Effects of Chemical Control of Aphid Vectors and of Cross-Protection on Increase and Spread of *Citrus tristeza virus*

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ABSTRACT. The recent spread of the brown citrus aphid *Toxoptera citricida* throughout Florida and the Caribbean has raised interest in the possibilities of reducing rate of spread of severe *Citrus tristeza virus* (CTV) through insecticidal control and cross protection. In order to evaluate these practices, six, 280-tree plots were established at the University of Puerto Rico farm at Isabella, to study the effect of various chemical control strategies on the spread of CTV where *T. citricida,* and other aphid vectors are present. Plots were established with healthy nursery trees, with a line-source of CTV-infected trees at one end of each plot to serve as inoculum sources. Tree disease status was determined prior to planting and assayed annually throughout the experiment by DAS-I ELISA. Planting materials were obtained locally and CTV-infected trees were determined to be of two isolate serotypes (positive or negative for antibody MCA13). Plots were untreated or treated with either Acephate or Imidacloprid. Aphid populations were visually counted on each tree and yellow traps were used to estimate flying aphid populations. CTV increase (the number of trees infected through time) and spread (the relative distances between infected trees and their spatial pattern) and aphid population dynamics were monitored for 5 yr from 1994 to 1999. Results indicated that insecticidal control of aphid populations with either Acephate or Imidacloprid had little effect on CTV increase and spread. In addition, Imidacloprid appeared to cause a secondary outbreak of citrus red mite, *Panonychus citri* (McGregor) and may also have exacerbated damage from the root weevil *Diaprepes abbreviatus* L. Acephate appeared to greatly reduce *Diaprepes* damage to roots and foliage, and thus promoted more vigorous trees that flushed more often. In addition, high winds associated with tropical storms occurred and plots next to a windbreak were also more vigorous. Faster-growing trees were more attractive to aphid vectors and resulted in a more rapid CTV increase in Acephate-treated plots. *T. citricida*, *Aphis gossypii*, and other vector species were noted either in colonies on the trees or in water traps. Sufficient migratory aphids apparently visited each plot, and transmitted CTV regardless of chemical treatment. Although aphid colonization was suppressed to some extent by chemical treatments, feeding activity by migratory aphids even on chemically treated trees occurred prior to death, and seemed to be sufficient for CTV acquisition and transmission. Thus, in these experiments, there was little benefit of vector population suppression via chemical control on CTV increase. The effect of cross-protection, by pre-immunization with local mild CTV isolates was also examined in the plots. Cross-protected plots did not demonstrate any inhibition of the increase or spatial spread of decline of CTV compared to non-protected plots.

Index words. Latency, inoculation, serological assay, vector chemical control, comparative epidemiology, cross protection.

The most efficient vector of *Citrus tristeza virus* (CTV), *Toxoptera citricida*, spread throughout the Caribbean and into Florida during the mid and late 1990s (27, 29). The vector is known to cause rapid increase and spread of CTV within a few years of its introduction (27, 29). In previous studies, CTV was monitored for 4 yr using monoclonal antibody probes and ELISA in four citrus orchards in northern Costa Rica and four orchards in the Dominican Republic following the introduction of *T. citricida* (9, 10, 15, 17).

It is well known that in spite of chemical control, viruliferous aphids can land on uninfected trees, probe and transmit the virus in relatively short feeding periods before the chemical kills the vector. What is not well understood is the effect of treating a citrus orchard with insecticides on CTV increase and spread. Traditionally, the effect of chemical control of vectors and/or their effect on virus populations has been studied by simply comparing statistically the effect of various chemical treatments relative to controls on

the vector populations and number of infected trees at points in time, often at the end of the growing season. A more informative approach is to examine the effect of various treatments on the population dynamics of vectors and the dynamics of spatial and temporal increase of virus infected trees through time.

The Gompertz nonlinear model, an asymmetrically sigmoid model, was selected in previous studies as the most appropriate in most cases to describe temporal increase of CTV (1, 6, 14, 15). More complex temporal models have also been described for situations where roguing of infected trees was used as a control strategy (7, 25). Various statistical assays are also used to examine the spatial arrangement of infected trees and can be applied to CTV to determine if various treatments affect spread and spatial patterns of the virus.

In previous studies 'Ordinary runs' analysis for association of CTV-infected trees failed to show a spatial relationship of virus status among immediately adjacent trees within- or across-row (15, 16, 17, 18). However, we can also look at associations of infected trees at larger spatial scales. The beta-binomial index of dispersion allows us to examine associations among groups of trees, and in previous studies has suggested aggregations of CTV-positive trees for various quadrat sizes for all plots within quadrat sizes tested (15, 16, 17, 18).

We can also look at associations of infected trees over longer distances via spatial autocorrelation analysis. Spatial autocorrelation (5) of proximity patterns has suggested that aggregation often exists among quadrats of various sizes up to four lag distances (distances between 2×2 groups of trees); however, significant lag positions discontinuous from the main proximity pattern were rare. Some asymmetry was also detected for some spatial autocorrelation proximity patterns (15). These results were interpreted to mean that although CTV-positive trees did not often influence the status of immediately adjacent trees, virus transmission was common within a local area of influence that extended two to eight trees in all directions. Where asymmetry was indicated, this area of influence was somewhat elliptical. The spatial and temporal analyses gave some insight into possible underlying processes of CTV spread in the presence of *T. citricida* and suggested CTV spread was predominantly to trees within a local area (15).

