at 405 nm (A_{405}) reading indicates the standard deviation for two replications per CTV isolate. Healthy = tissue of Madam Vinous sweet orange grown plants. Positive controls were citrus tissue infected with CTV isolates T26/T30, Valencia/Sour 2, T36, and T46. One plant was used per each CTV isolate. See Table 1 and Materials and Methods for details.

Fig. 2. Comparison of enzyme-linked immunosorbent assay results using goat T1 and rabbit C3 recombinant coat protein antibodies with Central California Tristeza Eradication Agency (CCTEA) antibodies, and a commercial detection kit from Agdia for the detection of *Citrus tristeza virus* (CTV) from 40 California CTV isolates. The upper bar of every optical density at 405 nm (A_{405}) reading indicates the standard deviation for two replications per CTV isolate. Test and TE = extraction buffer; Test Pos = positive control; Test Neg = negative control. Negative control consisted of a mix of tissue of Washington Navel and Valencia oranges, Murcott mandarin, Mexican Lime, and S-1 citron. Positive control consisted of tissue of Madam Vinous sweet orange samples infected with California CTV isolates numbers 107, 99402, and 575 (<http://www.cctea.org/web%20page%20page%20averaged%20rev.pdf>). See Materials and Methods for details.

Comparison of goat T1 and C3 rCP antibodies with the CCTEA protocol and a commercial CTV detection kit. The combination of goat T1 and rabbit C3 rCP antibodies was further evaluated in DASi-ELISA tests at the CCTEA facilities located at Tulare, CA, using a collection of 280 California CTV isolates maintained under greenhouse conditions. In the first test, the OD₄₀₅ readings of 40 CTV isolates were used to compare the T1 and C3 antibodies with the CCTEA antibodies and a commercial kit available from Agdia (Fig. 2). Each antibody source consistently distinguished the CTV-positive and -negative samples with OD₄₀₅ values between 0.250 and 1.850 for CTV-infected tissue, whereas OD₄₀₅ values for healthy tissue samples were less than 0.100 for all three antibody sources (Fig. 2). In addition, the CTV-positive and -negative samples were properly discriminated using any of the three CTV detection DASi-ELISA protocols (Fig. 2). In a second test, 240 additional California CTV isolates from the collection were evaluated in a comparable DASi-ELISA test by using the goat T1 and

rabbit C3 rCP antibodies and the CCTEA protocol. Again, for both DASi-ELISA protocols, there was a 100% concurrence of discriminating between CTV-positive and -negative samples (Table 2). Comparing the two DASi-ELISA protocols, 152 CTV isolates (63.33%) gave equal OD₄₀₅ values (less than 0.100 difference) for both protocols, 67 (27.91%) gave OD₄₀₅ values higher than 0.100 by the CCTEA protocol than the combination of goat T1 and rabbit C3 antibodies, and 21 (8.75%) gave OD₄₀₅ values higher than 0.100 by goat T1 and rabbit C3 than the CCTEA protocol (Table 2).

Evaluation of goat T1 and C3 rCP antibodies with field-collected samples. The combination of goat T1 and rabbit C3 rCP antibodies was evaluated in DASi-ELISA tests for its suitability for large-scale field indexing. In the first test, we evaluated five blocks planted with diverse mandarin cultivars with previous observations of atypically high OD₄₀₅ readings with DASi-ELISA during the spring of the regular monitoring program by the CCTEA (30,41). With the CCTEA proto-

col, the OD₄₀₅ readings of 428 field-grown mandarin samples were in the range of 0.083 to 0.213 (Table 3). In contrast, the OD₄₀₅ readings of the same 428 mandarin samples analyzed with the combination of T1 and C3 rCP antibodies were in the range of 0.056 to 0.097. In both assays, the mean OD₄₀₅ readings for healthy negative controls were consistently lower than 0.100. Likewise, CTV-infected positive controls ranged from 1.177 to 1.337 for the CCTEA protocol and 1.008 to 1.273 for the T1 and C3 rCP antibodies (Table 3). On the basis that reactions were considered positive (+) when the mean of OD₄₀₅ values were greater than two times that of the mean of the healthy controls, there were zero CTV positives detected with the combination of T1 and C3 rCP antibodies, whereas the CCTEA protocol resulted in 45 potentially CTV-positive samples in these four mandarin blocks (Table 3). In a separate study (41), samples from these suspect CTV-positive mandarin samples were proven to be CTV negative by immunocapture reverse-transcription polymerase chain reaction (IC-RT-PCR); hence, we concluded that they were false positives. We also evaluated the combination of T1 and C3 rCP antibodies with samples collected in commercial groves as part of the CCTEA's regular monitoring program. The evaluation included 41,195 samples from 301 commercial groves from districts 1, 2, and 3 of the California Central Valley. In total, 26 trees were found to be CTV positive, with the T1 and C3 rCP antibody combination resulting in 0.063% of CTV infected samples (Table 4).

