The Citrus Tristeza Situation in Central America[1]

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ABSTRACT. A survey was made in four Central American countries for citrus tristeza virus (CTV) and *Toxoptera citricida*, its efficient aphid vector. Using ELISA, CTV was detected in samples from Costa Rica, El Salvador and Nicaragua, but not from the single location sampled in Panama. *T. citricida* was identified in samples from Costa Rica, El Salvador and Panama, but not from Nicaragua. Previously CTV and *T. citricida* both were reported from Honduras. Serological analysis of CTV isolates from Costa Rica suggested that they were severe strains. The widespread presence of CTV and *T. citricida* throughout Central America poses a serious potential threat to the sour orange rootstock-based citrus industry of the region, and, ultimately, to Mexico and the United States.

Index words. Citrus tristeza virus, *Toxoptera citricida*, Latin America.

Citrus tristeza virus (CTV) is the most destructive virus disease of citrus (1, 3, 5). It occurs in most citrus growing areas of the world and has been especially destructive in Africa, Argentina, Brazil, Spain, the United States and Venezuela (1, 3, 4, 5). CTV was introduced into Argentina from Africa in the 1920's, and severe losses were incurred in 1930 in plantings of sweet orange trees on sour orange rootstock (3, 5). The presence of this scion/rootstock combination and *Toxoptera citricida* Kirk., the efficient aphid vector of CTV (8), facilitated the spread of CTV to Brazil and neighboring countries, where it caused the death of millions of citrus trees in the 1940's and 1950's (1, 5). In Venezuela, CTV was reported in 1960 in a citrus collection. It remained restricted there until 1975, when *T. citricida* appeared from neighboring countries (7). A destructive outbreak of CTV occurred in Venezuela in 1980, necessitating the replanting of a major portion of the citrus acreage (7). CTV also is widespread and serious in Colombia (9).

Citrus is becoming an increasingly important crop in Central America. Citrus acreage has been expanding rapidly to meet increased demands for local consumption and for export in response to recent freezes in the citrus growing regions of the United States. Much of the citrus in Central America is less than 10 years old, and it is expected that the acreage will increase rapidly during the next decade. Most of the citrus in Central America is on sour orange rootstock, making the plantings highly susceptible to CTV. These circumstances and recent reports of tree death in the major citrus growing region of Costa Rica prompted us to survey the region for the presence of CTV and *T. citricida*.

MATERIALS AND METHODS

Samples were collected from commercial citrus groves and from citrus germplasm collections at national experiment stations in Costa Rica, El Salvador, Nicaragua and Panama, with 15, 7, 10 and 1 sampling locations, respectively. Samples were taken from field trees with tristeza-like symptoms and at random in the germplasm collections to cover all possible sources of materials. Young flushes from opposite sides of the tree were collected and stored in plastic bags at 4C until processed.

CTV was detected by the double antibody sandwich method of the enzyme linked immunosorbent assay (ELISA) (2), using a commercially available kit (AGDIA, Inc. Elkhart, IN) or reagents kindly provided by Dr. R. F. Lee. Leaf or young bark samples were ground in a mortar and

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pestle at a 1/20 (wt/vol) dilution in 0.5M Tris-HCl buffer, pH 8.0. Samples (200 µl) were placed in previously coated plates which were incubated and rinsed and then successively reacted with the CTV IgG-alkaline phosphatase conjugate and the p-nitrophenyl phosphate substrate. Plates were read visually or with a spectrophotometer at 410 nm. Known CTV-free and infected tissue samples were included as controls. Samples with ELISA readings three times those of healthy tissues were considered positive for CTV.

Samples from known CTV-infected trees in Costa Rica were analyzed by western blotting with antibodies capable of distinguishing mild and severe CTV isolates (10, 11). A 1-cm square piece of young bark was finely diced with a scalpel and placed in a 1.5 ml microfuge tube containing 0.5 ml of extraction buffer [62.5 mM Tris buffer, pH 6.8, containing 2% sodium dodecyl sulfate (SDS) 10% glycerol and 5% β mercaptoethanol]. Tubes were closed, heated in boiling water for 90 sec and cooled to room temperature. Aliquots of 10-20 µl were subjected to electrophoresis on a 12% polyacrylamide gel containing 2% SDS, electroblotted to nitrocellulose and successively probed with a monoclonal antibody, MCA13 (kindly provided by Dr. T. A. Permar), which reacts predominantly with severe strains of CTV and a polyclonal antibody which reacts with mild and severe strains of CTV (10, 11). Tissues known to be CTV-free and infected with mild or severe strains of CTV were included as controls.

While sampling for CTV, various aphids on citrus were collected in vials containing a 70% ethanol solution. In the laboratory the aphids were mounted on glass slides, examined microscopically, and compared with photographs of known citrus colonizing aphid species for identification.