Patterns of longer distance spread were not detected within the confines of the plot sizes tested (15). Stochastic spatio-temporal models have been used to examine and differentiate the effects of local versus longer-distance sources of CTV on virus spatial patterns through time in pathosystems where *T. citricida* was or was not present (11, 12, 13, 18). Longer distance spread probably exists but may well be of a complexity beyond the detection ability of the spatial analysis methods employed or perhaps on a scale that is larger than the dimensions of the plots studied.

Methods for quantitative analysis of spatial patterns at a single point in time take advantage of the binary (i.e., presence or absence of CTV) data generated by ELISA methods. 'Ordinary runs' is a unidirectional analysis that can be used to assess aggregation within columns or rows in a population matrix of diseased plants (24). The beta-binomial discrete distribution is the most appropriate distribution to examine spatial patterns of disease incidence of binary data for the presence of aggregation within quadrats of different sizes (19, 20, 23).

The goal of the present study was to determine if chemical control of aphid vectors can reduce the rate of virus increase and spread of CTV in citrus plantings. In addition, we wanted to compare the rate of

increase and patterns of spread of mild (MCA13-negative) versus decline (MCA13-positive) strains of CTV and examine decline virus in a mild-isolate cross-protected plot to test the effect of cross-protection relative to chemical control using local CTV isolates from Puerto Rico.

MATERIALS AND METHODS

Plot design. Six 210-tree plots were established at the University of Puerto Rico farm at Isabella, with plot design, orientation, inoculation and treatments as summarized in Fig. 1. Plots were established with healthy, i.e., CTV-negative, nursery trees, Valencia sweet orange on Carrizo rootstock, with the addition of CTV-positive trees in specific locations to serve as inoculum sources. Tree status was determined prior to planting and assayed annually throughout the experiment by DAS-I ELISA. A line-source of infected trees, composed entirely of either mild- or decline-infected trees, was planted at one end of each plot (Fig. 1). Planting materials were obtained locally and CTV-infected trees were determined using a monoclonal antibody mixture (28). Decline-type isolates were differentiated from mild-type isolates by their DAS-I ELISA reaction to monoclonal antibody MCA13 (3, 26).

Insecticide treatments. Plots were untreated or treated as shown in Figure 1, as per manufacturer's recommendations with either Acephate (Orthene® 75 S/WSP) [O,S-dimethyl acetylphosphoramidothioate] a broad-spectrum organophosphate (Valent USA, Walnut Creek, CA] at 0.84 kg (ai)/ha (1.0 lb

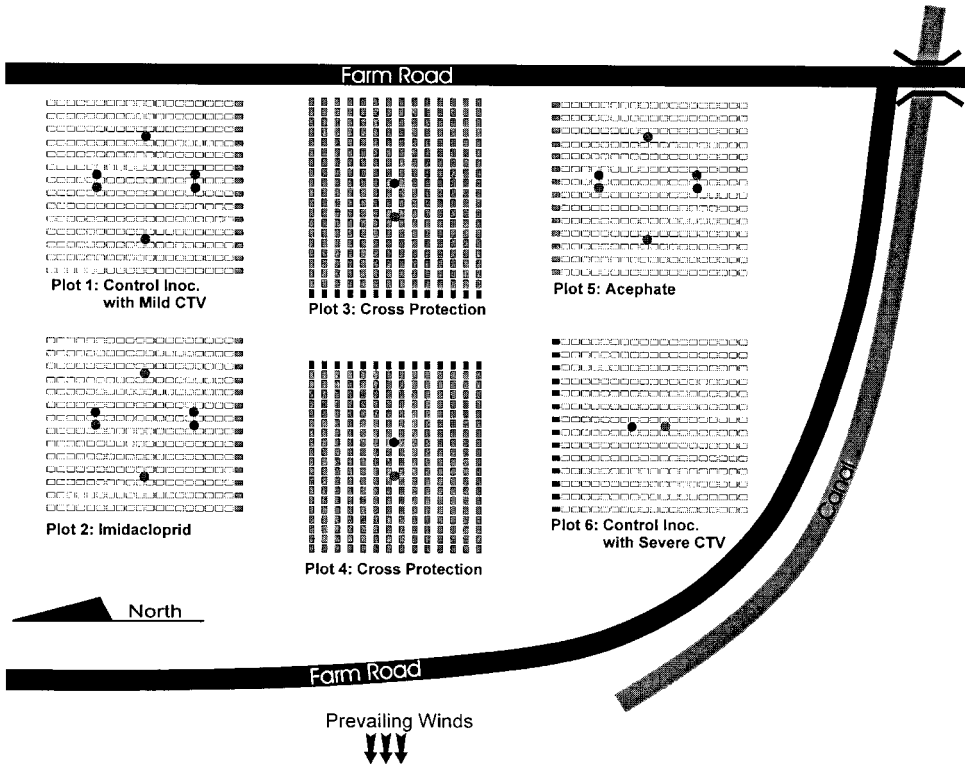

Fig. 1. Map of the experimental plots. Rectangles indicate relative placement of trees: White = not initially infected, Gray = infected with local mild CTV isolate, Black = infected with local decline (MCA13+) CTV isolate. Circles indicate relative positions of vector water-pan traps: Gray = yellow traps, Black = green traps.

product per acre) every 2-3 weeks or Imidacloprid (Admire® 2F) [1-{(6-Chloro-3-pyridinyl)methyl}-2-imidazolidinone], a selective neonicitinoid compound, manufactured by Bayer CropScience Research, Triangle Park, North Carolina as a soil drench of 1.13 g AI/1.8 l water per tree with four applications the first year and two applications annually in subsequent years.

