Emergence and Spread of Severe Strains of Citrus Tristeza Virus in the Dominican Republic

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ABSTRACT. Following the establishment of the brown citrus aphid, *Toxoptera citricida* (Kirkaldy), in the Dominican Republic, rapid diffusion of mild isolates of citrus tristeza virus (CTV) into all major commercial citrus areas was observed between 1992 and 1995. Decline and stem pitting isolates were not detected initially, but since 1996, isolates that react to the monoclonal antibody MCA-13 have been detected in several areas. An isolate causing decline in trees on sour orange rootstocks was discovered near Hato Mayor. An extensive survey of the area surrounding the original focus of infection of this decline isolate was conducted using a hierarchical sampling method. Increases in the area and the number of trees infected with decline isolates were documented. An isolate that severely affects Persian limes and causes stem pitting in grapefruit was also discovered in the Monte Plata area north of Santo Domingo. Biocharacterization tests and marker profiles using an immunocapture PCR protocol with selective primers indicated that this isolate is distinct from the decline-inducing isolate at Hato Mayor. While the mild isolate of CTV widely prevalent in the Dominican Republic appears similar to mild isolates from Florida, the MCA-13-positive isolates discovered do not have the marker profile associated with the typical Florida decline isolate T36. Hierarchical sampling methods and genotype-specific probes are being used to determine rates and patterns of movement of CTV and the probable origin of the MCA-13-positive isolates in new locations.

Early events in new epidemics of citrus tristeza virus (CTV), including rates and patterns of spread of the virus, are poorly understood. While CTV epidemics have been described in different locations (18), the presence of CTV was often not recognized until a number of trees were showing symptoms. By this time, important early details, such as the probable focus of initial infection, and spread associated with movement of infected nursery trees, were difficult to determine. Methods to rapidly identify infections in symptomless trees and to differentiate isolates were also lacking. A good opportunity to study the early phases of CTV spread in the presence of the brown citrus aphid (BrCA), *Toxoptera citricida*, has occurred in the Dominican Republic (DR). Shortly after the BrCA was established in 1992, surveys for CTV were made in the major commercial production areas (1, 5). These plantings had been established 4 to 8 yr earlier using primarily budwood sources imported from California that were virus-free. Some trees were also propagated with budwood from Florida of unknown CTV status. Most of the trees tested in 1992 were not infected. Those trees that were infected apparently carried a mild isolate (1). Repeated surveys were made in several different commercial citrus growing areas to document the rate and pattern of spread of CTV (5, 8). Rapid diffusion of CTV into commercial sweet orange plantings was observed in all areas between 1992 and 1995 while movement into grapefruit was much slower (5). None of these isolates tested reacted to the strain-selective monoclonal antibody MCA-13 (5), and no visible evidence for decline or stem pitting was found in these survey areas through 1995. Nearby Persian and Mexican lime trees were free of obvious vein clearing or stem pitting symptoms.

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A change in isolate severity was noted in 1996 with the appearance of decline symptoms in Valencia sweet orange trees on sour orange within a limited portion of a large commercial planting. This site is several km from one of our original survey locations near Hato Mayor in the eastern Dominican Republic (Fig. 1) (5). Isolates of CTV from these declining trees reacted to MCA-13, but normal appearing trees were either free of CTV or were infected with isolates that did not react to MCA-13. In addition, Persian lime trees with conspicuous vein clearing and stem pitting symptoms were discovered in the Monte Plata area north of Santo Domingo. This area, which is somewhat isolated from the major citrus areas we had previously surveyed, contains a number of small citrus plantings. Persian lime trees with conspicuous leaf symptoms reacted positively with MCA-13, and MCA-13-positive isolates were also found in nearby symptomless mandarin and sweet orange trees. These observations indicated dispersion of two distinct new isolates of CTV more severe than those detected previously. The apparent foci of these isolates were favorably located to study further local and long range movement.

Analysis of the pattern of spread of CTV in the Dominican Republic, Costa Rica, Florida, and Spain in the presence of two different aphid vectors demonstrated that the pattern of spread of CTV is influenced by the biology of the vectors (9). Aggregation of new infections near existing infected trees is common when BCA is the vector, while incidence appears more random when the melon or cotton aphid (*Aphis gossypii*) is the primary vector. While the dynamics of short range spread of CTV has been described (8, 10), information is still lacking on longer range spread of CTV by aphids between areas within large plantings, and between plantings in separate locations. It is frequently assumed that rapid spread of CTV is inevitable in the presence of the BCA, but some observations have indicated slow movement over relatively small distances between discrete plantings (4).

