Incidence of Brown Citrus Aphid and Citrus Tristeza Virus in Puerto Rico

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ABSTRACT. When the brown citrus aphid, *Toxoptera citricida* (Kirkaldy), was detected in Puerto Rico in 1992, citrus tristeza virus (CTV) incidence in field and dooryard citrus was low. Approximately 5% of the trees surveyed in 1992 gave a positive test in DAS-I ELISA and only 1.4% reacted with MCA13, a severe-strain discriminating monoclonal antibody. By 1995, incidence of CTV-infected trees had increased to 58% and the proportion MCA13 positive reached 26%. CTV was also detected in some budwood source trees and in nursery propagations. MCA13-negative and -positive isolates showed different patterns in single-stranded conformational polymorphism analysis of RT/PCR products from the p18 genome region of CTV. Glasshouse experiments confirmed single aphid transmission rates of 70% for isolate B14 from Brazil and 44% for isolate B23 from Israel. A one-year profile of winged aphid activity in a citrus plot at Isabela, Puerto Rico, indicated a spring and fall peak of aphid flight; however, additional smaller peaks, associated with citrus shoot growth, were observed for the brown citrus aphid. The brown citrus aphid was caught in low levels (1%) compared to the spirea aphid (56%) and the melon aphid (26%). Aphid colony counts on trees, however, showed that the spirea aphid and the brown citrus aphid were the principal colonizers. These data indicate that CTV in Puerto Rico has increased rapidly after the establishment of the brown citrus aphid and that at least two strains of CTV are being spread.

Index words. Epidemiology, ELISA, virus surveys, vector transmission, aphid traps.

Severe strains of citrus tristeza virus (CTV) vectored by the brown citrus aphid, *Toxoptera citricida* (Kirkaldy), caused decline and death of millions of citrus trees on sour orange rootstock during the 1930's and 1940's in Argentina and Brazil (15) and during the 1970's in Venezuela (12). Additional losses were sustained due to stem pitting in sensitive cultivars such as Pera sweet orange (Osb. (4). Until recently, however, citrus in Central America and the Caribbean islands has not been affected by CTV. Although several unverified reports indicated that the brown citrus aphid was collected many years ago in Bermuda (22), Trinidad (6), and the West Indies (24), it is apparent that widespread dissemination has occurred only in

recent years. Lastra et al. (16) reported finding reproducing populations of the brown citrus aphid on citrus in Costa Rica, El Salvador, and Panama in 1989. Subsequently, surveys were conducted in Central America and the Caribbean islands for presence of the brown citrus aphid and for incidence of CTV (17, 18). The brown citrus aphid was first detected in the Caribbean islands in 1992 (2, 28). Soon it was found on nearly all islands except the Bahamas. Information provided by growers and pest detection services indicated that these infestations were apparently the result of recent introductions. We now presume that earlier reports of the aphid in the Caribbean region were either from populations that did not become permanently established or were misidentified as suggested by Stoetzel and Hillman (23). The brown citrus aphid was not detected in Puerto Rico in 1990 in a thorough survey of the island, but by May 1992, it was present in high populations. This

paper reports results of surveys and experiments to evaluate the impact of the brown citrus aphid on incidence of CTV in Puerto Rico.

MATERIALS AND METHODS

Aphid surveys and identification. Suspect brown citrus aphids encountered in commercial groves, nurseries, and dooryards were collected in 70% alcohol and sent to the first author for identification. The melon aphid, *Aphis gossypii* Glover, and the spirea aphid, *A. spiraecola* Patch, were identified under magnification without clearing or mounting. Others, including the brown citrus aphid, were processed and identified as previously described (28).

CTV surveys. Since citrus is grown throughout Puerto Rico, all regions were included our survey (Fig. 1). In general, trees were sampled annually and were from the same groves or sites but not necessarily from the same tree from year to year. Citrus trees suspected of being on sour rootstock were examined for CTV decline symptoms including stunting, chlorosis, wilting and honeycombing at the budun-

ion. Mexican lime trees were examined for vein clearing, leaf cupping, and stem pitting. Bark was peeled from stems of sweet orange, grapefruit, and Chironja (grapefruit hybrid) to look for stem-pitting symptoms.