Sample collection. The increase and spatial spread of both CTV-isolate types were monitored from 1994 through 1999 by sampling and assaying all individual trees in each plot multiple times. Samples from each tree consisted of four young leaf petioles taken from young, nearly fully expanded leaves around the periphery of the tree. The four petioles from each tree were placed in a number-coded paper envelope and 20 individual envelopes corresponding to one row of trees were placed in sealable plastic bags, to which was added ca. 50 g silica gel, changed as needed to insure that the specimens dried completely. The dry samples were then transported to the USDA-ARS laboratory in Florida for processing.

ELISA. The four leaf petioles of each sample were placed in 5 ml of PBS-Tween buffer and pulverized for 30 sec in a Kleco tissue pulverizer. Extracts were assayed for CTV by double sandwich indirect (DAS-I) ELISA (2, 8). Isolates were differentiated into two serogroups, designated here as mild, i.e., non-decline-inducing, and potentially decline-inducing, based on reaction to two monoclonal probes. One was a mixture of the monoclonal antibodies 11B1 and 3E10, which in combination detect all known isolates of CTV (3, Garnsey et al. unpublished)). The second probe was monoclonal antibody MCA13, which reacts to the majority of decline-inducing and stem-pitting isolates of CTV, including those in Florida and Puerto Rico, but does not react to mild isolates found in Florida or Puerto Rico (9, 16). Maps were prepared for each plot by assessment date for total CTV-positive trees and for MCA13-positive trees only. Spatial and temporal analyses were conducted to determine the dynamics of virus spread.

Spatial analysis. To interpret the relationships among CTV-positive trees, the data were examined at two hierarchical levels: between adjacent individual trees and within quadrats. Ordinary runs analyses were performed on each data set to determine if aggregation existed between adjacent CTV-positive trees within- and/or across-rows with the use of a Visual Basic EXCEL macro (15, 24, Gottwald, unpublished software). A nonrandom pattern (i.e., aggregation) of CTV-positive trees was assumed for a particular row if the observed was less than the expected number of runs at $P = 0.05$.

To examine the data for the presence of aggregation at different spatial scales, the CTV incidence data from each block were partitioned into 2 × 2 quadrats with the use of a Visual Basic EXCEL macro (Gottwald, unpublished software). Aggregation within-quadrat was assessed via beta-binomial analysis. For the beta-binomial index (I_β), a large I_β (>1) combined with a small P (< 0.05) suggests aggregation of diseased trees (19, 23). The line source of inoculum trees was not included in the spatial or temporal assessments.

Temporal analysis. The virus incidence (number of CTV-infected trees divided by the total number of trees in the plot) of each plot was calculated for each year. The increase in virus incidence for all isolates and for MCA13-positive isolates was estimated by linear regression analysis of transformed disease incidence data.

The appropriateness of each model was determined by examining the coefficient of regression, the correlation coefficient of observed

vs. predicted values, and the plots of standardized residual values vs. predicted values. When the overall most appropriate linear model was selected, the data were then fitted by non-linear regression to the non-linear form of the model for predictive purposes (4, 21). Nonlinear regression analysis of nontransformed data from each plot was performed for the nonlinear form of the logistic models (SAS NLIN procedure using the DUD option, SAS Institute, Inc., Cary, NC, USA: version 6.04). General model types were selected initially on the shape of the disease progress curve. Models were further evaluated for the highest coefficient of correlation and were chosen as superior if no patterns were found in the residual plots (21, 22). CTV increase among plots was compared via *t*-test of nonlinear rates of virus increase of the most appropriate model to determine if there were significant differences in virus increase relative to host, virus isolate (all CTV and MCA13-positive) and location (22).

Vector population assessments. Two yellow (attractant) water pan traps were used to estimate aphid activity in each plot. Yellow traps present a spectral reflectance that is attractive to some aphid species but not all (Yokomi, unpublished) and are valuable only to monitor yearly changes in flight activity of aphids. The traps were examined weekly and aphids were identified to species level where possible. In addition, new flush was examined every 2 weeks to count the number of aphid colonies established in trees in each plot.

RESULTS

Vector populations. Traps attracted most aphids during the first year; then catches gradually became less, as the plants grew and became more attractive to the aphids, the traps becoming a relatively smaller target (Fig. 2). More than 30 species of aphids were captured during the first year but the most prevalent was *Aphis spiraecola*. The population proportions of the most abundant species were; *A. spiraecola* 62%, *A. gossypii* 23%, *T. citricida* 1%, and *T. aurantii* 0.1%).

