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The *Citrus tristeza virus* Epidemic in Bog Walk Valley, Jamaica

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ABSTRACT. In February 1997, a few trees in a grove of Valencia sweet orange on sour orange rootstock were found to be declining due to *Citrus tristeza virus* (CTV). This was the first grove in the Bog Walk Valley, Jamaica showing decline due to CTV. In the grove which originally had about 1,000 trees, 13 were dead and another 53 were in the final stages of decline. By August 1997, about 11% of the trees were dead and 5% were in the final stages of decline. By November 1997, an additional 25% of the trees had died, 43% were in the final stage of decline, and 25% were in early stages of decline. Only 38 trees in the original planting were rated as healthy; upon observation most of these trees had been unknowingly planted on CTV-tolerant rootstocks. By November 1997, isolated sweet orange trees on sour orange rootstock in neighboring groves were showing decline symptoms. In February 2000, the entire Bog Walk Valley was undergoing an epidemic of CTV decline on sour orange rootstock. Samples were collected from several declining trees in groves within 2.0 km of the original grove affected with CTV decline. The samples from the declining trees were tested by strain group specific probes (SGSP) and broad spectrum-, MCA13-, and orange stem pitting (OSP) - ELISA tests. All samples were broad spectrum and MCA13-ELISA positive, and several samples also reacted in the OSP-ELISA tests. In the SGSP tests, all isolates reacted with the universal probe for CTV, as well as several other probes indicating that the field isolates were composed of mixtures of CTV strains. Some of the SGSP probes which hybridized to the Jamaican isolates commonly cause stem pitting on grapefruit and/or sweet orange. OSP-ELISA tests also indicated the presence of CTV strains which commonly cause stem pitting in sweet orange indicator plants. *In planta* isolates are now established in the Collection of Exotic Citrus Pathogens, Beltsville, MD where further characterization will be done.

Index words. *Citrus tristeza virus*, strain differentiation, serology.

Citrus has been economically important in Jamaica. In 1967 an estimated 32,000 acres were grown in. However, there were only an estimated 24,000 acres in 1995, with about half of the total older than 9 yr (1). Until about 1996, almost all citrus was grown on sour orange (SO) or SO-type rootstocks, with some being on pummelo (shaddock) or rough lemon rootstock. Most of the older groves have 116 trees/acre, while newer plantings are at higher density, with about 232 trees/acre. The main citrus growing regions in Jamaica are St. Catherine, Manchester and St. James Parishes (1). The Bog Walk Valley is located in St. Catherine Parish.

Citrus tristeza virus (CTV) has probably been present in Jamaica for many years (6, 16). In surveys conducted early in 1993, it was found at about 15% incidence throughout all of the growing areas in Jamaica with about 10% of the

infected trees reacting with MCA13 in ELISA, suggesting the presence of strains capable of causing decline on SO rootstock (6, 16). *Toxoptera citricida*, the most efficient vector of CTV, was found in Jamaica in July 1993 (9). However through 1995, there was no evidence of trees declining on SO rootstock due to CTV (9). Growers began to question whether decline strains of CTV were really present or perhaps their SO-type rootstocks were CTV-tolerant, even though ELISA tests indicated that decline-inducing strains were probably present.

The Jamaican government, with the help of a Technical Cooperation Project (TCP) from the Food and Agricultural Organization (FAO) of the United Nations to provide technical assistance, began formulating a mandatory citrus certification program late in 1996, anticipating that losses would occur in trees on sour orange rootstock due to CTV (1). Fol-

lowing seminars early in 1997 to educate the citrus growers about the coming mandatory certification program, the first trees declining on SO rootstock due to CTV were found in one area of one grove, Enfield Block 172, located in Bog Walk Valley (7, 8).

We report here on the susceptibility of SO-type rootstocks to CTV, the progression of tree decline from the original infection focus, the economic impact that CTV has had on two farms in the Bog Walk Valley, and some of the molecular characteristics of some CTV isolates from Jamaica.

MATERIALS AND METHODS

Evaluation of rootstocks for susceptibility to CTV decline.

Seed from two SO-type rootstocks, bittersweet Seville from Jamaica and Barbour bittersweet from the Dominican Republic; Brazilian SO from California (as a true-to-type SO); and hog shaddock from Jamaica, were imported to Florida in 1993. The seed was grown and budded with Hamlin sweet orange scions when about 6 mo old. When the scions were well established, 12 budlings of each rootstock were shipped to the quarantine greenhouse of the Collection of Exotic Citrus Pathogens (CECP), Beltsville, MD (3). When the budlings were actively growing, six budlings of each rootstock were graft-inoculated with CTV isolate B18, a common CTV isolate from Brazil (collected by G. Müller) causing decline on SO and with strong seedling yellows (3). ELISA tests were performed on the new flush emerging after the graft inoculation to confirm CTV infection. The effect of CTV on the inoculated plants was evaluated 9 mo post-grafting by recording the symptoms present on the inoculated and uninoculated plants.