The original survey work in the Dominican Republic was based on individual testing of all trees in selected plots by ELISA (5). While this provided detailed information on CTV infections within these plots, surveys over larger areas were not possible with the limited technical

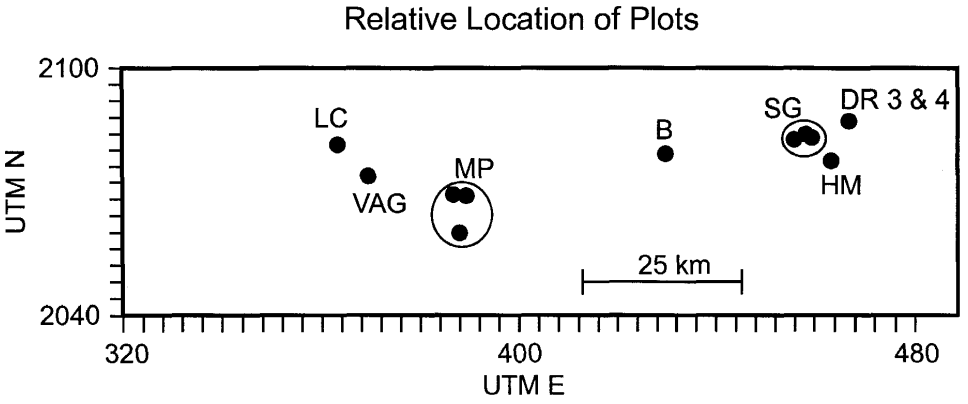

Fig. 1. Relative locations (±91 M) of test plots used to monitor spread of citrus tristeza virus in the Dominican Republic as determined by global positioning satellite (GPS) data and Universal transverse mercator (UTM) conversions. LC = Citricos La Cumbre, VAG = Villa Alta Gracia (DR1), MP = Monte Plata, B = Bayaguana, SG = Sabana Grande, HM = Hato Mayor, and DR3 and 4 = Citricos Don Juan, North of Hato Mayor.

resources available. The recent development of the hierarchical sampling (HS) method (14) provides a means to monitor changes in CTV infection within larger areas by collecting relatively moderate numbers of systematically arranged samples. It has also been adapted for survey use where non random incidence of CTV infections associated with vectoring by BrCA occurs (15). The HS method is especially useful in situations where CTV has been newly established and incidence of infection is low (6, 11, 14).

MCA-13 was used to distinguish putatively severe isolates of CTV from mild isolates of CTV in our initial studies. However, MCA-13 does not discriminate isolates that induce decline from those that cause stem pitting. A serological method to specifically identify isolates that cause stem pitting in sweet orange (OSP) has been recently described (16). Other approaches to discriminate CTV isolates based on molecular properties have also been described. These are based on differences in the conserved region of the CTV genome (2, 7) as well as differences in the more divergent 5' region of the CTV genome (12, 13).

This paper reports new information on the movement of distinct decline and stem-pitting isolates of CTV in the Dominican Republic obtained by use of hierarchical sampling methods and use of sequence-based markers to determine genetic relationships between isolates found in different hosts and locations.

METHODS AND MATERIALS

Plot locations. Test plots were located in five general locations arranged from east to west near Hato Mayor, Bayaguana, Monte Plata, Villa Altagracia, and La Cumbre. Most sites have been previously described (5), and the relative locations are shown in Fig. 1. Plot locations were mapped by global positioning satellite (GPS) instrumentation. Universal transverse mercator conversions were used to determine approximate (±91 M) distances between plots and locations. Extensive testing was done in an area near Hato Mayor called Sabana Grande that had not been surveyed previously. This area is approximately 14 km northwest of Hato Mayor, and 7 to 9 km west of two plots established as DR3 and DR4 (Fig. 1) in 1992. There are approximately 720 ha of citrus planted on rolling terrain in this location. Individual blocks are irregular in size and frequently separated for short distances by streams and/or forested areas (Fig. 2). A few declining trees on sour orange were noted in one block of trees on the southern edge of this large area in 1996. The samples collected from declining trees tested positively against MCA-13 and the whole block of trees was immediately removed by the owner. Sampling of individual trees nearby indicated that incidence of MCA-13-positive isolates in the general area was low.

