# Comparative Epidemiology of *Citrus tristeza virus* in Plantings of Various Citrus Species in Costa Rica, and Long Distance Spread by the Brown Citrus Aphid

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ABSTRACT. Five 400-tree plots were established to compare the virus increase and spread of *Citrus tristeza virus* (CTV) among grapefruit, orange and lemon plots in San Carlos and Nicoya citrus producing areas of Costa Rica. Tree disease status was assayed semiannually over a 5-yr period via DAS-I ELISA using a monoclonal mixture to detect all CTV isolates, and MCA13 to identify more severe isolates. Aphid population dynamics and species prevalence/diversity were monitored using yellow and green water traps to estimate flying aphid populations. Spatial and spatio-temporal analyses were conducted to determine the dynamics of virus spread. Virus increase was most rapid in the orange plot, much slower in the grapefruit plot and even slower in the lemon plots. Both grapefruit and orange plots in Boca de Arenal demonstrated some tree to adjacent tree associations of CTV-infected trees but none at the scale of groups of trees. This was reversed for the grapefruit plot in Nicoya for which no association existed among adjacent trees but aggregation did exist within groups of trees. Groups of trap trees were planted and maintained every 0.1 km along roadsides radiating away from the edges of a commercial citrus production area in San Carlos to detect long distance spread by events vector. Brown citrus aphid, *Toxoptera citricida*, colonies formed multiple times in the trap trees, and CTV-infected trap trees were found as far as 4.6 km from the nearest commercial source trees, indicating the ability of *T. citricida* to traverse and transmit CTV over considerable distances.

*Index words.* Virus transmission, CTV spread, CTV increase, comparative epidemiology, *Toxoptera citricida*.

*Citrus tristeza virus* (CTV) can be spread by human dissemination of infected plant materials and by vector transmission from plant to plant. The first occurrence of CTV isolates in previously non-infested areas is almost exclusively by introduction of infected budwood or whole plants. Depending on the dissemination of these materials, CTV can be widespread or localized at the onset, but initial virus incidence is usually very low because proportionally few infected plants are introduced into a large population of preexisting virus-free trees. Such was likely the case in Costa Rica when CTV was first introduced.

The diversity of virus isolates introduced is directly related to that present at the source of the propagating material. The diversity of CTV isolates present in Costa Rica is both relatively low and similar to that found in Florida. (10) From discussion with plantation managers, it is believed that the majority of the planting materials used to establish the Costa Rican commercial citrus plantations came from certified virus-free stock from California. However, both mild T30-type isolates and decline T36-like isolates are common in Costa Rica and indicate introductions of uncertified propagation materials from Florida (11, & Gottwald pers. comm. with Costa Rican production managers).

T30-type isolates are often referred to as 'mild' because they are asymptomatic in most commercial plantings. Conversely, T36-type isolates are often associated with sweet orange-on-sour orange decline, and where diversity in an area is low such as in Florida or Costa Rica, can be discriminated from T30-type mild isolates by the use of monoclonal antibody MCA13 (7, 22). Even so, T36-induced decline is rare in Costa Rica due to the prevalence of rootstocks other than sour orange. How-

ever, in contrast to Florida, Costa Rica has a relatively new citrus industry and CTV isolates are still comparatively low in incidence (11). Thus, commercial plantings in Costa Rica are suitable for investigating CTV increase and spread, whereas older industries such as in Florida either have high CTV incidence or ongoing eradication efforts, either of which is less suited to such studies.

Spatial and temporal analyses of CTV epidemics have been conducted previously but mostly from limited data and almost exclusively for CTV decline for which *Aphis gossypii* was believed to be the major vector (6, 9, 10, 21). Using data from intensely-mapped multi-year studies of CTV incidence in eastern Spain and Florida, we found that for CTV epidemics where *A. gossypii* was the predominant CTV vector, CTV incidence progressed from low levels (~0.05) to high levels (~0.95) in 8 to 15 yr (10, 13, Gottwald, Garnsey and Irey, unpublished). Similar examination of data from Costa Rica and the Dominican Republic showed that although CTV-positive trees did not often influence immediately adjacent trees, virus transmission was common within an area that extended two to eight trees in all directions (11). 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. Patterns of longer distance spread were not detected within the confines of the plot sizes tested. 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 (11).

For the Spanish data there was little evidence for aggregation of CTV-infected trees, and the spatial patterns of CTV-infected trees could not be distinguished from random (10). Virus spread did not occur preferentially to trees adjacent to those already infected. Rather, new infections probably arose from both inter- and intra-plot transmissions (10). Gibson (8) recently reevaluated the same data from Eastern Spain and Gottwald et al. (10) examined CTV data from Costa Rica and the Dominican Republic. Using all of the Spanish CTV data collectively, Hughes and Madden (14) found that the data could be fitted by binomial distributions with a separate mean incidence for each assessment, but with a common aggregation parameter, equal to 0.03. The low value of the aggregation parameter, and the proximity of the data points to the theoretical binomial line in a plot of observed variance against binomial variance, are indicative of a random pattern at the quadrat scale (15). Gibson (8) and Gottwald et al. (12) examined the data using a spatio-temporal stochastic model based on Markov chain, Monte Carlo integration methods. These stochastic model analyses reinforced the inter- and intra-plot transmission theory and provided some evidence that one component of spread was likely due to short-distance transmissions from nearby trees, which was not apparent from the analytical methods used by Gottwald et al. (9, 10, 11, 13).