T. citricida populations on new shoots were abundant in all plots except for the Imidacloprid plot (Fig. 3). The vector populations on new shoots increased through time as the trees established a larger canopy (Fig. 4). Chemical control did suppress *T. citricida* populations. The percentage of new citrus shoots infested with *T. citricida* populations increased in 1997 and 1998 with the exception of the Imidacloprid-treated plot. However, the two chemical control plots did show reduced numbers of aphid colonies. Unfortunately, during the experi-

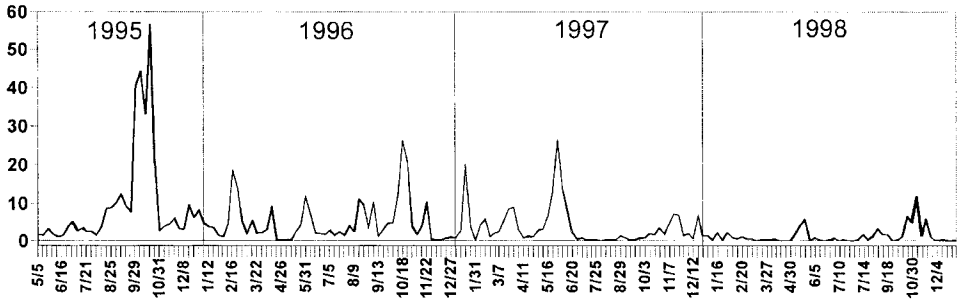

Fig. 2. Average number of alate aphids per week (all species) captured in water pan traps in the experimental plots. The traps attracted more aphids during the first year; then catches grew less, as more plant material grew and became more attractive to the aphids than the traps.

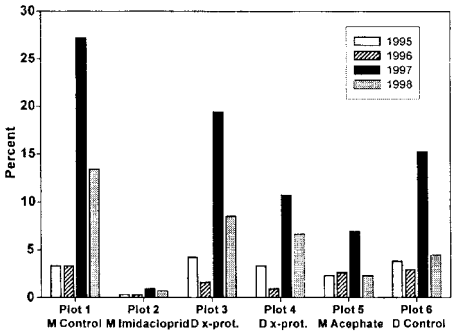

Fig. 3. Yearly percentage of new shoots infested by *Toxoptera citricida* in the experimental plots, by treatment. *T. citricida* populations on new citrus shoots were prevalent in all plots except for the Imidacloprid plot. The greatest number of *T. citricida* colonies occurred in 1997. NTN = Imidacloprid. M = plot established with CTV-free trees, then challenged with mild-type CTV isolate; D = plot established with CTV-free trees, then challenged with decline-type CTV isolate; and x-prot = cross-protected plot, i.e., plot initially established with mild-type isolate infected plants and challenged through time with decline-isolate inoculum from line source of infected trees.

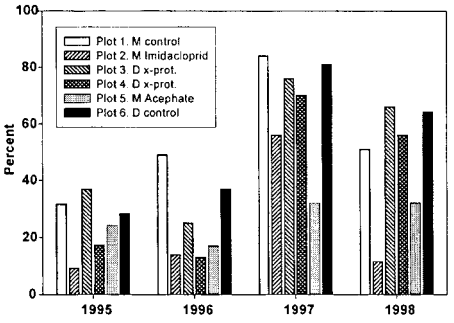

Fig. 4. Yearly percentage of new shoots infested with *Toxoptera citricida* in the experimental plots. *T. citricida* populations on new citrus shoots increased through time as the trees established a larger canopy. Chemical control did depress *T. citricida* populations. M = plot established with CTV-free trees then challenged with mild-type CTV isolate; D = plot established with CTV-free trees then challenged with decline-type CTV isolate; and x-prot = cross-protected plot, i.e., plot initially established with mild-type isolate infected plants and challenged through time with decline-isolate inoculum from line source of infected trees.

ment, *Diaprepes* weevil heavily infested the plots and likely caused a general reduction in tree growth. Weevil populations were heavy because the Isabella station is on land previously planted to sugarcane that had been infested with the weevil. In addition, the one plot treated with Imidacloprid became heavily infested with red mites, *Panonychus citri*, and plants had reduced growth and vigor.

Spatial Analyses. Ordinary runs for all CTV isolates combined resulted in very little indication of aggregation within or across rows, i.e., at the individual adjacent tree scale, and beta-binomial analyses resulted in even less indication of aggregation at the group scale (Tables 1 and 2). Ordinary runs for MCA13-positive isolates also indicated little aggregation within or across rows. However, beta-binomial analyses resulted in more indication of aggregation at the group scale, i.e., groups of four trees in quadrats compared to all CTV isolates combined (Tables 1 and 2).

Temporal Analyses. Of the linear models tested, data for all CTV isolates combined was best represented by the Logistic model, $dy/dt = rLy(1-y)$, although the Gompertz model $dy/dt = rGy(-\ln(y))$ was a close second (Table 3). This trend was also shown by data for MCA13-positive isolates, that is, the Logistic model was the best overall fit. However, the Exponential model, $dy/dt = rEy$, and the Gompertz model, were close second and third choices, respectively. The logistic model was chosen to make comparisons among treatments, and the non-linear form of the logistic model was used for predictive purposes. Because the logistic model was chosen to make comparisons among treatments, the non-linear form of the logistic model was also fitted to the data (Table 4).

Comparison of logistic rates of increase for all virus isolates com-

TABLE 1
ORDINARY RUNS AND BETA BINOMIAL INDEX OF DISPERSION (I) ANALYSES FOR ALL ISOLATES OF *CITRUS TRISTEZA VIRUS* (CTV)