The Monte Plata area lies between survey areas previously established at Villa Altagracia and Bayaguana (Fig. 1) and has not been previously surveyed. Samples were taken at six locations in the area.

Unless otherwise noted, all samples were collected from commercial plantings. Most plots were in plantings of Valencia sweet orange, but some samples were taken from grapefruit, mandarin, and Persian lime trees.

Plot design and analysis. Two types of plots were used. The first was intensively sampled plots (referred to in the remainder of the paper as IS plots) in which all trees in a 20 by 20 tree area were sampled. This is the same protocol described for an earlier survey (5). The second type was plots sampled by the hierarchical method (hereafter called HS plots) in which 25 composite samples were taken in a 20×20 tree area. Each composite sample con-

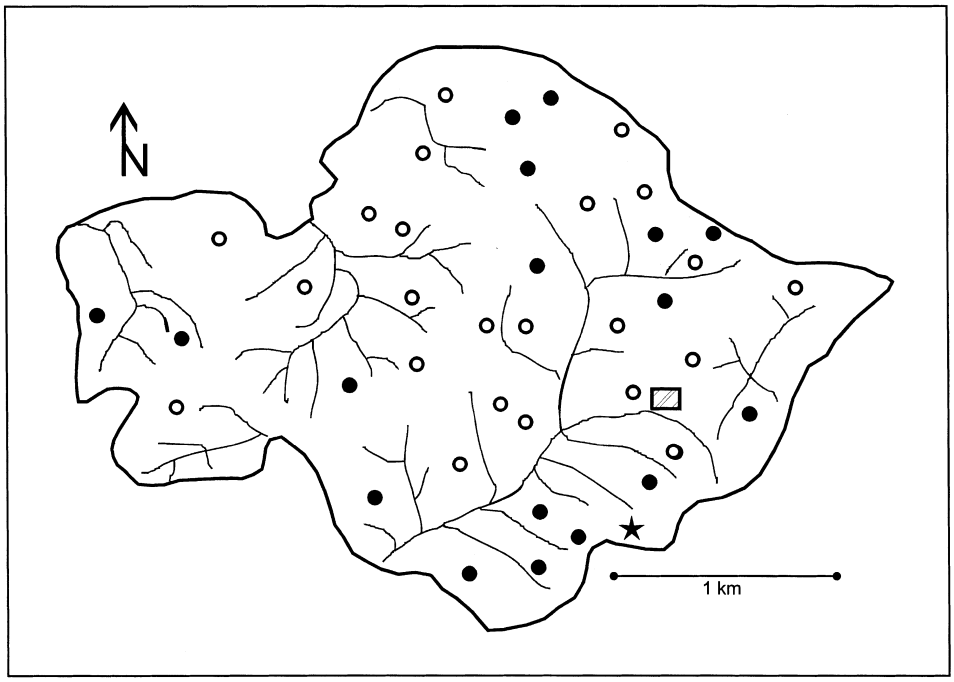

Fig. 2. Location of survey sites in the Sabana Grande area. Circles indicate location of plots sampled by hierarchical methods (HS plots). Solid circles indicate plots with trees that tested positively with MCA-13. The rectangular box indicates location of a 400 tree plot in which each tree was sampled individually (IS plot), and the star indicates the site where MCA-13-positive isolates were first discovered in the Sabana Grande area.

sisted of tissue taken from four adjacent trees in a two by two configuration. The sampling sites were arranged systematically within the 400 tree area (11, 15). Calculation of estimated infection levels on an individual tree basis in HS plots from composite sample data was as previously described. When all composites tested positive individual tree infection levels above 60% were assumed (15).

Sample collection and processing. Samples for ELISA from IS plots were collected as previously described (5), and consisted of petioles from young leaves or pedicel bark from young fruit. For HS plots, two petioles were collected from each of the four trees that comprised the sample and were pooled. Tissue samples were placed in paper envelopes and dried over silica gel (5). Samples collected for immunocapture PCR analysis were collected and processed in the same manner, except that 1 to 5 g quantities of tissue were collected from each tree. Samples for biological assay were collected as short pieces of budwood, surface sterilized, and sent to the quarantine facility at Beltsville, MD, under permit (3).