In the present study, we took advantage of the Costa Rica situation to investigate two previously unexamined factors of CTV biology: 1) Comparison of CTV increase and spread in commercial plantings of various citrus species, (orange, grapefruit and lemon); and 2) determination of distances of spread of CTV when *T. citricida* is the prevalent vector.

## MATERIALS AND METHODS

**Sample collection.** The increase and spatial spread of both mild and decline-type isolates were monitored

over 5 yr from 1997 to 2001 by sampling and assaying each tree in each plot via ELISA in spring and fall each year. Samples consisted of four young leaf petioles taken from young, nearly fully expanded leaves around the periphery of the tree. Samples were placed in a number-coded paper envelope; 20 envelopes corresponding to one row of trees were placed in sealable plastic bags, to which was added ca. 50 g of a moisture-indicating silica gel. The dry samples were then transported to either the University of Costa Rica in San José or the USDA-ARS laboratory in Florida for processing.

**ELISA processing.** Each sample was placed in 5 ml of PBS-Tween buffer and pulverized for 30 s in a Kleco tissue pulverizer. Extracts were assayed for presence of CTV via double sandwich indirect (DAS-I) ELISA (3, 7, 24, 25). CTV isolates were differentiated into *mild*, i.e., non-decline-inducing, and potentially *decline-inducing*, isolates, based on differential reaction to two monoclonal probes. The first probe consisted of a mixture of the monoclonal antibodies 11B1 and 3E10, which in combination act as a universal probe for all known isolates (3, 7, Garnsey et al. unpublished). The second probe was the single monoclonal antibody MCA13, which reacts to the majority of decline-inducing and stem-pitting isolates of CTV but not to mild isolates found in Florida (7, 22). Thus, a sample reacting to the universal probe but not MCA13 was designated as *mild-type*, whereas a sample reacting to both probes was designated as *decline-type.* 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.

**Long Distance Spread of CTV.** Two citrus plantations with CTV infestations, Tico Frut Finca Uno and Finca Chavarria, were identified at the edge of the citrus zone near Muelle, San Carlos. No other citrus existed in the vicinity. A collection of sweet orange trees to be used as trap trees were assayed for CTV via ELISA while still in the nursery. Uninfected trees were moved to a greenhouse at the University of Costa Rica and maintained in isolation to ensure they remained virus free. Groups of four trap trees were planted along roadsides every 0.1 km radiating away from these commercial citrus plantings to detect long distance spread by vector (Fig. 1). Locations of the outer edges of the infested plantations and of each group of trap trees were recorded via a hand-held Global Positioning System (GPS) receiver (Model XL-12, Garmin Corp.). All trees were assayed individually by ELISA approximately every 3 mo. Trees that assayed positive for CTV were removed, replanted with virus-free trees, and the experiment reassayed 11 times at three-month intervals. Distances of new infections from infested commercial plantings, were determined by GPS. The occurrence of all CTV infections in trap trees was regressed against distance to determine if new infections were more prevalent near versus far from the source.

**Spatial and temporal spread of CTV.** Five, 400-tree plots were established in 1997 to compare the virus increase and spread of CTV among grapefruit, orange and lemon plots in Boca de Arenal, San Carlos and Península de Nicoya, Guanacaste commercial citrus producing areas of Costa Rica. In the Boca de Arenal area, two plots, one each of orange and grapefruit, were established in approximately 10-yr-old commercial plantings. In the Nicoya peninsula area, one grapefruit plot (Plot 7), and two lemon plots (Plots 8 and 9) were established in approximately 5-yr-old commercial plantings. Virus status was assayed semiannually throughout the experiment via ELISA to detect all CTV