Plot	Date	Disease incidence	Ordinary runs[a]				Beta Binomial[b]	
			Row	Column	Row (all)	Col (all)	I_β	P
1	Feb 95	0.0414	1/8	0/6	R	R	1.525	0.040
	July 95	0.0608	1/12	0/9	R	R	1.241	0.060
	Jan 96	0.0608	1/12	0/9	R	R	1.241	0.060
	July 96	0.0772	1/14	0/11	R	R	1.114	‘0.078
	Feb 97	0.1163	0/17	0/13	R	R	1.207	0.115
	Nov 97	0.7093	2/18	5/14	N	N	1.398	0.722
	July 98	0.9609	1/7	1/5	R	N	1.563	0.963
	June 99	0.9922	0/2	0/2	R	R	0.951	0.992
2	Feb 95	0.0263	0/7	0/6	R	R	0.929	0.028
	July 95	0.0321	0/6	0/7	R	R	0.970	0.034
	Jan 96	0.0444	0/8	0/9	R	R	0.924	0.047
	July 96	0.0526	0/9	0/11	R	R	1.052	0.056
	Feb 97	0.0857	0/12	0/14	R	R	0.847	0.091
	Nov 97	0.2571	0/18	0/14	R	R	1.275	0.261
	July 98	0.6368	2/19	0/14	N	N	1.235	0.636
	June 99	0.8087	0/17	0/10	R	N	1.207	0.808
3	Feb 95	0.8985	0/14	0/12	R	R	1.312	0.901
	July 95	0.9084	0/12	0/12	R	R	1.311	0.917
	Jan 96	0.9080	0/12	0/12	R	R	1.318	0.911
	July 96	0.9055	0/12	0/12	R	R	1.304	0.909
	Feb 97	0.9091	0/12	0/12	R	R	1.048	0.913
	Nov 97	0.9605	0/6	0/5	R	R	1.252	0.962
	July 98	0.9880	0/3	0/3	R	R	1.030	0.987
	June 99	1.0000	N/A	N/A	N/A	N/A	N/A	1.000
4	Feb 95	0.9286	0/12	0/13	R	R	0.768	0.925
	July 95	0.9506	0/10	0/9	R	R	0.838	0.948
	Jan 96	.09540	0/9	0/9	R	R	0.872	0.951
	July 96	0.9614	0/9	0/7	R	R	0.896	0.959
	Feb 97	0.9608	0/9	0/7	R	R	0.947	0.959
	Nov 97	0.9647	0/8	0/7	R	R	1.252	0.962
	July 98	1.0000	N/A	N/A	N/A	N/A	N/A	1.000
	June 99	1.0000	N/A	N/A	N/A	N/A	N/A	1.000
5	Feb 95	0.0752	0/11	0/12	R	R	0.903	0.071
	July 95	0.0792	0/12	0/13	R	R	0.999	0.075
	Jan 96	0.1057	0/16	0/14	R	R	1.014	0.103
	July 96	0.1477	0/17	0/14	R	R	1.129	0.147
	Feb 97	0.2235	1/19	0/14	R	R	1.024	0.223
	Nov 97	0.3939	1/19	1/14	R	R	0.981	0.382
	July 98	0.7786	0/17	0/14	R	R	1.007	0.767
	June 99	0.9542	0/9	0/7	R	R	1.027	0.952
6	Feb 95	0.0188	0/5	0/4	R	R	0.954	0.020
	July 95	0.0340	0/9	0/7	R	R	0.903	0.036
	Jan 96	0.0377	0/10	0/7	R	R	1.102	0.029
	July 96	0.0528	0/11	0/8	R	R	0.991	0.056
	Feb 97	0.1208	1/17	1/13	R	R	1.095	0.115
	Nov 97	0.4642	1/19	0/14	R	R	1.117	0.468
	July 98	0.9042	1/13	1/11	N	R	1.236	0.911
	June 99	0.9579	1/6	0/7	N	R	1.082	0.956

[a]Values shown for each plot represent the number of rows with significant aggregation ($P = 0.05$) over the total number of rows tested within each row or column (across row). Not all rows or across rows had CTV within the row, and thus were not tested. Row (all) and Col (all) tests were conducted by treating the planting as a single long row. N = nonrandom and R = random.

[b]Index of dispersion (I_β) and associated probability (P) values for plots infected with CTV, where I_β = observed variance/binomial variance and P = probability. P-values were calculated by comparison of df × I_β with the chi-squared distribution. Values of I_β not significantly different from 1 ($0.95 > P > 0.05$) indicate that the pattern of diseased trees is indistinguishable from random. A large (>1.0) I_β and a small P (≤0.05) suggest rejection of H_0: random pattern of virus-infected trees, in favor of H_1: aggregated pattern of virus-infected trees.

TABLE 2
ORDINARY RUNS ANALYSIS AND BETA BINOMIAL INDEX OF DISPERSION (*I*) ANALYSES FOR DECLINE-INDUCING ISOLATES OF *CITRUS TRISTEZA VIRUS* (CTV)