Surveys. Trees in Enfield Block 172 of Bog Walk Valley were rated on a scale of 0 to 3 whereby 0 = healthy appearance; 1 = off-color

green, slight leaf wilt; 2 = off-color green, twig dieback, canopy thin, small fruit; and 3 = dead or in the final stages of decline with fallen leaves but fruit remaining. The presence of decline strains of CTV was confirmed by ELISA tests using MCA13 (15).

ELISA. Double antibody sandwich indirect (DASI) ELISA assays were performed as previously described (2). Rabbit polyclonal antibodies CREC 1052 or CREC 1027 were used for coating of ELISA plates, and goat polyclonal antibody 604, made from a fusion p25 coat protein (CP) gene of CTV isolate B227 expressed in *Escherichia coli* (10), was used as the secondary antibody for broad spectrum detection (BSD)-ELISA of all CTV strains. For selective detection of severe isolates, rabbit polyclonal antibodies CREC 1052 or CREC 1027 were used for coating, with the monoclonal MCA13 as secondary antibody for MCA13-ELISA (15). Anti-goat and anti-mouse antibody-alkaline phosphatase conjugates (Sigma, St. Louis, MO) were used as tertiary antibodies, as appropriate. For the BSD- and MCA13-ELISA tests, OD_{405nm} values three times those of the healthy controls were considered to be positive. The OD_{405nm} values for the healthy controls ranged from 0.050 to 0.200. The orange stem pitting (OSP)-ELISA was performed as described by Nikolaeva et al. (11) using the polyclonal antibody system. In these tests, samples were considered to be positive for OSP if the OD values were at least 10 times the values of the control plants which were infected with a mild or a decline inducing strain of CTV (these control values ranged from 0.200 to 0.300) and if the BSD tests indicated high CTV titer in the sample.

Strain group-specific probes.

The CTV CP gene was amplified by reverse transcription (RT) PCR using RNA extracts from fresh tissue obtained by phenol/chloroform

extraction and spin-column chromatography in Sephadex G-50 as described by Pappu et al. (14) or by the immunocapture RT PCR method described by Ochoa et al. (12). The specific primers used for PCR of the CTV CP gene were (CN119 (5' AGATCTACCATGGACGACGAAACAAAG3') and CN120 (5'GAATTCGCGGCCGCTCAACGTGTGTTA AATTTCC3') (13). The CP gene PCR products were blotted onto a nylon membrane and cross-linked by UV.

Hybridization of the PCR products with the biotin labeled strain group-specific probes (SGSP) and visualization were as described by Ochoa et al. (12). The membranes were stripped and re-probed with the next SGSP probe following each hybridization, with the SGSP universal probe 0 being used first for hybridization and then tested once more, following hybridization with SGSP probes 1 through 7. This repeat hybridization with the first probe used ensured that the target DNA had remained on the membrane throughout the procedure.

RESULTS AND DISCUSSION

Rootstock susceptibility to CTV decline strains. Following the first finding of the *T. citricida* in the Caribbean Basin, workshops were held to educate farmers, nurserymen, and regulatory personnel about the expected spread of the aphid and anticipated effect of tristeza decline of trees on SO rootstock (4, 5, 6, 9). In 1993 growers in the Dominican Republic and Jamaica were convinced that their SO-type and shaddock rootstocks were tolerant to CTV-induced decline. In Jamaica, bittersweet Seville, a SO-type rootstock, was being used for some propagations, with other plants being propagated on the vigorous hog shaddock rootstock. In the Dominican Republic, Babour bittersweet was being used as a SO-type rootstock. The growers reasoned that since ELISA tests

indicated the presence of decline strains but no decline was apparent, their rootstocks must be CTV-tolerant. Seed was obtained, budlings produced and some budlings were CTV-challenged by grafting in the CECF quarantine greenhouse, Beltsville, MD. At 9 mo post-challenge, all of the CTV-infected budlings on Brazilian SO (used as the type clone of SO), Babour bittersweet, and bittersweet Seville were either showing severe dwarfing and chlorosis or had already died due to CTV-induced decline. The budlings on hog shaddock did not die, but the rootstock had severe stem pitting, and the scions were very chlorotic and stunted. The uninoculated budlings were all growing vigorously without symptoms. Thus by 1995, although there was no evidence in the field in Jamaica of CTV-induced decline on SO rootstock, it had been demonstrated that the SO-type and an alternative rootstock, hog shaddock, were susceptible to CTV-decline strains.