ELISA. The dried tissue samples were rehydrated in extraction buffer (PBST), pulverized with a Kleco tissue pulverizer (Kinetic Laboratory Equipt. Co. Visalia, CA 93292) and the extracts were tested in a double antibody indirect (DAS-I) ELISA, as previously described (5). A mixture of two broadly reactive monoclonal antibodies (3E10 and 11B1) was used as the intermediate antibody for general detection of all isolates (5). Extracts that tested positively were also tested using the selective Mab MCA-13 (17) as the intermedi-

ate antibody. Select samples were also tested with a combination of trapping and intermediate antibodies selective for CTV isolates that cause sweet orange stem pitting (16). The percentage of individual trees infected in the HS plots was calculated from the number of composite samples that tested positive for CTV as previously described (11, 15).

Immunocapture PCR. Samples to be evaluated for genetic grouping by PCR were extracted as indicated for ELISA and incubated in the presence of paramagnetic beads (Dynal, Inc., Lake Success, NY 11042) that had been pre-coated with CTV-specific polyclonal antibodies (12, 13). Complementary DNA (cDNA) was synthesized using random hexamers and RNA associated with virions trapped by the antibody coated beads. Analysis by PCR was done using primer pairs designed to amplify a general CTV molecular marker (the capsid protein gene) and by primers designed to amplify sequence-specific markers for different CTV molecular groups (12, 13). Isolates were differentiated by their respective marker profiles.

RESULTS

Diffusion of MCA-13-positive isolates in Sabana Grande. From June through December 1997, 43 HS plots were sampled within 1-3 km of the site where decline was first noted in 1996 (Fig. 2). In 36 of the 42 plots sampled by HS, 100% of the pooled samples tested positively against the Mab mix. Individual tree infection levels exceeding 60% were assumed for these plots (11) and calculated infection levels in the remaining plots ranged from 12 to 56%. In 25 of the HS plots, all pooled samples tested negatively against MCA-13. In 16 plots the maximum incidence of pooled samples that tested MCA-13-positive was 8% (equivalent to individual tree incidence of 2% or less). One plot had a calculated individual tree incidence of MCA-13-positive isolates of 5.4%. The distribution of HS plots containing MCA-13-positive isolates is shown in Fig. 2. In June of 1998, we sampled six HS plots in the same area. The calculated incidence of MCA-13-positive trees ranged from 3.1 to 20.5%.

An IS plot with 400 trees was established about 0.5 km north of the original decline site (Fig. 2). In June, 1997, 344 of the 400 trees tested positively for CTV infection and six tested positively against MCA-13. Five of these six trees were removed, but by June, 1998, the number of infected trees was 394 and the number of MCA-13-positive trees had increased to 20. The data from this plot indicated that the infection levels estimated from the HS plots in the surrounding area are realistic. An HS plot established several km farther north tested free of MCA-13-positive isolates both years.

A few declining trees were observed in 1997 in blocks that were adjacent to the original 1996 site of decline. By June 1998, large numbers of trees were in various stages of decline. Trees in more advanced stages of decline typically showed inverse pitting (honey combing) (19) in the inner face of the bark immediately below the budunion, and sometimes a yellowish stain was also present (Fig. 3c). No quick decline symptoms were observed. While most trees in this location are on sour orange, some blocks contain trees on Alemow rootstock. Stunted or unthrifty trees were also observed on Alemow, and these typically have extensive stem pitting in the trunk below the bud union.

MCA-13-positive isolates in prior test locations. No MCA-13-positive isolates have yet been recovered in the original DR3 and DR4 IS plots that are 5 to 8 km east of the Sabana Grande location (Fig. 1). However, MCA-13-positive isolates have recently been found in a nearby HS plot and infection levels were comparable to those in the Sa-