Plot	Date	Disease incidence	Ordinary runs[a]				Beta binomial[b]	
			Row	Column	Row (all)	Col (all)	I_β	*P*
1	Feb 95	0.0000	N/A	N/A	N/A	N/A	N/A	0.000
	July 95	0.0000	N/A	N/A	N/A	N/A	N/A	0.000
	Jan 96	0.0000	N/A	N/A	N/A	N/A	N/A	0.000
	July 96	0.0000	N/A	N/A	N/A	N/A	N/A	0.000
	Feb 97	0.0233	0/6	0/5	R	R	1.021	0.025
	Nov 97	0.0659	0/12	0/9	R	R	0.949	0.070
	July 98	0.1992	1/17	1/14	N	N	1.647	0.210
	June 99	0.4766	2/19	3/14	N	N	1.390	0.484
2	Feb 95	0.0000	N/A	N/A	N/A	N/A	N/A	0.000
	July 95	0.0000	N/A	N/A	N/A	N/A	N/A	0.000
	Jan 96	0.0000	N/A	N/A	N/A	N/A	N/A	0.000
	July 96	0.0000	N/A	N/A	N/A	N/A	N/A	0.000
	Feb 97	0.0082	0/2	0/2	R	R	1.059	0.009
	Nov 97	0.0163	0/4	0/4	R	R	1.162	0.018
	July 98	0.0556	0/9	0/10	R	R	1.316	0.056
	June 99	0.1304	0/15	0/12	R	R	0.917	0.129
3	Feb 95	0.0226	0/4	0/4	R	N	1.289	0.024
	July 95	0.0267	0/5	0/4	R	R	1.210	0.028
	Jan 96	0.0268	0/5	0/4	R	R	1.206	0.028
	July 96	0.0394	0/7	0/6	N	N	1.267	0.041
	Feb 97	0.0435	0/8	0/7	N	N	1.212	0.045
	Nov 97	0.0830	0/11	0/10	N	R	1.209	0.086
	July 98	0.1928	0/17	2/14	R	R	1.316	0.056
	June 99	0.4280	1/19	5/14	R	R	1.464	0.431
4	Feb 95	0.0150	0/4	0/2	R	R	1.483	0.016
	July 95	0.0152	0/4	0/2	R	R	1.471	0.016
	Jan 96	0.0153	0/4	0/2	R	R	1.463	0.016
	July 96	0.0154	0/4	0/2	R	R	1.455	0.016
	Feb 97	0.0157	0/4	0/2	R	R	1.437	0.016
	Nov 97	0.0471	0/9	0/7	R	R	1.425	0.045
	July 98	0.3640	0/19	0/13	R	N	1.514	0.360
	June 99	0.4656	2/19	1/14	R	N	1.272	0.458
5	Feb 95	0.0075	0/2	0/1	R	R	0.992	0.008
	July 95	0.0075	0/2	0/1	R	R	0.992	0.008
	Jan 96	0.0075	0/2	0/1	R	R	0.992	0.008
	July 96	0.0189	0/4	0/4	R	R	0.950	0.020
	Feb 97	0.0303	0/7	0/7	R	R	0.925	0.028
	Nov 97	0.0682	0/13	0/11	R	R	0.792	0.068
	July 98	0.1985	1/18	1/14	R	R	1.142	0.197
	June 99	0.3511	0/19	1/14	R	N	1.455	0.350
6	Feb 95	0.0038	0/1	0/1	R	R	1.004	0.004
	July 95	0.0038	0/1	0/1	R	R	1.004	0.004
	Jan 96	0.0038	0/1	0/1	R	R	1.004	0.004
	July 96	0.0075	0/2	0/2	R	R	0.992	0.008
	Feb 97	0.0264	0/5	0/6	R	R	0.967	0.016
	Nov 97	0.0755	0/14	1/11	R	R	0.924	0.067
	July 98	0.3372	1/19	1/14	R	N	1.091	0.334
	June 99	0.5556	0/19	1/14	N	R	1.135	0.556

[a]Values shown for each plot represent the number of rows with significant aggregation ($P = 0.05$) over the total number of rows tested within each row or column (across row). Not all rows or across rows had CTV within the row, and thus were not tested. Row (all) and Col (all) tests were conducted by treating the planting as a single long row. N = nonrandom and R = random.

[b]Index of dispersion (I_β) and associated probability (P) values for plots infected with CTV, where I_β = observed variance/binomial variance and P = probability. P-values were calculated by comparison of df × I_β with the chi-squared distribution. Values of I_β not significantly different from 1 ($0.95 > P > 0.05$) indicate that the pattern of diseased trees is indistinguishable from random. A large (>1.0) I_β and a small P (≤0.05) suggest rejection of H_0: random pattern of virus-infected trees, in favor of H_1: aggregated pattern of virus-infected trees.

TABLE 3
RESULTS OF LINEAR REGRESSION OF TRANSFORMED DATA ON *CITRUS TRISTEZA VIRUS* (CTV) INCREASE IN PLOTS IN ISABELA, PUERTO RICO

Isolate	Plot	Model	Parameter estimate	Standard error	Adjusted R^2	ρ	Residual pattern
All CTV	1	Exponential (E)	0.00235	0.00032	0.8861	0.86282	+, -
		Monomolecular (M)	0.00299	0.00061	0.7661	0.66780	+
		Logistic (L)	0.00535	0.00073	0.8840	0.96434*	+
		Gompertz (G)	0.00389	0.00066	0.8292	0.90577	+
	2	E	0.00240	0.00020	0.9555	0.95164	+, -
		M	0.00097	0.00022	0.7333	0.81866	+
		L	0.00337	0.00034	0.9336	0.98469*	+, -
		G	0.00181	0.00028	0.8590	0.95932	+
	5	E	0.00179	0.00011	0.9770	0.96960	+, -
		M	0.00167	0.00040	0.7034	0.79127	+
		L	0.00347	0.00040	0.9122	0.97179*	+
		G	0.00238	0.00042	0.8198	0.93544	+
	6	E	0.00278	0.00025	0.9444	0.88120	+, -
		M	0.00199	0.00043	0.7452	0.74589	+
		L	0.00477	0.00049	0.9313	0.98514*	+, -
		G	0.00299	0.00047	0.8502	0.94414	+, -
MCA13+	1	E	0.00361	0.00029	0.9807	0.99219	-
		M	0.00034	0.00010	0.6220	0.81086	+
		L	0.00435	0.00018	0.9952	0.99862*	-
		G	0.00194	0.00019	0.9705	0.99682	+
	2	E	0.00339	0.00034	0.9706	0.99339	-
		M	0.00008	0.00002	0.6661	0.84232	+
		L	0.00355	0.00034	0.9729	0.99493	-
		G	0.00105	0.00011	0.9665	0.99831*	-
	3	E	0.00186	0.00020	0.9260	0.98633*	+
		M	0.00028	0.00008	0.6359	0.82198	+
		L	0.00214	0.00026	0.9023	0.97740	+
		G	0.00087	0.00015	0.8207	0.94442	+
	4	E	0.00241	0.00050	0.7642	0.93611	+, -
		M	0.00037	0.00010	0.6544	0.80244	+
		L	0.00279	0.00059	0.7558	0.94643*	+, -
		G	0.00112	0.00026	0.7199	0.94065	+, -
	5	E	0.00272	0.00021	0.9605	0.98650	-
		M	0.00024	0.00006	0.7161	0.86003	+
		L	0.00296	0.00023	0.9579	0.99282*	-
		G	0.00100	0.00012	0.9109	0.98325	+, -
	6	E	0.00365	0.00034	0.9439	0.95621	+, -
		M	0.00045	0.00012	0.6703	0.81796	+
		L	0.00410	0.00038	0.9421	0.98236*	+, -
		G	0.00147	0.00021	0.8755	0.97886	+