Increased incidence of CTV in Enfield Grove 172. The first location in Jamaica where trees on SO rootstock declined and died due to decline strains of CTV was Enfield Block 172, in the heart of Bog Walk Valley, St. Catherine Parish. Enfield Block 172 was planted with 976 Valencia sweet orange trees on SO rootstock in 1976. The trees were mapped annually beginning in 1990 using the rating of 0 to 3 described above. These data, along with the annual number of replants and average yield, expressed as 90 pound boxes/acre, are summarized in Table 1. Following the final evaluation of the block in November 1997, it was entirely replanted. Examination of the 38 apparently healthy trees in November 1997 (Table 1) revealed that 17 were on a trifoliolate-type rootstock. The others were probably on rough lemon or Cleopatra mandarin rootstock, as honeycombing was not observed on bark flaps opened immediately

TABLE 1
TRISTEZA DISEASE RATING, AVERAGE PRODUCTION (BOXES PER ACRE), AND ANNUAL NUMBER OF REPLANTS FOR ENFIELD BLOCK 172, BOG WALK VALLEY, JAMAICA

| Year | 0 Rating | 1 Rating | 2 Rating | 3 Rating | Total Replants | Yield boxes/acre |
|---------|----------|----------|----------|----------|----------------|------------------|
| 1990 | 970 | 0 | 0 | 0 | 6 | 486 |
| 1991 | 955 | 0 | 0 | 0 | 21 | 387 |
| 1992 | 949 | 0 | 0 | 0 | 27 | 313 |
| 1993 | 943 | 0 | 0 | 0 | 33 | 392 |
| 1994 | 937 | 0 | 0 | 0 | 39 | 276 |
| 1995 | 931 | 0 | 0 | 0 | 45 | 499 |
| 1996 | 875 | 50 | 0 | 0 | 51 | 493 |
| 01/1997 | 511 | 328 | 76 | 10 | * | * |
| 07/1997 | 597 | 210 | 44 | 102 | * | * |
| 10/1997 | 38 | 254 | 388 | 228 | 51 | 423 |

*Yield data for 1997 reported for 10/1997.

below the bud union. All the surviving trees were positive in MCA13-ELISA tests.

In November 1997, clusters of declining trees were apparent in groves surrounding the original block. A total of 17 samples were collected from typical declining trees in these areas. The furthest locations were approximately 2 km from Enfield Block 172. All samples collected in November 1997 were subjected to BSD-ELISA, MCA13-ELISA, OSP-ELISA, and analyzed by SGSP.

All samples were positive for CTV in BSD-ELISA, positive in MCA13-ELISA, and hybridized with Probe 0 (universal). One sample did

not react to any other probe suggesting that it may have carried a unique CP sequence not previously characterized. Two samples were positive by OSP-ELISA, and hybridized with probe 3 suggesting that the strain was B185-like (Table 2). Two samples hybridized with probes 2, 5, 6 and 7 suggesting they were a mixture of mild and decline/stem pitting strains with B53, B249, T30, and B188 as type strains (Table 2). Seven samples hybridized with probe 3 suggesting a B185-like CTV strain. Four samples hybridized with probes 2 and 3 suggesting a mixture of B53- and B185-like CTV strains. One sample hybridized with probe 1 suggesting the presence of a

TABLE 2
SUMMARY OF THE BIOLOGICAL ACTIVITY FOR THE TYPE STRAINS OF *CITRUS TRISTEZA VIRUS* DETECTED BY THE DIFFERENT STRAIN GROUP-SPECIFIC PROBES

| Strain group-specific probe | Type strain | Biological activity |
|-----------------------------|-------------|---|
| Probe 0 | Universal | Recognizes all strains of <i>Citrus tristeza virus</i> |
| Probe 1 | T36 | Decline on sour orange, seedling yellows |
| Probe 2 | B53 | Decline on sour orange, seedling yellows, stem pitting on grapefruit and sweet orange |
| Probe 3 | B185 | Decline on sour orange, seedling yellows, stem pitting on grapefruit and sweet orange |
| Probe 4 | T3 | Decline on sour orange, seedling yellows, stem pitting on sweet orange |
| Probe 5 | B249 | Decline on sour orange, seedling yellows, stem pitting on grapefruit and sweet orange |
| Probe 6 | T30 | Mild strains (from Florida) |
| Probe 7 | B188 | Mild strains from Florida and other parts of the world |

T36-like strain causing decline on sour orange without stem pitting. One sample hybridized with probe 2 suggesting a B53-like strain of CTV. One sample hybridized with probes 6 and 7 suggesting possibly a mixture of mild strains that are T30 and/or B188-like.

Thus, testing of samples collected directly from the field indicated that stem pitting strains of CTV may occur in Jamaica. *In planta* cultures from isolates collected in February 2001 have been established in the CCEP quarantine greenhouse in Beltsville, MD where their biological and molecular characteristics are being evaluated. By May 2002, stem pitting apparently due to CTV was occurring in young grapefruit trees

on CTV-tolerant rootstocks planted in the Bog Walk Valley.

