Comparison of Biological Indexing and Immunological Assays for Identifying Severe Florida Isolates of Citrus Tristeza Virus

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ABSTRACT. One hundred fifty-six isolates of citrus tristeza virus (CTV) were collected from sites in all the major citrus-growing areas of Florida. These isolates came primarily from sweet orange and grapefruit budded on sour orange rootstock and included trees with obvious stunting or decline symptoms and vigorous trees without CTV symptoms. Sixty-eight isolates were indexed by graft inoculation to greenhouse-grown Mexican lime and sweet orange on sour orange indicator plants. Crude extracts from all the greenhouse host plants were assayed by double antibody sandwich (DAS) ELISA using broad spectrum anti-CTV polyclonal and four monoclonal antibodies developed to different epitopes of CTV. The reactivity of one of these monoclonal antibodies, CTV-MCA13, was closely related to isolate severity as determined by biological index plants. A comparison of the 68 CTV isolates tested by both biological indexing and the CTV-MCA13-based immunoassays showed a greater than 95% agreement between these two assays for determining CTV isolate severity. The other monoclonal antibodies tested did not discriminate between Florida CTV isolates.

Many strains of citrus tristeza virus (CTV) have been described which vary markedly in severity. Identification of strains and analysis of strain severity have been done by time-consuming bioassays on citrus indicator plants (4). Rapid immunological assays for detecting CTV have been available for several years using polyclonal antisera-based enzyme linked immunosorbent assays (ELISA) (1, 2, 3). These assays accurately detected infection, but did not indicate isolate severity. Limitations inherent in the use of polyclonal antisera led Vela *et al.* (11, 12) to develop monoclonal antibodies (MCA) to CTV. However, these also reacted to all CTV isolates tested. Since mild isolates of CTV cause little or no damage and may provide some protection against infection by severe isolates in many citrus cultivars, a need has developed to differentiate CTV isolates (7). Recently, an MCA designated CTV-MCA13 was developed which discriminated between CTV isolates. It reacted only with severe isolates of virus in preliminary tests (10), and work by Irey *et al.* (8) demonstrated the effectiveness of the CTV-MCA13-based ELISA assay for identifying trees infected by severe CTV isolates in the field. The number of different CTV isolate sources tested to date, however, has been limited. The objective of this study was to determine the serological diversity of an extensive collection of Florida CTV isolates using four different monoclonal antibodies and to compare the CTV-MCA13-based ELISA assay with the classic biological evaluation of CTV isolate severity.

METHODS AND MATERIALS

Virus Isolates. CTV isolates used in this study were collected from all the major citrus-growing areas of Florida. Approximately 130 of the 156 isolates were taken from sweet orange budded to sour orange rootstock. Sweet orange cultivars included Valencia, Pineapple, navel, and Parson Brown. Nineteen isolates were from Marsh grapefruit on sour orange or alemow rootstocks and the remaining isolates came from Meyer lemon, Mexican lime, and other miscellaneous sources. Trees selected for testing were vigorous trees, young

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stunted tree, and mature trees in various stages of decline. Field isolates were maintained by graft inoculation to glasshouse-grown citrus plants, usually sweet orange or Mexican lime.

Biological Indexing. Sixty-eight of the 156 CTV isolates collected were graft-inoculated to two or more Mexican lime on alemow and five or more grafted combinations of sweet orange on sour orange rootstock (6). Symptoms of vein clearing, leaf cupping, stem pitting, or stunting on Mexican lime were ranked as mild, moderate, or strong 6 months after plant inoculation. Symptoms of chlorosis and stunting in sweet orange on sour orange were ranked as negative, mild, moderate, or strong between 6 and 12 months after plant inoculation. CTV isolates causing mild or moderate symptoms in Mexican lime, only, were considered mild isolates. Isolates causing noticeable symptoms in sweet orange on sour orange plants were considered severe isolates.

Immunological Assays. Preparation of lyophilized plant extracts and the direct and indirect double antibody sandwich (DAS) ELISA assays used were as described by Permar *et al.* (10). In the direct DAS ELISA, a polyclonal antiserum (1052) developed to Florida CTV isolate T-36 was the source of coating antibody. The secondary antibody was 1052 IgG conjugated with alkaline phosphatase. In the indirect DAS ELISA, polyclonal antibodies (1052) were used for coating. The secondary monoclonal antibodies were CTV-MCA13 and CTV-MCA14 developed to Florida CTV isolate T-36 and 3DF1 and 3CA5 developed to the Spanish CTV isolate T-308 (11). All four monoclonal antibodies reacted to different CTV epitopes (5). Each ELISA plate contained four sets of controls; extracts from plants infected with a known severe Florida CTV isolate (T-36) (10), a known mild Florida CTV isolate (T-30) (9), extract from a healthy plant, and extraction buffer alone. Samples were considered positive when A_{405} values exceeded five times the average of the healthy and buffer control values. All 156 CTV isolates collected were tested against the five antibody sources. Plant extracts that tested positive with 3DF1 and CTV-MCA13 monoclonal antibodies were considered positive for severe CTV isolates. Those that tested positive only with 3DF1 were considered positive for mild CTV isolates.