Adjusted coefficients of correlation of observed versus predicted values (R2) and rates of virus increase (b) were estimated by linear regression of transformed disease incidence over time. Disease incidence values were transformed by, ln(y), ln(1/(1-y)), ln (y/(1-y)), and -ln(-ln(y)) for exponential, monomolecular, logistic, and Gompertz transformations, respectively. Correlation coefficients (ρ) of predicted values against observed values and the presence or absence of patterns in the plots of residual values were examined for appropriateness of models.

bined were made via *t*-test using model parameters determined by nonlinear regression of the logistic model. Both Imidacloprid and Acephate treatments depressed the rate of virus incidence below that of the untreated control (Table 5). There was no significant difference between rates for Imidacloprid and Acephate treatments. Also the Imidacloprid-treated plot (Plot 2) was significantly different from the

TABLE 4
NONLINEAR LOGISTIC REGRESSION ANALYSIS OF THE VIRUS INCIDENCE OF *CITRUS TRISTEZA VIRUS* EXPERIMENTAL PLOTS

Isolate	Plot	Parameter Estimate r	Asymptotic standard error	Asymptotic 95% confidence limits		ρ	Residual pattern
				Lower	Upper		
All CTV	1	0.00761	0.000194	0.00715	0.00807	0.99404	-
	2	0.00585	0.000128	0.00555	0.00615	0.99466	-
	5	0.00658	0.000251	0.00599	0.00718	0.99515	-
	6	0.00688	0.000110	0.00662	0.00714	0.99679	-
MCA13+	1	0.00433	0.000020	0.00428	0.00438	0.99865	-
	2	0.00317	0.000023	0.00312	0.00323	0.99678	-
	3	0.00423	0.000059	0.00409	0.00437	0.99852	-
	4	0.00447	0.000150	0.00412	0.00482	0.93662	-
	5	0.00405	0.000068	0.00389	0.00421	0.98222	-
	6	0.00464	0.000090	0.00443	0.00485	0.98175	-

Model parameters were estimated by nonlinear regression of the integrated equations $y = 1/[1 + \exp^{-(ln(y_0/1-y_0)) + rt}]$, for the logistic, where r is rate parameter, y is disease incidence of trees, and t is time in days. Correlation coefficients of observed versus predicted values (r^{*2}) and the presence/absence of patterns in plots of residual values (data not shown) were examined for the appropriateness of the model.

decline-type isolate control plot (Plot 6). The Acephate-treated plot (Plot 5) was not significantly different from the decline control plot.

Nonlinear logistic rates of virus increase of decline isolates were also compared via *t*-test. Decline isolates progressed only slightly more rapidly in the control plot containing a source of decline inoculum (Plot 6) compared to the control plot with a mild, T30 inoculum source (Plot 1). However this difference was slight, indicating that proximity to decline isolates was

TABLE 5
PAIRED *T*-TEST COMPARISON OF NONLINEAR LOGISTIC RATE PARAMETERS OF *CITRUS TRISTEZA VIRUS* ISOLATES IN DIFFERENT PLOTS

Isolate	Plot	Parameter estimate	Standard error	df	t-values				
					Plot 2	Plot 5	Plot 6		
All CTV	1	0.00761	0.00019	7	7.5724**	3.2468**	3.2733**		
	2	0.00585	0.00013	7		2.5909*	6.1029**		
	5	0.00658	0.00025	7			1.0947		
	6	0.00688	0.00011	7					
					t-value				
					Plot 2	Plot 3	Plot 4	Plot 5	Plot 6
MCA13+	1	0.00433	0.00002	3	38.0584**	1.6052	0.9251	3.9503**	3.3624**
	2	0.00317	0.00002	3		16.7392**	8.5665**	12.2589**	15.8248**
	3	0.00423	0.00006	7			1.4890	1.9994*	3.8099**
	4	0.00447	0.00015	7				2.5502*	0.9718
	5	0.00405	0.00007	7					5.2305**
	6	0.00464	0.00009	7					

The degrees of freedom (df) associated with each plot are shown. The df associated with each *t*-test is equivalent to the sum of the df associated with the two plots being compared -2. For example, the df associated with a comparison of Plot 2 vs. Plot 5 is 3 + 7 -2 = 8. * and ** indicate differences detected by *t*-test for $P = 0.05$ and 0.01, respectively.