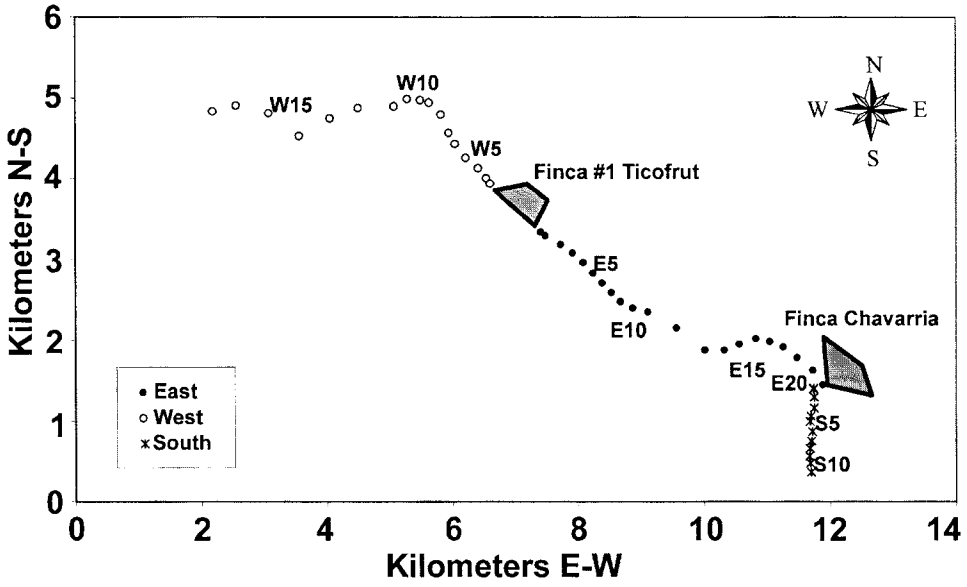


**Fig. 1. Spatial positions of *Citrus tristeza virus* (CTV) trap trees in the Muelle Area of San Carlos, Costa Rica in relationship to two CTV-infected citrus plantations. Groups of four, CTV-free trap trees were planted along roadsides radiating away from these plantations to detect long distance spread. CTV-positive trees were replaced with new CTV-free trees and the experiment repeated 11 times and 3 mo intervals. Distances of new CTV infections from infected commercial plantings, were determined via GPS.**

isolates and to discriminate decline isolates. Spatial pattern maps were prepared for each plot on each assessment date based on ELISA results.

**Spatial analysis.** To interpret the relationships among CTV-positive trees, CTV data were examined at discrete 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 (11, 18, 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 $P = 0.05$ (18).

To detect aggregation at different spatial scales, the CTV incidence data from each block were examined at the individual tree scale or partitioned into 2 × 2 quadrats (groups of trees) with the use of a Visual Basic EXCEL macro (Gottwald, unpublished software). Aggregation within quadrat was assessed by beta-binomial analysis. For the beta-binomial index, a large $I_\beta$ (>1) combined with a small $P$ (< 0.05) suggests aggregation of diseased trees (15, 17).

**Temporal analysis.** The virus incidence (number of CTV-infected trees divided by the total number of trees) of each plot was calculated for each assessment. The increase in virus incidence for all CTV isolates and of MCA13-positive isolates was assessed 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 (14, 16). When the overall most appropriate linear model was selected, considering all

data sets, the data were then fitted by non-linear regression to the non-linear form of the model for predictive purposes. Nonlinear regression analysis of untransformed 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 based 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 (1, 16). CTV increase among plots was compared via *t*-test of rates of virus increase determined by linear regression of the most appropriate model to determine if there were significant difference in virus increase relative to host, virus isolate type (all CTV and MCA13-positive) and location.

**Vector population dynamics.** Aphid population dynamics and species prevalence/diversity were monitored using yellow and green water traps to estimate flying aphid populations. Traps were located in commercial plantings at Finca Chavarría, Boca de Arenal, and Tico Frut Finca

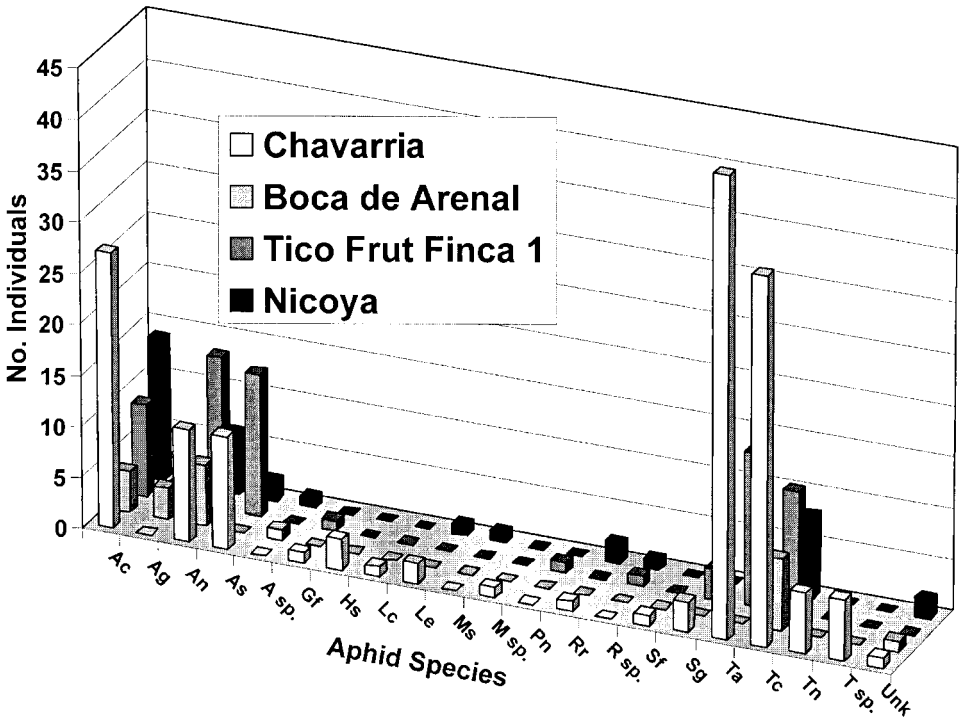


**Fig. 2. Aphid species caught at each location over duration of study, using yellow and green water traps to estimate flying aphid populations. Traps were located in commercial plantings at Finca Chavarría, Boca de Arenal, and Tico Frut Finca 1, at San Carlos and Peninsula Nicoya in Guanacaste and monitored every two weeks. Aphid species were identified where possible and grouped as follows: Ag = *Aphis gossypii*, An = *A. nerii*, As = *A. spiraecola*, A spp. = *Aphis* spp., Gf = *Geophenfigus floccosus*, Hs = *Hysteroneura setariae*, Lc = *Lyzerus cermelii*, Le = *Lipaphis erysimi*, Ms = *Melanaphis sacchariii*, M sp. = *Macrosiphum* spp., Pn = *Pentalonia nigronervosa*, Rr = *Rhopalosiphum rufiabdominalis*, R. sp. = *Rhopalosiphum* spp., Sf = *Sipha flava*, Sg = *Schizaphis graminum*, Ta = *Toxoptera aurantii*, Tc = *T. citricida*, Tn = *Tetraneura nigriabdominalis*, T sp. = *Toxoptera* spp., and Unk = unknown Species. Due to relatively low aphid catches, populations are expressed as total catches of each aphid species/group over the duration of the study.**