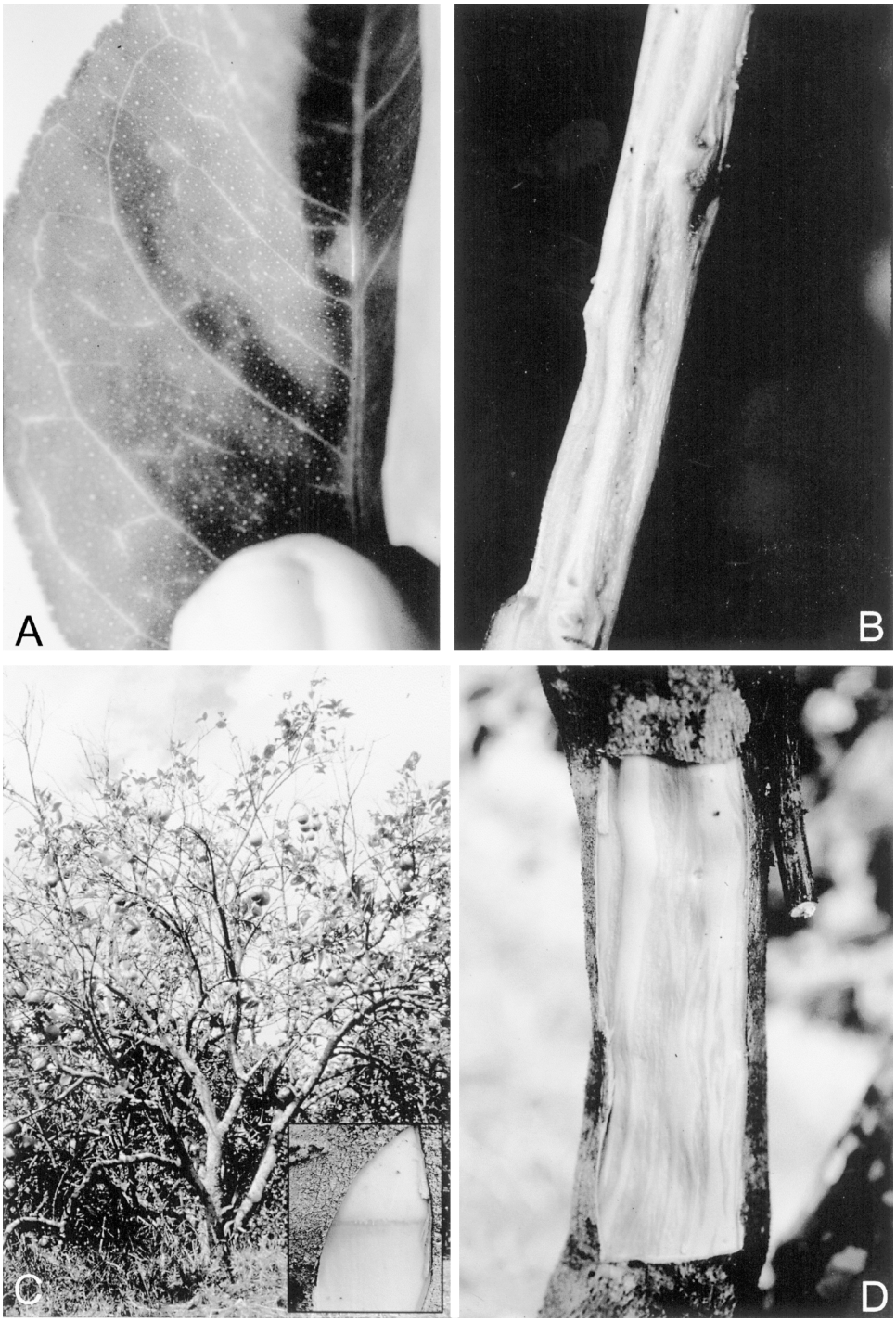

Fig. 3. Symptoms of CTV infection in different locations in the Dominican Republic A) Vein clearing in Persian lime leaf from tree in Monte Plata area. B) Stem pitting in twig from Persian lime tree at Monte Plata. C) Decline symptoms in Valencia orange on sour orange rootstock at Sabana Grande (inset shows budunion symptoms). D) Stem pitting in limb of naturally-infected red grapefruit tree in Monte Plata area.

bana Grande location. The DR4 plot has been sampled for MCA-13-positive isolates by the HS method since 1996 when the plot was essentially fully infected by mild isolates. The DR3 plot, which is in a grapefruit block, has been sampled as an IS plot continuously since 1992. The overall CTV infection level has climbed from 0.5% in 1995 to 13% in 1998, but no MCA-13-positive trees have been detected to date.