Citrus tissue samples for serological assays were collected from field and nursery trees and processed as previously reported (9, 28). Composite samples were taken in some groves where CTV incidence was expected to be low or absent; these consisted of one leaf petiole from each of 5-10 trees combined as one sample.

Samples of citrus tissue were assayed for presence of CTV by enzyme-linked immunosorbent assay (ELISA). Double antibody sandwich-indirect (DAS-I) ELISA (using a polyclonal trapping antibody and a mixture of broad spectrum monoclonal antibodies) or DAS ELISA (using the polyclonal antibody 1052 for trapping and conjugate) were used for detection of all isolates (3, 8, 9). Samples which tested positive for CTV were then tested with the severe-strain discriminating monoclonal antibody, MCA13, in DAS-I ELISA as a pre-

Distribution of Citrus Tristeza Virus in Puerto Rico
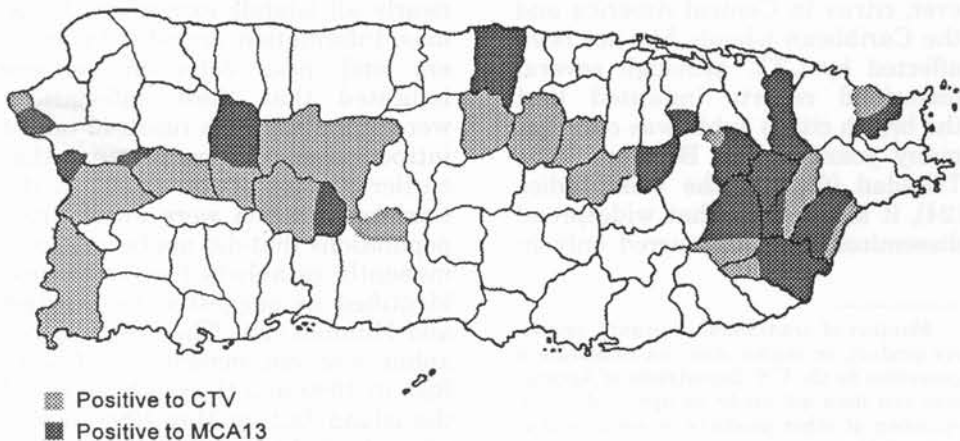

▨ Positive to CTV
▩ Positive to MCA13

Fig. 1. Map of Puerto Rico with generalized location of mild (MCA13- negative) and potentially severe (MCA13-positive) strains of CTV based on serology as of July 1995.

sumptive diagnosis for potentially severe strains of CTV (20).

CTV isolate characterization. Three MCA13-positive source plants from Puerto Rico were graft inoculated into CTV indicator hosts in a quarantine glasshouse in Beltsville, MD to determine isolate severity (11). Additionally, four MCA13-negative and two MCA13-positive trees from Isabela, Puerto Rico, were tested by reverse transcription polymerase chain reaction (RT/PCR) using p27 and p18 region primers of the genome of T36 isolate of CTV (19). RT-PCR products were examined electrophoretically for single-stranded conformation polymorphism (SSCP) (7).

Vector transmission efficiency. Brown citrus aphids from Puerto Rico were reared and tested for CTV transmission efficiency in quarantine at the Foreign Disease-Weed Science Research Unit, USDA-ARS, Ft. Detrick, Frederick, MD. Experimental conditions were as previously described (28). A highly efficient vector population of brown citrus aphid from Hawaii was used as a standard for comparison. B14, a stem pitting isolate from Capão Bonito, Brazil, and B23, a seedling yellows isolate from Israel (10), were the CTV isolates used for the transmission experiments. Transmission efficiencies were tested using 1, 5, and 10 aphids per Mexican lime receptor plants. Each treatment had either 5 or 10 replications and single aphid transmission (SAT) rates were calculated by the formula $P = 1 - (1-I)^{1/k}$ where P = probability of SAT; I = proportion of infected plants; and k = number of vectors per plant (28).