1, in San Carlos and Península de Nicoya in Guanacaste and were monitored every 2 weeks. Aphid species were identified where possible and segregated into the following groups: Ag = *Aphis gossypii*, An = *A. nerii*, As = *A. spiraecola*, A spp. = *Aphis* spp., Gf = *Geophenfigus floccosus*, Hs = *Hysteroneura setariae*, Lc = *Lyzerus cermelii*, Le = *Lipaphis erysimi*, Ms = *Melanaphis saccharii*, M sp. = *Macrosiphum* spp., Pn = *Pentalonia nigronervosa*, Rr = *Rhopalosiphum rufiabdominalis*, R. sp. = *Rhopalosiphum* spp., Sf = *Sipha flava*, Sg = *Schizaphis graminum*, Ta = *Toxoptera aurantii*, Tc = *T. citricida*, Tn = *Tetraneura nigriabdominalis*, T sp. = *Toxoptera* spp., and Unk = unknown Species. Due to relatively low aphid catches, populations were expressed as total catches of each aphid species/group over the duration of the study.

## RESULTS

**Aphid populations.** Catches were low for all locations, making estimations of population dynamics infeasible. Therefore, only the totals for each species at each plot are presented (Figs. 2 and 3). Total of all aphids caught over all years and all locations are also presented, separated into catches from yellow versus green water pan traps. As expected *Aphis* spp. and *Toxoptera* spp. were the most prevalent in the citrus plantings and *T. citricida* was the most prevalent aphid species overall.

**Long-distance spread.** The spatial positions of groups of four trap-trees dispersed each ca. 0.1 km along area roads in the Muelle area of San Carlos, Costa Rica are shown (Fig. 1). Virus transmission was first detected at a distance of 1.1 km away from the commercial citrus

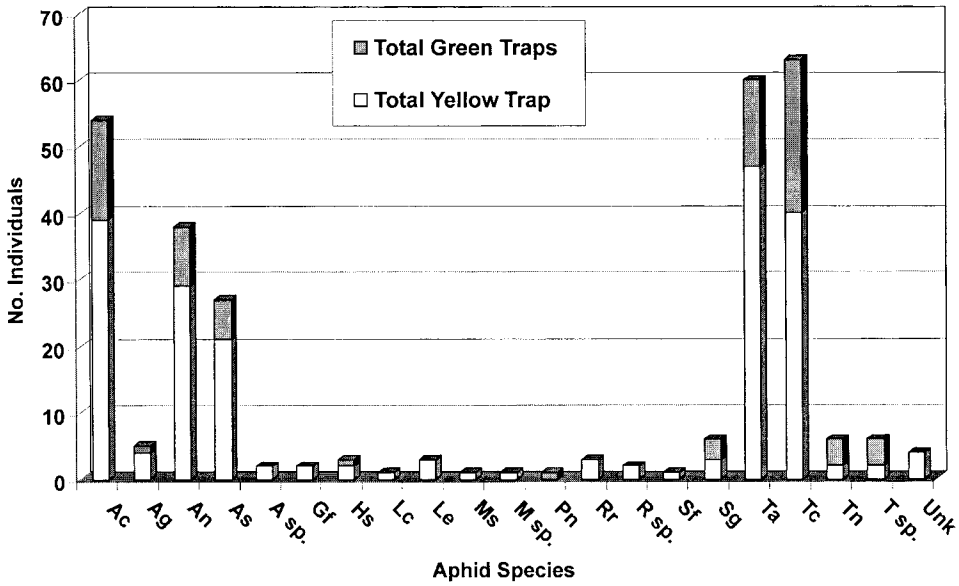


**Fig. 3. Total aphids caught in yellow+green water pan traps, to estimate flying aphid populations. Traps were monitored every two weeks and trap data was combined over all years and locations. Aphid species were identified where possible and grouped as: Ag = *Aphis gossypii*, An = *A. nerii*, As = *A. spiraecola*, A spp. = *Aphis* spp., Gf = *Geophenfigus floccosus*, Hs = *Hysteroneura setariae*, Lc = *Lyzerus cermelii*, Le = *Lipaphis erysimi*, Ms = *Melanaphis saccharii*, M sp. = *Macrosiphum* spp., Pn = *Pentalonia nigronervosa*, Rr = *Rhopalosiphum rufiabdominalis*, R. sp. = *Rhopalosiphum* spp., Sf = *Sipha flava*, Sg = *Schizaphis graminum*, Ta = *Toxoptera aurantii*, Tc = *T. citricida*, Tn = *Tetraneura nigriabdominalis*, T sp. = *Toxoptera* spp., and Unk = unknown Species.**