No MCA-13-positive samples were collected in the original DR2 plot at Bayaguana through 1997. Sampling in 1996 and 1997 to detect MCA-13-positive isolates was by the HS method after incidence of mild isolates exceeded 90%. An additional HS plot (HS3) was established about 1 km south of DR2 in 1997, and it also tested negatively for MCA-13-positive isolates. In June, 1998, one composite sample in DR2 and three in HS3 tested positively by MCA-13 (individual tree infection levels of 1 and 3% respectively). Neither plot is on sour orange, but trees on sour orange in nearby blocks have remained free of decline symptoms.

The DR1 plot at Villa Altagracia, approximately 45 km west of Bayaguana (Fig. 1), remained free of MCA-13-positive isolates until 1997. Two MCA-13-positive trees were discovered in the IS plot survey made in June, 1997, and seven were found in December, 1997. These infected trees were removed in the spring of 1998, but seven new infections with MCA-13-positive isolates were found in June of 1998. Three of the four HS plots established nearby in March of 1998 also contained MCA-13-positive samples and the calculated MCA-13-positive virus incidence was from 1 to 4%. An HS plot approximately 3 km west of DR2 was negative in 1997 for MCA-13-positive isolates, but had a calculated single tree infection level of 10% in 1998. HS plots at the La Cumbre location, which is 10 km north of Villa Altagracia, and separated by some hills, remained free of MCA-13-positive isolates through 1998.

Stem pitting isolates in Monte Plata. Surveys for CTV among scattered smaller commercial citrus plantings in the Monte Plata region were made for the first time in 1997. This is north of Santo Domingo and between the Bayaguana and Villa Altagracia locations (Fig. 1). Pronounced vein clearing and stem pitting symptoms were seen in Persian lime trees in six different plantings (Fig. 3a and b) and tree vigor was reduced, even though most infections were apparently relatively recent. Some unaffected trees could still be found, and growers reported that young trees obtained from nurseries in other locations grew normally when first planted and then developed symptoms. All Persian lime trees with pronounced vein clearing symptoms indexed positively by MCA-13. Most samples collected from nearby symptomless sweet orange and mandarin trees also tested positively with MCA-13. Additional surveys were made in the Monte Plata area in 1998. Stem pitting was found in both grafted and seedling grapefruit trees near affected Persian lime trees (Fig. 3). Valencia trees grafted on sour orange were found with mild stem pitting in the scion and a slight honeycombing reaction at the budunion, but no distinct decline symptoms were seen. All eight MCA-13-positive samples from the Monte Plata area that were tested using the serological test for sweet orange stem pitting isolates gave a positive reaction. Four samples infected with MCA-13-negative isolates and two infected with MCA-13-positive decline isolates did not react.

Bio-indexing results: Limited testing has been done on selected Dominican Republic isolates that had been established in the collection of exotic CTV isolates at Belts-

ville. Two MCA-13-negative isolates collected from IS test plots between 1993 and 1995 produced a mild reaction in limes and no stem pitting in orange or grapefruit seedlings. Two isolates from declining trees at Sabana Grande did not produce a strong effect on grafted combinations of sweet orange on sour orange or seedling yellows (SY) in sour orange seedlings, but produced a mild stem pitting in grapefruit. Neither isolate caused stem pitting in Madam Vinous sweet orange seedlings. Isolates from five Persian limes and one Valencia sweet orange at Monte Plata all induced a strong reaction in Mexican lime, strong stem pitting in grapefruit, and moderate to strong stem pitting in Madam Vinous seedlings. None produced strong SY in sour orange and only one isolate produced definite decline effects in grafted sweet/sour indicators. Propagations of several other isolates from this area produced stem pitting in the Madam Vinous seedlings used to maintain the isolates.