DISCUSSION

In this work, we report the advantages of using of goat T1 as the capture (trapping) and rabbit C3 as the intermediate (detecting) rCP antibodies as a DASi-

Table 2. Comparison of goat T1 and rabbit C3 recombinant coat protein antibodies with the Central California Tristeza Eradication Agency (CCTEA) protocol for the detection of *Citrus tristeza virus* (CTV) with 260 California CTV isolates^a

CTV isolates	No. of samples (%)			Total
	Equal OD ₄₀₅ between CCTEA versus T1 and C3 ^b	OD values >0.100 by CCTEA than by T1 and C3	OD values >0.100 by T1 and C3 than by CCTEA	
41-88	18 (37.50)	23 (48.00)	7 (14.58)	48 (100)
89-136	33 (68.75)	10 (20.83)	5 (10.41)	48 (100)
137-184	34 (70.84)	9 (18.75)	5 (10.41)	48 (100)
185-232	37 (77.08)	7 (14.58)	4 (8.33)	48 (100)
233-280	30 (62.50)	18 (37.50)	0 (0%)	48 (100)
Total	152	67	21	240 (100)

^a See Materials and Methods for details.

^b Equal optical density at 405 nm (OD₄₀₅) values (<0.100 difference); OD₄₀₅ readings were ≤0.100 different among them.

Table 3. Serological comparison of the Central California Tristeza Eradication Agency (CCTEA) protocol and T1 and C3 recombinant coat protein antibodies with field-collected mandarin samples and known positive and negative *Citrus tristeza virus* (CTV)-infected controls in double-antibody sandwich indirect enzyme-linked immunosorbent assay (DASi ELISA)^a

Block	Cultivar	Total samples	Mean OD ₄₀₅ ^b	Range OD ₄₀₅	No. of positive samples ^c	Healthy control OD ₄₀₅	Positive control OD ₄₀₅
CCTEA protocol							
45A	Unknown mandarin	144	0.139	0.207–0.101	20	0.083	1.337
04B	Unknown young citrus	93	0.117	0.183–0.079	19	0.079	1.177
06 ^a	Unknown young citrus	117	0.112	0.213–0.078	19	0.079	1.216
04F	Unknown mandarin	42	0.120	0.182–0.087	3	0.083	1.323
04G	Unknown mandarin	42	0.121	0.163–0.083	4	0.074	1.206
Total	...	428	...	...	45	...	...
T1 and C3 antibodies							
45A	Unknown mandarin	144	0.065	0.097–0.061	0	0.064	1.273
04B	Unknown young citrus	93	0.061	0.065–0.060	0	0.059	1.175
06 ^a	Unknown young citrus	117	0.062	0.074–0.057	0	0.060	1.166
04F	Unknown mandarin	42	0.057	0.070–0.053	0	0.053	1.008
04G	Unknown mandarin	42	0.061	0.070–0.056	0	0.060	1.044
Total	...	428	...	...	0	...	...

^a See Materials and Methods for details.

^b Optical density at 405 nm (OD₄₀₅) after 60 min of substrate reaction. Mean of two replications per ELISA plate. Reactions were considered positive (+) when the mean of OD₄₀₅ was higher than two times the mean of healthy controls or 0.100, whichever was greater. Reactions with lower values were considered negatives (–).

^c The number of “positive samples” with the CCTEA protocol are, in fact, false positives.