planting (CTV infection source) during the December 1998 assessment (Table 1). If any of the four trap trees tested positive, the location was considered CTV infected. The first detection was concurrent with visual confirmation of a *T. citricida* colony on one of the four trap trees at that location. The nearest source of *T. citricida* was also the same commercial planting. Numerous (64) virus transmission events were recorded over the duration of the 11 assessments (Table 1). The longest distance of virus transmission recorded was 4.61 km, and represented the farthest set of trap trees from the north western CTV-infected commercial planting. Detections at this location occurred on two occasions over the duration of the study. Three other detections were made at 4.26 km, indicating that spread over 4 km via aphid transmission is not uncommon. Regression analysis of occurrence of CTV infections in groups of trap trees over distance indicated that the slope of the regression line was not significantly different from 0 (Fig. 4). This means that there was no prevalence of spread over any particular distance and distant trees were just as likely to become infected as those near the sources of infection.

**Spatial Analyses.** Virus increase was most rapid in the orange plot and much slower in the grapefruit plot and in the lemon plots, with little discernible difference in spatial patterns. Ordinary Runs analysis and beta binomial analyses of total CTV in grapefruit in Boca de Arenal, Plot 5, demonstrated some but infrequent adjacent tree associations but no spatial associations at 2 × 2 quadrat (group) scale (Table 2). For the orange planting in Boca de Arenal, Plot 6, a few spatial associations were demonstrated at the adjacent tree scale but none at the group scale (Table 2). For the grapefruit in Península de Nicoya, Plot 7, no Ordinary Runs assays revealed adjacent tree associations but beta-binomial analysis assays did demonstrate within group associations (Table 2).

If we reexamine the same plots by Ordinary Runs and beta-bino-

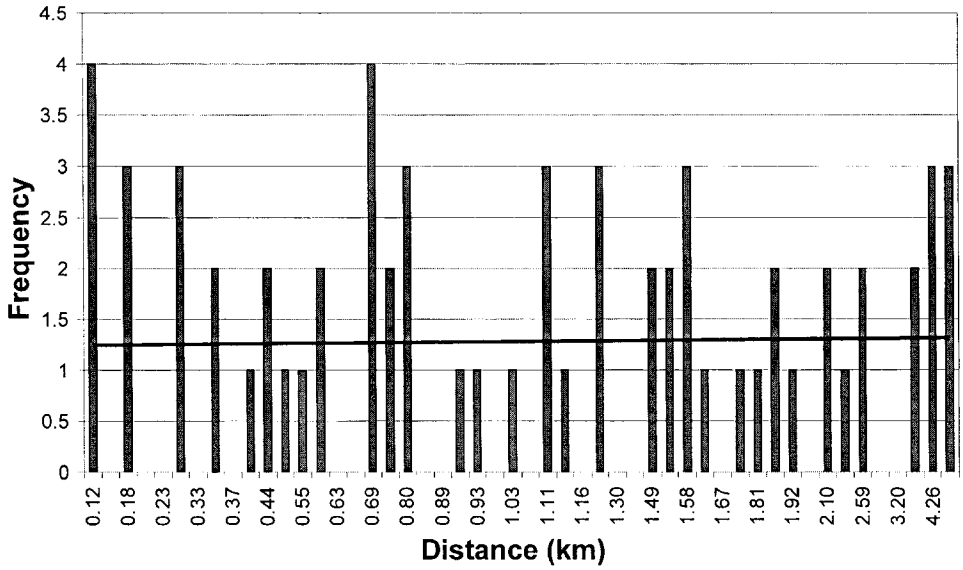


**Fig. 4. Incidence of *Citrus tristeza virus* in trap trees in relation to distance from the source of virus. The slope (*b* = 0.149) of the linear regression (black line across histogram) was not significantly different from 0, (regression coefficient $r^2$ = 0.016) indicating no preference for spread near versus far from the source.**