Estimation of aphid activity. Winged aphid activity was monitored by utilizing a panel (10.8 × 10.8 cm) made from yellow acrylic (#2037 Farco Plastics, Orlando, FL) to attract aphids into a water tray (25, 26). A total of 9 yellow traps were placed at mid-canopy height (approximately 0.75 m) in the center of 6 296-tree plots in Isabela, PR; 3 plots each had 3 traps and 3 plots each had one trap. Traps were serviced 5 times per week. Visual inspection of 20 trees per plot was conducted biweekly and aphid species and colony size were determined from October 1994 through November 1995.

RESULTS

Aphid surveys. USDA-APHIS-PPQ inspectors in Puerto Rico were alerted to search for brown citrus aphids in April 1992 following a report by Aubert et al. (2) that the aphid had recently been found in several other Caribbean islands. The inspectors immediately found aphids that were identified as brown citrus aphid. Immediately thereafter, a survey was conducted to determine the extent of brown citrus aphid infestation and incidence of CTV. The survey indicated that the aphid was widespread and was the predominant aphid colonizing citrus on the island. The brown citrus aphid was also found colonizing acerola or West Indian Cherry. Searches of azalea and Surinam Cherry, failed to reveal brown citrus aphid colonies even though these non-citrus plants were nearby citrus plants with large populations of the aphid (28).

CTV surveys. In 1992, the incidence of CTV among 221 field and dooryard citrus trees in Puerto Rico was determined to be 5% and 1.4% of these trees tested positive with MCA13 (Table 1). CTV-infected trees were found among limited samples from a single nursery. From 1993 to 1995, incidence of CTV increased to approximately 58% and MCA13-reactive trees increased to 26%, based on samples from 1,818 trees. CTV incidence among 2,000 trees from citrus nurseries in 1995 was 38%, and 3.3% of the trees were MCA13 positive. The general distribution of CTV in Puerto Rico follows the hilly central belt across the

TABLE 1
CTV SURVEYS IN COMMERCIAL CITRUS GROVES AND DOORYARDS IN PUERTO RICO

| Sample period | | Proportion of trees infected[z] | |
	n^y	% + CTV	% + MCA13
1992	221	5.4	1.4
1993	1,180	14.7	6.8
1994	177	33.9	—[x]
1995	240	57.9	26
Total	1,818		

[z]Enzyme-linked immunosorbent assay (ELISA) was used for determine CTV infection. General CTV detection was done with polyclonal or a mixture of monoclonal antibodies (3); MCA13 was used as a severe-strain discriminating monoclonal antibody (20).
[y]n = number of trees sampled and examined for CTV infection by ELISA.
[x]Data not taken.

island were most commercial citrus is grown and MCA13-positive strains of CTV were more widespread in the eastern region (Fig. 1).

CTV isolate characterization. In the glasshouse, two of the three isolates tested caused mild decline on sweet orange trees on sour orange rootstock and induced some stem pitting in grapefruit seedlings; whereas one isolate caused no symptoms in sweet orange trees on sour orange rootstock or in grapefruit. The PCR products from the 6 isolates tested showed similar SSCP pattern for the p27 genomic region, but 2 different patterns for the p18 genomic region. The four MCA13 negative isolates showed a pattern similar to the T30 mild isolate from Florida, and the 2 MCA13-positive isolates had a different SSCP pattern (data not shown).

Vector transmission. Brown citrus aphids from Puerto Rico transmitted both B14 and B23 CTV isolates with high efficiency. Single aphid transmission rates for the Puerto Rican populations were 70% for isolate B14 and 44% for isolate B23, while comparable values for the Hawaiian populations were 85% and 64%, respectively (Table 2). The low SATs for B23 with five aphids and for B14 with 10 aphids are apparent abnormalities.