ELISA complete CTV kit. The detection of CTV antigens was consistent and the discrimination of healthy tissue samples from samples of diverse citrus plants was more than satisfactory. Goat T1 and rabbit C3 rCP antibodies showed 100% virus detection (Fig. 1) with a collection of 69 CTV isolates that cause a broad spectrum of disease symptoms in different citrus hosts, such as decline, seedling yellows, orange stem pitting, grapefruit stem pitting, or mild symptoms, as well as combinations of these symptoms. Likewise, these CTV isolates represent virus populations from 18 countries on five continents. OD₄₀₅ readings in the range of 0.500 to 2.200 were consistent in 93% of the CTV isolates tested; only six CTV isolates gave OD₄₀₅ in the range of 0.200 and 0.500, which was considered still adequate for virus detection when the OD₄₀₅ values for the healthy controls were less than 0.100 (Fig. 1). The differences in OD₄₀₅ readings showed by all CTV isolates from the international USDA collection must have been related to differences in virus titer in the test plants rather than differential specificity to the rCP antibodies.

The combination of goat T1 and rabbit C3 rCP antibodies also showed 100% virus detection with the 280 CTV isolates from California (Fig 2; Table 2). These CTV isolates also exhibited a wide range of CTV symptoms in different citrus hosts and are representative of the CTV isolates that have been found in California citrus groves since 1993 (29). The results obtained with goat T1 and rabbit C3 rCP antibodies with the California CTV isolates were comparable with the level of detection achieved with the CCTEA's protocol and Agdia's detection kit, respectively, with 100% detection of CTV by the three sources of antibodies. There were some slight differences in the OD₄₀₅ values obtained by goat T1 and rabbit C3 rCP antibodies compared with the CCTEA's protocol and Agdia's detection kit (Fig 2; Table 2); however, these differences were similar to those that are normally found when several commercially available serological kits are compared for CTV detection (21) and can be explained on the basis of the nature of the source of antisera (21), where a combination of antibody concentration and specificity must be involved.

In addition, goat T1 and rabbit C3 rCP antibodies were useful for large-scale in-

dexing of citrus trees (Tables 3 and 4), even with field-grown mandarin cultivars exhibiting atypically high OD₄₀₅ values in DASI-ELISA tests performed with the CCTEA's protocol (30,41). The OD₄₀₅ readings with field-grown mandarin samples evaluated were in the range of 0.083 to 0.213 with the CCTEA's antibodies, whereas the OD₄₀₅ readings with the same mandarin samples analyzed with the T1 and C3 rCP antibodies were in the range of 0.056 to 0.097 (Table 3). The reason why this phenomenon occurred with the CCTEA's protocol and not with the T1 and C3 rCP antibodies is unknown. It has been hypothesized that a stress-related protein present in field-grown mandarin cultivars under some adverse environmental conditions is involved in the atypically high OD₄₀₅ readings in DASI-ELISA tests (30). However, this hypothesis warrants further verification.

CTV is a quarantine pathogen in many countries and several million trees are analyzed every year worldwide to detect the presence of CTV to prevent further spread of the virus, either from valuable, commercial budwood sources registered through certification programs or natural spread in the field. Results of this work provide evidence that antibodies developed against the rCP of CTV are a viable option for large-scale indexing of citrus trees. According to our system, the key points are to have the cultures of *E. coli* transformed with the p25 CP gene of CTV, then to purify the corresponding untagged recombinant CP through SDS-PAGE followed by extensive dialysis to eliminate SDS from the gel slices containing the rCP prior its use as an immunogen, and to have a reliable system for the massive production and evaluation of the CTV-specific antisera. These features may guarantee a permanent supply of CTV antibodies to support the continuous activities of any large CTV-monitoring program worldwide. Currently, several serological detection kits are commercially available for CTV, mostly from the United States, Spain, Switzerland, and Italy. Although these serological kits are efficient for CTV detection, they are relatively expensive and their costs significantly increase when they have to be imported into other countries. Additionally, their availability is not always immediate (21); these features, consequently, limit their usefulness for large-

scale indexing, especially for those citrus industries where the serological detection kits must be imported from foreign sources.

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Table 4. Detection of *Citrus tristeza virus* (CTV) with T1 and C3 recombinant coat protein antibodies from field samples of young plantings (<5 years) of sweet orange and mandarin cultivars collected from March to June 2007 in three pest control districts in three counties of California's Central San Joaquin Valley

District	No. of groves monitored for CTV	No. of samples monitored for CTV	No. of samples positive for CTV	CTV detected samples (%)
No. 1	54	5,268	0	0.000
No. 2	233	34,559	15	0.043
No. 3	14	1,368	11	0.804
Total	301	41,195	26	0.063

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