Immunocapture PCR results: Tests were run with tissue collected from infected plants established in the CTV collection at Beltsville and from field-collected tissues. Samples from test plots infected with isolates which do not react to MCA-13 yielded reaction products similar to those expected for the T30 mild isolate from Florida (14). No amplification products were obtained with T36 or T3 specific primers (see Table 1, Samples 15 to 17). Isolates from declining trees on sour orange at Sa-

TABLE 1
SUMMARY OF MARKER PROFILES OF SELECTED ISOLATES OF CITRUS TRISTEZA VIRUS FROM THE DOMINICAN REPUBLIC USING SELECTIVE PRIMERS AND IMMUNOCAPTURE PCR

			Primers[x]							
Sample	Host[z]	Location[y]	VT Pol	VT k17	T30 Pol	T30 k17	T36 Pol	T36 k17	T3 K17	MCA 13
1	P. lime	MP-1	+++	0	+	+++	0	0	+++	+
2	Grapefruit	MP-1	+++	0	0	+++	0	0	+++	+
3	Valencia	MP-1	+++	+	+++	+++	0	0	++	+
4	Mandarin 1	MP-1	+++	0	+++	++	0	0	++	+
5	Mandarin 2	MP-1	+++	0	0	++	0	0	++	+
6	P. lime	MP-2	+++	+	+++	+++	0	0	+++	+
7	P. lime	MP-3	+++	NT	+++	NT	0	0	+++	+
8	P. lime	MP-4	+++	NT	0	NT	0	0	+++	+
9	Valencia	SG-1	++	NT	+++	NT	0	0	0	+
10	Valencia	SG-2	+++	NT	+++	NT	0	0	0	+
11	Valencia	SG-3	0	0	+++	+++	0	0	0	+
12	Valencia	Sg-4	+	+	+++	+++	0	0	0	+
13	Valencia	HM-DA	+++	0	0	+++	0	0	++	+
14	Navel	HM-DA	+++	0	+++	+++	0	0	0	+
15	Valencia	DR1	0	NT	+++	NT	0	0	0	0
16	Valencia	DR2	+	NT	+++	NT	0	0	0	0
17	Valencia	DR4	0	NT	+++	NT	0	0	0	0

[z]P. lime is Persian lime, mandarin 1 and mandarin 2 are two different unidentified mandarins.
[y]MP = Monte Plata area, SG = Sabana Grande, HM-DA = nursery at Hato Mayor, DR1 = test plot at Villa Alta Gracia, DR2 = test plot at Bayaguana, and DR4 = test plot at Hato Mayor. See Fig. 1 for relative locations.
[x]Primers were designed from polymerase (pol) and k17 regions of the genomes of isolates T3, T30, T36, and VT as described by Hilf et al. (12, 13). Relative amounts of amplification product, as determined by electrophoresis of PCR reaction products, are indicated by +, ++, and +++.
0 indicates no product formed, and NT indicates no test was made.

bana Grande yielded amplification products with VT and or T30 primers, but did not yield amplification products with either T3 or T36 primers (Table 1, Samples 9 to 12).

In contrast, isolates from symptomatic Persian limes, and also from mandarin, grapefruit, and sweet orange trees in the Monte Plata area yielded a reaction product to the T3 k17 and VT pol primers (Table 1, Samples 1 to 8). Some of these samples also yielded an amplification product with T30 primers indicating a probable dual infection. No amplification product was obtained for any of the Monte Plata samples with the T36 primers. Similar reaction products were obtained from isolates from Persian lime, grapefruit, two types of mandarin, and sweet orange collected from a single location in the Monte Plata area (Table 1, Samples 1 to 5). Samples from Persian lime at four different locations in the Monte Plata area yielded similar VT and T3 primer amplification profiles (Table 1, Samples 1 and 6 to 8). Samples which gave an amplification product to the T3 k17 primer also reacted positively with an antibody combination that reacts selectively with CTV isolates that induce stem pitting in sweet orange (data not presented). Two different T3 and VT primer product profiles were seen among samples from an old Department of Agriculture nursery block near Hato Mayor (Table 1, Samples 13, 14). All samples yielded a product to VT pol, but only two samples yielded a product to T3 k17.

DISCUSSION

The results obtained indicate that following the previously reported widespread dissemination of benign isolates of CTV in commercial plantings (5), at least two new and distinctly different isolates of CTV are now also spreading into commercial plantings. Although the apparent foci of these isolates were not included in the earlier surveys, ingress of these isolates into commercial plantings has apparently been a recent event. The levels of infection at Sabana Grande are still relatively low and there was no prior history of decline of trees on sour orange in this area. The increase of decline isolates (based on MCA-13 reactivity) near the original focus discovered in 1996, and the failure to find MCA-13-positive isolates several km to the north, suggests that spread has occurred from a localized source. The original source of inoculum is unknown and occurrence of primary infections from an undetermined location outside the immediate area may also be involved. Although the MCA-13-positive isolates found in Monte Plata were already widespread when our first survey was made in that area in 1997, field observations suggest that much of this movement has occurred recently, especially in Persian lime.