Aphid counts. Only 1% of the winged aphids caught in yellow traps at Isabela were brown citrus aphids (Table 3). The most frequently trapped aphids trapped were spirea aphid (56%) and melon aphid (29%). Other aphids trapped included poplar aphids, *Pemphigus* sp. Hartig, (4%), corn leaf aphids, *Rhopalosiphum maidis* (Fitch) (4%), and cowpea aphids, *Aphis craccivora* Koch, (1%). Only a few black citrus aphids, *T. aurantii* (Boyer de Fonscolombe) (0.1%) were collected. There was a small peak of total aphid flight activity in March and a larger peak in September-October (Fig. 2). Small peaks of brown citrus aphid flights were observed from winter-spring and in fall when citrus flush was more abundant. Spirea aphid colonies comprised 57% of total; the brown citrus aphid accounted for 31%; melon aphids 11%; and black citrus aphids 0.3%.

DISCUSSION

Since 1989, there has been an ingress of the brown citrus aphid into the southern portion of Central America and most of the islands in the Caribbean including Puerto Rico (2, 16, 28). The aphid's establishment in Puerto Rico probably occurred in 1991. The exact time of

TABLE 2
COMPARISON OF TRANSMISSION EFFICIENCIES FOR TWO ISOLATES OF CTV BY
BROWN CITRUS APHIDS FROM PUERTO RICO AND HAWAII[z]

No. aphids per plant	n^y	CTV isolate					
		B14			B23		
		Hawaii	Puerto Rico		Hawaii	Puerto Rico	
		SAT[x]	SAT	Avg.[w]	SAT	SAT	Avg.
1	5	40	60	50	20	20	20
5	10	100	100	100	37	13	25
10	10	100	21	61	100	100	100
20	5	100	100	100	100	—	—
Avg.[v]		85	70	78	64	44	61

[z]Tests were conducted under glasshouse conditions at Frederick, MD. Donor plants were sweet orange and receptor plants were Mexican lime (27). CTV isolates used in these experiments were B14 and B23 (10).
[y]n = Number of test plants.
[x]SAT = Single aphid transmission (SAT) rates were calculated by the formula $P = 1 - (1-I)^{1/k}$ where P = probability of SAT; I = proportion of infected plants; and k = number of vectors per plant (27). Test aphids were originally from Hawaii or Puerto Rico.
[w]Avg. = Average transmission efficiency in the row is combined for aphid populations per isolate.
[v]Avg. = Average transmission efficiencies in a column are combined for aphid all levels per aphid per isolate.

the aphid's establishment in Puerto Rico is unknown. Although it was not found during extensive surveys in May 1990, detection of colonies was widespread by May 1992. We did not find the aphid on non-rutaceous hosts reported elsewhere (1, 5).

The low incidence (5%) of CTV in Puerto Rico in 1992 is consistent with incidences reported for citrus in other Caribbean countries during this time period (2, 16, 28). The rapid increase in incidence of CTV after introduction of the brown citrus aphid agrees with our laboratory data showing high vector efficiency for CTV by the aphid population from Puerto Rico. The SAT efficiencies determined for the Puerto Rican source of the brown citrus aphid agree with those previously reported (28). The rate of spread of CTV in Puerto Rico is comparable with those being observed in the Dominican Republic (9), Costa Rica (13), and other areas where brown citrus aphid is established (14). CTV incidence in Puerto Rico observed in

1994-95 corroborates data published by Rodríguez and Escudero (21).

Although no tristeza decline has been observed, the increase of MCA13-positive trees indicate that decline or stem pitting strains of CTV may be spreading in Puerto Rico. Biological characterization tests at Beltsville indicate that some Puerto Rican isolates do cause CTV decline and some stem pitting in grapefruit (11), however, the observed reactions are relatively mild compared to isolates from many other countries. Since CTV-infected budwood source trees and nursery stock were found in Puerto Rico as early as 1992, spread has occurred both by the aphid and by propagation. The increase in CTV incidence, however, cannot be attributed only to propagation since the survey trees where infection has increased were planted prior to 1992.