RESULTS

Samples collected in 33 locations in Central America were assayed for CTV by ELISA. Of 484 samples, 90 were found to be infected (Table 1, Fig. 1). In Costa Rica, CTV was detected at the experiment station and in two groves in San Carlos, the major citrus producing region adjacent to Nicaragua. In El Salvador, CTV was detected at the experiment station and in one grove. In Nicaragua, CTV was detected only at the experiment station. CTV was not detected in 10 samples from a single location in the major citrus growing area of Panama.

Bark samples from four known CTV-infected trees in Costa Rica were analyzed by western blotting. All four samples reacted strongly with the MCA13 monoclonal antibody and with the polyclonal antibody, indicting that all are probable severe strains. Biological assessments are in progress.

Toxoptera citricida was found in Costa Rica, El Salvador and Panama,

TABLE 1
DETECTION OF CITRUS TRISTEZA VIRUS AND *TOXOPTERA CITRICIDA* IN CENTRAL AMERICA

Country surveyed	Hectarage of citrus (approx.)	No. sites sampled	Detection of CTV by ELISA[z]	Detection of *T. citricida*
Costa Rica	5,000	15	74/290	+
El Salvador	4,000	7	14/91	+
Nicaragua	7,000	10	2/93	–
Panama	3,000	1	0/10	+
TOTALS	19,000	33	90/484	

[z]Number of positive samples/number of samples tested.

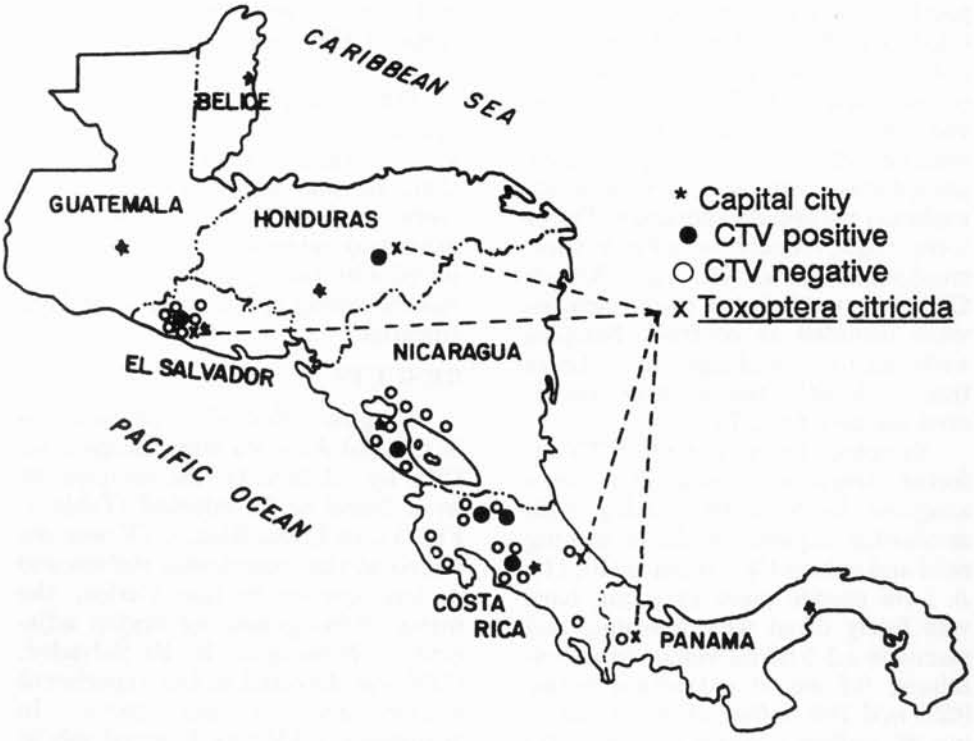

Fig. 1. Map of Central America showing the countries surveyed and the distribution of citrus tristeza virus (CTV) and *Toxoptera citricida*. Data for Honduras are from reference 6.

but not in Nicaragua. However, sampling was performed during the rainy season when aphid populations are greatly reduced. Other aphids found colonizing citrus were *Toxoptera aurantii*, *Aphis gossypii* and *Pentalonia nigronervosa*, all of which occurred in much greater abundance than *T. citricids*.

DISCUSSION

Citrus tristeza virus was detected in Costa Rica, El Salvador and Nicaragua, but not in the single location sampled in Panama. Previously CTV was reported in Honduras (6). Incidence in Costa Rica was widespread, but incidence in El Salvador and Nicaragua was restricted to or in the vicinity of experiment stations. Thus caution must be observed in these countries when selecting domestic budwood sources for propagation to meet the increasing need for citrus trees. Further analysis of four samples from Costa Rica indicated infection by severe strains of CTV.

Toxoptera citricida was found to occur in Costa Rica, El Salvador and Panama, but it was not found in Nicaragua. Previously it was reported in Honduras (6). Thus, in limited surveys. CTV and its efficient aphid vector, *T. citricida*, have each been found in four of five contiguous countries of Central America. Since most of the citrus trees in these countries currently are grafted on sour orange rootstock, this poses a serious potential threat to citrus production in these countries, and ultimately in Mexico and the United States, if *T. citricida* becomes widespread as occurred previously in South America.

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