Appearance of MCA-13-reactive isolates at the Bayaguana and Villa Altagracia locations during the last year is further indication of increased movement of MCA-13-positive isolates. It is unlikely that these isolates would have remained undetected during the repeated earlier surveys and no symptoms in limes in these areas were seen earlier.

Differences in symptom expression, reaction in the OSP serological test, and marker profiles obtained by IC-PCR analysis suggest that the MCA-13-positive isolates found in the Monte Plata area are different from the isolates discovered at Sabana Grande. The difference in response to the T3 k17 primer between isolates from these two locations is especially clear. The exact origin of isolates with the T3 k17 marker in the Monte Plata area remains undetermined, but the only other location where we have found a T3 k17 marker is in the old nursery site at Hato Mayor where some

imported mandarin budwood was reportedly propagated approximately 15 yr ago. These trees were reportedly destroyed because of concerns about CTV.

Marker profiles suggest that the MCA-13-positive isolates tested from five different cultivars at Monte Plata may have a similar origin. This indicates that one of these cultivars is the primary source of infection or that all have been infected by a similar isolate from an unknown source. It is unlikely that either the Persian lime or grapefruit plants were the primary source, since testing and examination of Persian lime trees in numerous locations had indicated that most trees were free of CTV or carried only a mild isolate. Grower observations that nursery trees shipped from other areas grow vigorously at first and then develop symptoms, is a further indication that the Persian lime trees were not an original source. CTV infection in grapefruit by any isolate of CTV is relatively uncommon at other locations in the Dominican Republic, and no stem pitting symptoms have been seen elsewhere.

The detection of MCA-13-positive isolates in Hato Mayor, Bayaguana, and Villa Altagracia indicates that the initial stages of natural spread of these isolates is now beginning to occur. It is unlikely that these isolates were present earlier but went undetected. Tests are in progress to determine if the MCA-13-positive isolates discovered at Bayaguana and Villa Altagracia are related to those discovered at the Sabana Grande and Monte Plata locations. While introduction of these isolates via infected nursery stock into these areas is possible, trees for the commercial plantings where the plots are located are grown on site from existing budwood source trees.

The presence of two distinct sources of challenge infection and the ability to monitor movement of these isolates into plots within an immediate area, and also between spatially well separated plantings provides a unique opportunity to learn more about patterns and rates of spread of CTV. In contrast to the situation in most areas where CTV has moved into uninfected trees, we have the opportunity to observe the pattern of super infection by a challenge isolate in trees with pre-existing infections of a mild isolate.

The results presented clearly indicate the potential usefulness of CTV group-specific primers for epidemiological applications. We were able to determine a probable difference between MCA-13-positive isolates in several locations, the relationship of isolates from different hosts at a single location and indications for mixed infections. The similarity of primer reaction between the Dominican Republic mild isolates and the Florida mild isolate T30 further supports the previous suggestion that the original source of inoculum may have originated in Florida. Failure to find MCA-13-positive isolates that were amplified by the T36 primers suggests that Florida was probably not the origin of either of the two MCA-13-positive isolates discovered. Several isolates of CTV from Japan have yielded biocharacterization, serological, and marker profiles similar to those observed with the Monte Plata isolates.

The spread of more severe isolates of CTV is a matter of concern for the citrus industry of the Dominican Republic and increased losses of trees on sour orange are anticipated. While many of the newer plantings are on decline-tolerant rootstocks, the isolates discovered in the Monte Plata area can cause significant injury to Persian lime and grapefruit plantings regardless of rootstock. Some adverse effects on fruit size may also occur in sweet orange. The increased incidence of trees infected with decline isolates at Hato Mayor over a short time frame, plus models generated from previous studies on

movement of CTV by BrCA indicate that rapid spread of these isolates is likely. Initial field observations suggest that the presence of a pre-existing infection by benign isolates has not provided useful levels of cross protection. Occurrence of super infection by new isolates over existing mild isolates is also indicated by IC-PCR analysis.

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