TABLE 1
DISTANCE OF SPREAD OF *CITRUS TRISTEZA VIRUS* BY *TOXOPTERA CITRICIDA* IN EASTERN COSTA RICA

| Transect | Assessment date | Tree position [#] and distance to nearest source (in km) | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| East (E) | | [1] 0.14 | [2] 0.23 | [3] 0.49 | [4] 0.71 | [5] 0.91 | [6] 1.11 | [7] 1.30 | [8] 1.49 | [9] 1.67 | [10] 1.88 | [11] 2.10 | [12] 2.60 | [13] 2.09 | [14] 1.79 | [15] 1.58 | [16] 1.36 | [17] 1.14 | [18] 0.67 | [19] 0.67 | [20] 0.37 | [21] 0.18 |
| | 12/1/1998 | | | | | | X | | | | | | | | | | | | | | | X |
| | 3/30/1999 | | | | X | | | | | | | | | | | | | | | | | |
| | 6/30/1999 | | | | X | | | | | | X | X | | | | | | | | | | X |
| | 9/30/1999 | | | | | | | | | | | | | | | | | | | | | X |
| | 12/30/1999 | | | | | | | | | | | | | | | | | | | | | |
| | 3/30/2000 | | | | | | | | | | | | X | | | X | | | | | | |
| | 6/30/2000 | | | | | | | | X | | | | | | | X | | | | | | |
| | 9/29/2000 | | | | | | | | X | | | | | | | | | X | | | | |
| | 12/30/2000 | | | X | | X | | | | | | | | | | | | | | | | |
| | 4/6/2001 | | | | | | X | | | | | | X | | | | | | | | | |
| | 7/11/2001 | | | | | | X | | | | X | X | | | X | X | | | | X | | |
| West (W) | | [1] 0.12 | [2] 0.21 | [3] 0.39 | [4] 0.63 | [5] 0.86 | [6] 1.03 | [7] 1.28 | [8] 1.52 | [9] 1.64 | [10] 1.81 | [11] 1.92 | [12] 2.41 | [13] 2.78 | [14] 3.20 | [15] 3.73 | [16] 4.26 | [17] 1.28 | | | | |
| | 12/1/1998 | | | X | | | | | | | | | | | | | | | | | | |
| | 3/30/1999 | | | | | | | | | X | | | | | | X | | X | | | | |
| | 6/30/1999 | X | | | | | | | | | | | | | | | | X | | | | |
| | 9/30/1999 | | | | | | | | | | | | | | | | | X | | | | |
| | 12/30/1999 | | | | | | | | | | | | | | | | | | | | | |
| | 3/30/2000 | X | | | | | | X | | | | | | | | | X | | | | | |
| | 6/30/2000 | | | | | | | X | | | X | | | | | | | | | | | |
| | 9/29/2000 | | | | | | X | X | | | | | | | | X | | | | | | |
| | 12/30/2000 | | | | | | | | X | | | | | | | | X | | | | | |

Groups of four virus-free trap trees, were planted every 0.1 km on roadsides radiating away from CTV-infected commercial citrus plantings to detect long distance spread vector events (Fig. 1). Locations of the edges of the infected plantations and of each group of trap trees were recorded via GPS. Each tree was assayed individually by ELISA approximately every three months. CTV-positive trees were replaced with CTV-free trees when detected. Refer to Fig. 1 for spatial position of trap trees, i.e., W15 in the Figure corresponds to West tree #15 located 3.73 km from nearest source of CTV and vectors.

TABLE 1 (CONTINUED)
DISTANCE OF SPREAD OF *CITRUS TRISTEZA VIRUS* BY *TOXOPTERA CITRICIDA* IN EASTERN COSTA RICA

| Transect | Assessment date | Tree position [#] and distance to nearest source (in km) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 4/6/2001 | X | | | | | | | X | | | | X |
| | 7/11/2001 | X | | | | | | | | | X | X | |
| South (S) | | [1] 0.33 | [2] 0.33 | [3] 0.36 | [4] 0.44 | [5] 0.55 | [6] 0.60 | [7] 0.69 | [8] 0.80 | [9] 0.89 | [10] 0.98 | [11] 1.06 | [12] 1.16 |
| | 12/1/1998 | | | | | | | X | | | | | |
| | 3/30/1999 | | X | | | | | X | | | | | |
| | 6/30/1999 | | X | | | | | | X | | | | |
| | 9/30/1999 | | | | | | | | X | | | | |
| | 12/30/1999 | | | | | | | | | | | | |
| | 3/30/2000 | | | | X | | | | | | | | |
| | 6/30/2000 | | | | | | | | | | | | |
| | 9/29/2000 | | X | X | | | X | | | | | | |
| | 12/30/2000 | | | | | | | X | X | | | | |
| | 4/6/2001 | | | X | X | | | | | | | | |
| | 7/11/2001 | | | | | X | X | X | | | | | |

Groups of four virus-free trap trees, were planted every 0.1 km on roadsides radiating away from CTV-infected commercial citrus plantings to detect long distance spread vector events (Fig. 1). Locations of the edges of the infected plantations and of each group of trap trees were recorded via GPS. Each tree was assayed individually by ELISA approximately every three months. CTV-positive trees were replaced with CTV-free trees when detected. Refer to Fig. 1 for spatial position of trap trees, i.e., W15 in the Figure corresponds to West tree #15 located 3.73 km from nearest source of CTV and vectors.

TABLE 2
ORDINARY RUNS ANALYSIS AND BETA BINOMIAL INDEX OF DISPERSION ($I_\beta$) ANALYSES FOR *CITRUS TRISTEZA VIRUS* (CTV) IN COSTA RICA