Puerto Rico is an excellent study site for research on CTV disease dynamics. The brown citrus aphid is

TABLE 3

APHID SPECIES DETECTED BY TWO METHODS IN A YOUNG CITRUS GROVE IN ISABELA, PUERTO RICO

Species	Common name	Percent of total number	
		Alates in yellow traps[z]	Colonies on trees[y]
Aphis craccivora	Black legume or cowpea aphid	1	0
A. gossypii	Melon or cotton aphid	29	11
A. spiraecola	Spirea or green citrus aphid	56	57
Lipaphis erysimi	Turnip or mustard aphid	2	0
Pemphigus spp.	Poplar-lettuce aphid	4	0
Rhopalosiphum maidis	Corn leaf aphid	4	0
Toxoptera citricida	Brown citrus aphid	1	31
T. aurantii	Black citrus aphid	0.1	0.3
Other aphids	—	3	0

[z]Data from 9 traps collected January to December, 1995.
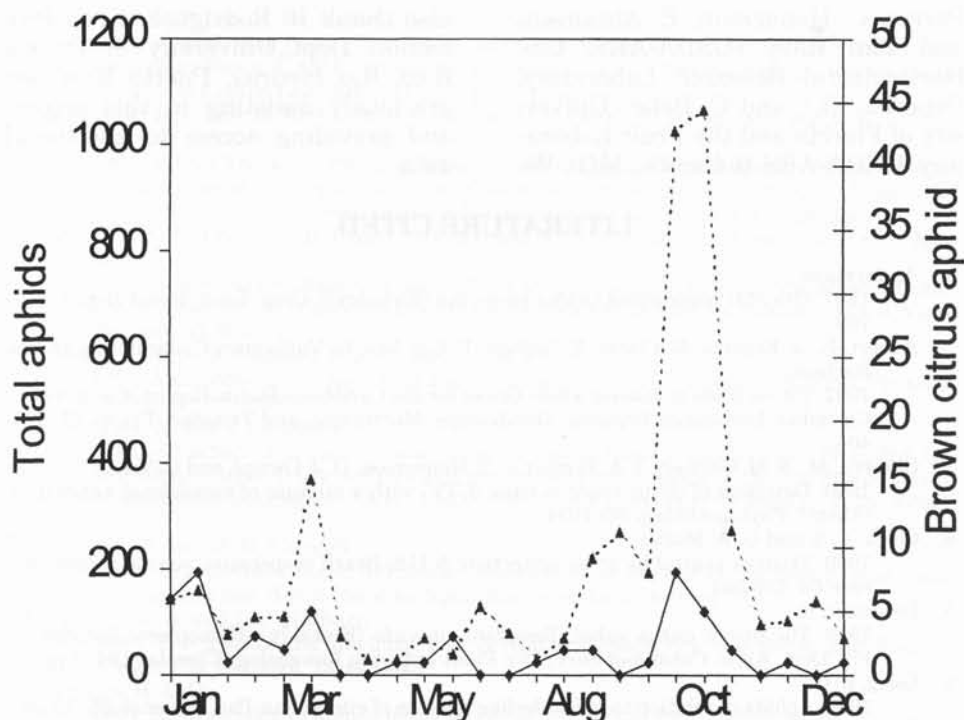[y]Data from October, 1994 to November, 1995.

Fig. 2. Total seasonal catches of winged aphids in yellow water traps in a citrus plot in Isabela, Puerto Rico. Aphids collected from January to December, 1995. Legend ---▲--- Total aphids; ___♦___ Brown citrus aphid.

present in areas with different climatic conditions. At least two isolates of CTV are present whose movements can be traced with differential serological or molecular probes. Sites with abandoned citrus plantings and areas with little or no citrus are available. Plots were established to evaluate the increase and spread of the disease relative to colonization and seasonal winged activity of the brown citrus aphid and the effect of vector control strategies on movement of CTV. The low proportion of the yellow trap catches of the brown citrus aphid in relation to numbers actually colonizing citrus in our plots may indicate some limitations on the use of traps in assessing relative population levels of brown citrus aphid. For example, both the spirea aphid and the melon aphid have large host ranges and likely have natural populations which are greater than brown citrus

aphid in the environment. Furthermore, our plot was in a windy location and the trees were young and isolated from other citrus. In a commercial citrus planting, the brown citrus aphid is expected to be the most abundant aphid as was previously observed (27, 28). Continuation of this study should provide insights on the dynamics of CTV spread and help determine the most accurate methods to measure vector populations relative to disease progress.

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