Fruit yields from two farms in Bog Walk Valley. Yield losses in groves on SO rootstock in Bog Walk Valley were first apparent by the end of 1997 and worsened in subsequent years. The expected and actual fruit yields for two large farms, Enfield Farm with 541 acres and New Works Farm with 618 acres, in Bog Walk Valley are summarized in Table 3. Yield is based on standard 90 pound boxes. While the exchange rate of the Jamaican dollar to the U.S. dollar has varied, as well as the average price per box of citrus, for this comparison we have valued a box of fruit at US\$4.35 and used an exchange rate

TABLE 3
SUMMARY OF YIELD, EXPECTED YIELD, AND ECONOMIC LOSSES DUE TO *CITRUS TRISTEZA VIRUS* (CTV) FOR TWO FARMS IN BOG WALK VALLEY, JAMAICA

| Year | Acres | Expected yield/acre | Actual yield/acre | Total expected production | Total actual production | Boxes lost due to CTV | Jamaican dollars lost to CTV |
|-----------------|-------|---------------------|-------------------|---------------------------|-------------------------|-----------------------|------------------------------|
| Enfield Farm: | | | | | | | |
| 1991 | 526 | 71 | 71 | 37,210 | 37,210 | 0 | 0 |
| 1992 | 541 | 75 | 75 | 40,700 | 40,700 | 0 | 0 |
| 1993 | 541 | 75 | 75 | 40,606 | 40,606 | 0 | 0 |
| 1994 | 541 | 79 | 79 | 42,862 | 42,862 | 0 | 0 |
| 1995 | 541 | 143 | 143 | 77,226 | 77,226 | 0 | 0 |
| 1996 | 541 | 241 | 241 | 130,557 | 130,557 | 0 | 0 |
| 1997 | 541 | 300 | 219 | 162,300 | 118,281 | 44,019 | 8,803,800 |
| 1998 | 541 | 350 | 189 | 189,350 | 102,245 | 87,105 | 17,421,000 |
| 1999 | 541 | 350 | 189 | 189,350 | 102,281 | 87,069 | 17,413,800 |
| 2000 | 541 | 350 | 118 | 189,350 | 63,634 | 125,716 | 25,143,200 |
| 2001 | 541 | 350 | 80 | 189,350 | 43,508 | 145,842 | 29,168,400 |
| | | | | | | 489,751 | 97,950,200 |
| New Works Farm: | | | | | | | |
| 1991 | 547 | 218 | 218 | 119,267 | 119,267 | 0 | 0 |
| 1992 | 548 | 300 | 300 | 164,269 | 164,269 | 0 | 0 |
| 1993 | 618 | 312 | 312 | 192,648 | 192,648 | 0 | 0 |
| 1994 | 618 | 217 | 217 | 134,001 | 134,001 | 0 | 0 |
| 1995 | 618 | 366 | 366 | 226,342 | 226,342 | 0 | 0 |
| 1996 | 618 | 394 | 394 | 243,422 | 243,422 | 0 | 0 |
| 1997 | 618 | 400 | 314 | 247,200 | 193,908 | 53,292 | 10,658,400 |
| 1998 | 618 | 425 | 300 | 262,650 | 185,539 | 77,111 | 15,422,200 |
| 1999 | 618 | 425 | 312 | 262,650 | 192,656 | 69,994 | 13,998,800 |
| 2000 | 618 | 425 | 219 | 262,650 | 135,066 | 127,584 | 25,516,800 |
| 2001 | 618 | 425 | 106 | 262,650 | 65,247 | 197,404 | 39,480,600 |
| | | | | | | 525,384 | 105,076,800 |



Fig. 1. Four-year-old trees on sour orange rootstock at the New Works Farm, Bog Walk Valley, Jamaica, undergoing decline due to *Citrus tristeza virus* in February 2001.

of 46 Jamaican dollars to 1 US dollar. The expected yield is based on past performance for the variety considering age, weather, and soil type. In February 2001, it was evident that New Works Farm where the trees were younger, was losing trees rapidly due to CTV. Few trees showing symptoms of CTV would survive until harvest (Fig. 1). At Enfield Farm where the trees were older before CTV decline commenced, losses were also high but in some instances the trees looked as if they might survive until the fruit could be picked. By 2001, the total loss at Enfield Farm was US\$2,129,352 while that at New Works Farm was US\$2,284,278. In total for the 1,159 acres of citrus at both farms, the accumulated loss due to CTV in November 2001 was US\$4,413,630.

Other farms located in Bog Walk Valley have incurred similar losses. The epidemic of CTV-induced decline on SO rootstock has now spread to the citrus-producing areas of the central valley and the northern coast in Jamaica.

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