| Plot | Date | Disease incidence | Ordinary runs[a] | | | | Beta binomial[b] | |
|---|---|---|---|---|---|---|---|---|
| | | | Row | Column | Row (all) | Col (all) | $I_\beta$ | P |
| 5 | Nov 97 | 0.2282 | 6/20 | 1/18 | N | N | 1.485 | 0.230 |
| | June 98 | 0.2418 | 4/20 | 1/18 | N | N | 1.438 | 0.242 |
| | Sept 98 | 0.2690 | 3/20 | 0/19 | N | N | 1.338 | 0.270 |
| | Nov 98 | 0.2834 | 4/20 | 0/19 | N | N | 1.356 | 0.284 |
| | Aug 99 | 0.2885 | 4/20 | 1/19 | N | N | 1.413 | 0.290 |
| | Dec 99 | 0.2853 | 4/20 | 1/19 | N | N | 1.394 | 0.286 |
| | July 00 | 0.2853 | 4/20 | 1/19 | N | N | 1.394 | 0.286 |
| | Nov 00 | — | — | — | — | — | — | — |
| | June 01 | — | — | — | — | — | — | — |
| 6 | Nov 97 | 0.2590 | 1/20 | 1/20 | R | R | 1.273 | 0.259 |
| | June 98 | 0.2923 | 1/20 | 1/20 | R | N | 1.221 | 0.292 |
| | Sept 98 | 0.3949 | 0/20 | 2/20 | R | N | 1.138 | 0.395 |
| | Nov 98 | 0.4308 | 0/20 | 2/20 | R | R | 1.070 | 0.431 |
| | Aug 99 | 0.4692 | 0/20 | 1/20 | R | R | 1.100 | 0.469 |
| | Dec 99 | 0.4820 | 0/20 | 1/20 | R | N | N/A | N/A |
| | July 00 | 0.4871 | 0/20 | 1/20 | R | R | N/A | N/A |
| | Nov 00 | 0.4897 | 0/20 | 2/20 | R | N | N/A | N/A |
| | June 01 | 0.6289 | 0/20 | 3/20 | R | N | N/A | N/A |
| 7 | Nov 97 | 0.0405 | 0/14 | 0/9 | R | R | 1.133 | 0.040 |
| | June 98 | — | — | — | — | — | — | — |
| | Sept 98 | 0.0405 | 0/13 | 0/9 | R | R | 1.133 | 0.040 |
| | Nov 98 | 0.0406 | 0/13 | 0/9 | R | R | 1.130 | 0.041 |
| | Aug 99 | 0.0406 | 0/13 | 0/9 | R | R | 1.130 | 0.041 |
| | Dec 99 | 0.0410 | 0/13 | 0/9 | R | R | 1.118 | 0.041 |
| | July 00 | 0.0390 | 0/11 | 0/9 | N | R | N/A | N/A |
| | Nov 00 | 0.0360 | 0/10 | 0/9 | R | R | N/A | N/A |
| | June 01 | 0.0435 | 0/10 | 0/8 | R | R | N/A | N/A |

[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 disease 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 by following a serpentine pattern throughout the entire planting, where N = nonrandom and R = random. [b]Index of dispersion ($I_\beta$) and associated probability ($P$) values for plots infected with PPV, where $I_\beta$ = observed variance/binomial variance and $P$ = probability. $P$-values were calculated by comparison of df × $I_\beta$ with the chi-squared distribution. Values of $I_\beta$ 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) $I$ 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. CTV-positive trees were determined by ELISA using a monoclonal antibody.

mial analyses but restricting the analyses to only MCA13 decline-type isolates, Boca de Arenal grapefruit, Plot 5, and orange, Plot 6, demonstrated a few spatial associations at the adjacent tree scale but none at the group scale (Table 3). Grapefruit, Plot 7, was not examined spatially because of low CTV incidence throughout the study. Lemons in Península de Nicoya, Plots 8 and 9, were also not examined by spatial analyses due to tree loss from *Phytophthora*.

**CTV temporal increase.** This was examined by linear and non-linear modeling. For linear models, even though the monomolecular model was the most appropriate when estimated via linear regression, it does not make sense biologically for a perennial virus disease such as citrus tristeza. The logistic model, dy/dt = rLy(1-y), also provid-

TABLE 3
ORDINARY RUNS ANALYSIS AND BETA BINOMIAL INDEX OF DISPERSION (*I* ) ANALYSES FOR MCA13-POSITIVE STRAINS OF *CITRUS TRISTEZA VIRUS* IN COSTA RICA

| Plot | Date | Disease incidence | Ordinary runs[a] | | | | Beta binomial[b] | |
|---|---|---|---|---|---|---|---|---|
| | | | Row | Column | Row (all) | Col (all) | $I_\beta$ | P |
| 5 | Nov 97 | 0.1576 | 2/20 | 1/16 | N | N | 1.401 | 0.160 |
| | June 98 | 0.1793 | 2/20 | 1/17 | N | N | 1.478 | 0.181 |
| | Sept 98 | 0.2554 | 4/20 | 0/18 | N | N | 1.372 | 0.257 |
| | Nov 98 | 0.2670 | 4/20 | 0/18 | N | N | 1.447 | 0.268 |
| | Aug 99 | 0.2775 | 3/20 | 1/18 | N | N | 1.458 | 0.279 |
| | Dec 99 | 0.2799 | 3/20 | 1/19 | N | N | 1.392 | 0.281 |
| | July 00 | 0.2826 | 4/20 | 1/19 | N | N | 1.393 | 0.284 |
| | Nov 00 | — | — | — | — | — | — | — |
| | June 01 | — | — | — | — | — | — | — |
| 6 | Nov 97 | 0.0795 | 1/15 | 2/16 | R | N | 1.499 | 0.079 |
| | June 98 | 0.0949 | 1/15 | 2/17 | N | N | 1.469 | 0.095 |
| | Sept 98 | 0.1256 | 0/16 | 1/18 | N | N | 1.430 | 0.127 |
| | Nov 98 | 0.1590 | 0/17 | 2/18 | N | N | 1.228 | 0.160 |
| | Aug 99 | 0.2051 | 1/18 | 3/18 | N | N | 1.432 | 0.206 |
| | Dec 99 | 0.2062 | 1/18 | 2/18 | N | N | N/A | N/A |
| | July 00 | 0.2191 | 2/19 | 0/18 | N | N | N/A | N/A |
| | Nov 00 | 0.2216 | 2/19 | 0/18 | N | N | N/A | N/A |
| | June 01 | 0.3170 | 2/19 | 0/19 | N | N | N/A | N/A |