RESULTS

Immunoassay of plant tissue samples showed that only CTV-MCA13 could discriminate between Florida isolates of CTV. CTV-MCA13 reacted with 67 samples while the other four antibody sources reacted to all 156 samples surveyed. Sixty-eight of the 156 samples surveyed were also indexed on Mexican lime and sweet on sour orange host plants. All 68 isolates caused CTV symptoms in Mexican lime, and 42 isolates showed symptoms in sweet on sour orange host plants. Field symptoms of the original host plants were generally in agreement with biological assays and CTV-MCA13-based immunoassays. Vigorous trees contained mild CTV isolates and stunted or decline trees were infected with severe CTV isolates.

In immunological assays with 3DF1 antibody, all plant extracts reacted with A_{405} values greater than 20 times the average value of the negative control (A_{405} = 0.023). There was a well-defined separation of positive and negative ELISA reactions using CTV-MCA13 antibody with positive reaction values greater than eight times the average negative control (A_{405} = 0.031) and negative reactions very close to the negative control value.

Results of the biological indexing assays showed that 66 of the 68 CTV isolates tested could be categorized into two groups (Fig. 1). In one group were isolates that caused mild reactions in Mexican lime and no reaction

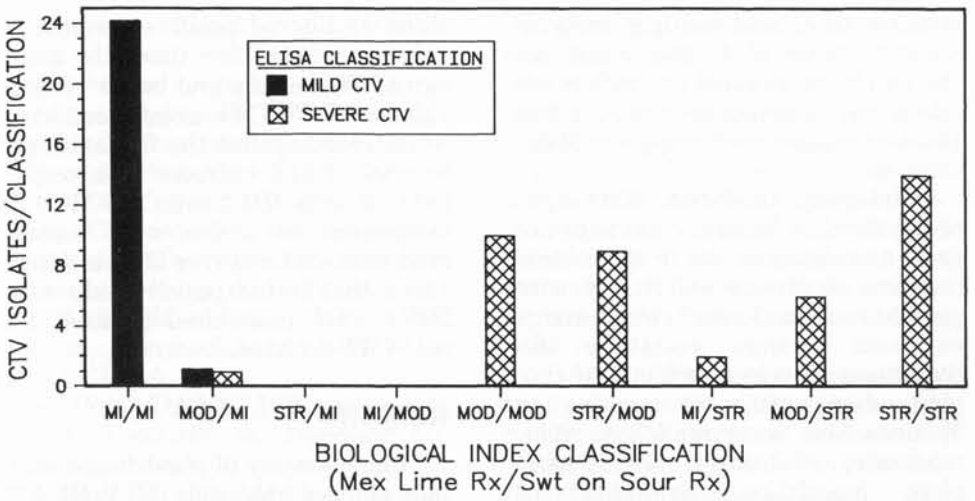

Fig. 1. Comparison of biological indexing and ELISA assays for detecting severe CTV isolates: MI = negative or mild reaction (Rx), MOD = moderate reaction, STR = strong reaction.

in sweet orange on sour orange rootstock. The other group included those isolates that caused a moderate to strong reaction with both sweet orange on sour orange and Mexican lime indexing plants. Data presented in Fig. 1 also shows that all of the CTV isolates tested as mild by ELISA, except one, were included in the first biological indexing group and all but one isolate determined to be severe by ELISA were included in the second biological index group.

DISCUSSION

An apparent low level of serological diversity has been demonstrated among Florida isolates of CTV. One polyclonal and four monoclonal antibodies known to react with different epitopes of CTV (5) were tested against isolates from 156 trees. CTV-MCA13 alone discriminated between Florida CTV isolates, the other four antibody sources reacted to all the isolates tested.

Data presented in this study from a wide variety of citrus cultivars in all the major citrus-growing areas of Florida has shown a greater than 95% agreement between biological indexing and immunoassays for determining CTV isolate severity. These results are supported by previous work. Permar *et al.* (10) reported a monoclonal antibody CTV-MCA13 developed to a severe Florida CTV isolate that could discriminate strains of CTV. An extensive study by Irey *et al.* (8) used CTV-MCA13 in an ELISA assay to detect severe CTV isolates in young citrus field plantings. These authors found a greater than 90% agreement between visual plant symptoms and ELISA test results. Even with large numbers of samples taken over a wide area of the state, the possibility still exists that there may be mild Florida isolates that react with CTV-MCA13 or severe isolates that do not. The apparent low serological diversity among Florida isolates makes this possibility less likely. This combined evidence strongly suggests that the CTV-MCA13-based ELISA is a viable alternative to standard biological indexing assays for detecting severe CTV isolate infection in Florida. Application of this monoclonal antibody to testing of commercial budwood sources in Florida could greatly reduce the spread of severe isolates by propagation. Another application would be an assay to rapidly determine the cause of stunting in young citrus trees. Stunting caused by severe CTV isolates is often misdiagnosed as a horticultural problem (8).

The obvious advantages of the immunoassay over biological indexing are speed, lower expense, and suitability for large-scale tests. Biological indexing assays require establishing grafted indicator plants and maintenance of these plants for 6 to 12 months after inoculation. A minimum of five plants per assay are needed. Another advantage is the ease of interpreting immunoassay results compared to biological indexing. Biological indexing assays have employed up to nine different index host plants (4). Valid interpretation of CTV isolate severity requires familiarity with all possible CTV-induced symptoms in each of the different index host plants and a subjective evaluation of the level of symptom expression. Mild stunting effects are difficult to recognize. In the immunoassay, only a threshold value between positive and negative reactions need be established to separate mild from severe CTV isolates. Biological indexing, however, will remain the ultimate test for determining biological activity of a specific CTV isolate.

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