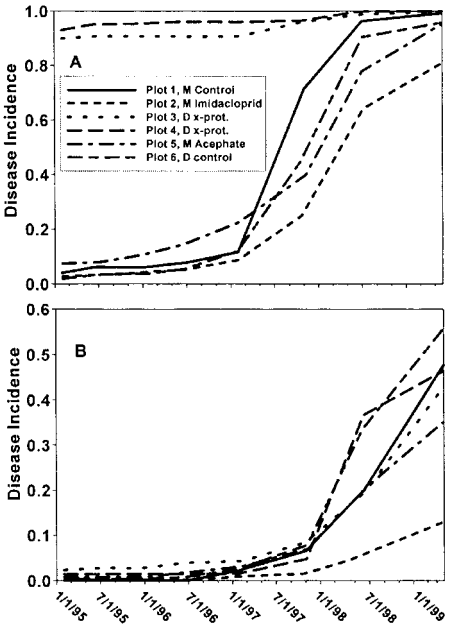

Fig. 5. Increase in CTV incidence in the experimental plots. A. All isolates; B. Decline isolates (MCA13+).

not very important. There was no significant difference in the rate of increase of decline isolates between the two cross-protection plots (Table 5). For the untreated control plots, decline isolates progressed more rapidly in Plot 1, that had a line source of mild isolate-inoculated trees, than in Plot 6, that had a line-source of trees infected with the decline isolate. Thus, proximity to decline did not seem to affect increase of the decline inducing isolate.

The rates of increase of decline isolates in the cross-protection plots were intermediate, i.e., ranged between those for mild and severe sources of inoculum. Thus no benefit of mild strain cross-protection was noted for the isolates that existed in Puerto Rico at the time of the study. The rate of decline-isolate increase for the Imidacloprid-treated plot was less than the Acephate treated plot. Imidacloprid and Acephate plots were intermediate in rate of decline isolate increase between the two untreated control plots. The Imidacloprid and Acephate plots both had rates of decline-isolate increase slightly but significantly less than untreated control plots.

DISCUSSION

Vector populations. Colonizing vector populations were easily determined by counting colonies and determining the vector species. However, the dynamics and estimation of migratory vector population numbers were undoubtedly poorly predicted by the water pan trap results. The relatively few numbers of trapped aphids were not a good estimate of true population numbers or valuable to estimate or compare species prevalence. Even though population estimates were not good, traps did reveal the presence of several potential vector species and a general trend toward an increase in population numbers as trees continued to grow and canopy volume increased, making the plots more attractive to vectors through time.

Spatial aggregation. Spatial associations of infected trees over distance were present but not prevalent. Associations among adjacent infected trees were rare. Associations within groups of infected trees occurred only in relatively few instances. These results parallel those previously found in plots in Costa Rica and the Dominican Republic and were indicative of the type of spread of CTV pathosystems where *T. citricida* and other vectors occur in combination (11, 14, 15, 16, 18). That is, spread caused by *T. citricida* is local, to nearby trees as well as over longer distances among trees placed at some distance to each other. As with previous studies, the size of the plots was too small to easily demonstrate spatial relationships over longer distances.

Rates of virus increase. Overall, the logistic temporal model provided a good representation of CTV increase. The logistic model is relatively simple and for the purposes of

this study a more complex model was not needed to adequately describe CTV increase and compare the effects of various treatments. Therefore comparisons among plots could be accomplished by direct comparison of their nonlinear logistic rates of virus increase. This method demonstrated that both Imidacloprid and Acephate treatments depressed rates of virus increase slightly below that of the untreated control plots. In addition, the Acephate-treated plot had a slight but significantly greater rate of virus increase compared to the Imidacloprid-treated plot. Thus, both chemical treatments decreased the rate of virus spread slightly, but probably not enough to justify the expense.

However, if chemical control of vector populations is justified for other reasons, or when insecticides are applied to the orchard to target other insect pests but also affect aphid populations, some indirect benefit on reduction of virus increase may be realized. With chemical control, viruliferous aphids can still land on uninfected trees, probe and transmit the virus before the chemical kills the vector. Therefore, if chemical control, which generally is highly efficient, did not sufficiently reduce vector populations, then biocontrol would likely have even less impact on CTV epidemics, since biocontrol acts less rapidly, often requiring several days, and allowing plenty of time for virus transmission to occur. Both chemical and biocontrol agents can reduce resident vector populations, but have little effect on immigration of viruliferous vectors from outside; these can transmit viruses before being affected.

The CTV-decline isolate progressed only slightly more rapidly in the control plot with a decline source than in the control plot with a non-decline source. Thus, immediate proximity to decline isolates may not be very important for increase and spread of decline isolates to nearby trees. There was no significant difference in the rate of increase of decline isolates between the two cross-protection plots, and their rates of increase of decline isolates were intermediate between the two non-cross-protected control plots. Thus no benefit from cross-protection was observed when the T30-like, non-decline strains were distributed all trees in all trees prior to planting as a potential cross-protecting strategy. Thus, in our tests, cross-protection had no benefit and led to no inhibition of decline isolates. In a previous test in Costa Rica, mild, T30-like isolates of CTV appeared to have no effect on the ingress of decline-type isolates (17). This is consistent with our findings.

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