[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 disease 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 by following a serpentine pattern throughout the entire planting, where N = nonrandom and R = random. [b]Index of dispersion ($I_\beta$) and associated probability (*P*) values for plots infected with PPV, where $I_\beta$ = observed variance/binomial variance and *P* = probability. *P*-values were calculated by comparison of df × $I_\beta$ with the chi-squared distribution. Values of $I_\beta$ 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* 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. CTV-positive trees were determined by ELISA using a monoclonal antibody.

ing a good fit overall, is more easily explained biologically, and was therefore used (Table 4). Only the orange Plot 6 in Boca de Arenal area, expressed appreciable virus increase, whereas grapefruit in this area and in Península de Nicoya had very little increase as expected. Lemon plots also had very few new CTV infections over the duration of the study. For linear models of MCA13-positive decline-type isolates, the monomolecular model was again the most appropriate but the logistic model was used for the reasons given above (Table 4 and Fig. 5). Decline isolates increased in both Boca de Arenal orange and grapefruit plots. The grapefruit plot was eliminated by the grower prior to end of the study and therefore had fewer assessments.

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 5). However, the nonlinear regression analyses resulted in relatively low coefficients of correlation of observed versus predicted values in several cases. Therefore, the nonlinear models were not used further.

Rates of virus increase were compared via *t*-test using linear model parameters. If we consider all CTV isolates, in the Boca de Arenal area, virus increase was significantly less for grapefruit, Plot 5, compared to orange, Plot 6 (Table 6). In the Península de Nicoya area, the rate of virus increase for grapefruit, Plot 7, was very low resulting in a flat

TABLE 4
LINEAR REGRESSION ANALYSES OF THE INCIDENCE OF *CITRUS TRISTEZA VIRUS* DETECTED BY ELISA IN SIX CITRUS PLOTS IN COSTA RICA

| Assay | Plot | Model | Parameter estimate | Standard error | Adjusted $R^2$ | r | Residual pattern |
|---|---|---|---|---|---|---|---|
| All CTV | 5 | Exponential (E) | 0.00023 | 0.00007 | 0.6163 | 0.81318 | + |
| | | Monomolecular (M) | 0.00008 | 0.00002 | 0.6260 | 0.83361* | + |
| | | Logistic (L) | 0.00031 | 0.00009 | 0.6190 | 0.81902 | + |
| | | Gompertz (G) | 0.00017 | 0.00005 | 0.6218 | 0.82497 | + |
| | 6 | E | 0.00057 | 0.00010 | 0.7986 | 0.91559 | - |
| | | M | 0.00043 | 0.00007 | 0.8373 | 0.93412* | - |
| | | L | 0.00100 | 0.00016 | 0.8321 | 0.92356 | - |
| | | G | 0.00067 | 0.00010 | 0.8407 | 0.92843 | - |
| | 7 | E | -0.00001 | 0.00005 | -0.1570 | 0.06533* | + |
| | | M | -3.17E-07 | 0.00000 | -0.1619 | 0.06475 | + |
| | | L | -0.00001 | 0.00005 | -0.1572 | 0.06530 | + |
| | | G | -3.11E-06 | 0.00002 | -0.1586 | 0.06512 | + |
| | 8 | E | 0.00081 | 0.00029 | 0.4865 | 0.76352* | - |
| | | M | 0.00001 | 0.00000 | 0.4912 | 0.75060 | - |
| | | L | 0.00083 | 0.00030 | 0.4869 | 0.76333 | - |
| | | G | 0.00020 | 0.00007 | 0.4902 | 0.76086 | - |
| | 9 | E | 0.00055 | 0.00014 | 0.6782 | 0.88528* | - |
| | | M | 0.00001 | 0.00000 | 0.7123 | 0.84344 | - |
| | | L | 0.00056 | 0.00014 | 0.6781 | 0.88468 | - |
| | | G | 0.00014 | 0.00003 | 0.6775 | 0.87650 | - |
| MCA13 only | 5 | E | 0.00058 | 0.00018 | 0.6070 | 0.79862 | + |
| | | M | 0.00017 | 0.00005 | 0.6468 | 0.84475* | + |
| | | L | 0.00075 | 0.00023 | 0.6161 | 0.81147 | + |
| | | G | 0.00039 | 0.00012 | 0.6277 | 0.82544 | + |
| | 6 | E | 0.00096 | 0.00012 | 0.8818 | 0.94941 | - |
| | | M | 0.00020 | 0.00002 | 0.8980 | 0.95850* | - |
| | | L | 0.00116 | 0.00014 | 0.8945 | 0.95199 | - |
| | | G | 0.00054 | 0.00006 | 0.9079 | 0.95596 | - |

Adjusted coefficients of correlation of observed versus predicted values ($R^2$) 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 ($\rho$) of predicted values against observed values and the presence or absence of patterns in residual plots were examined for appropriateness of models.

progress curve, whereas the two lemon plots, Plots 8 and 9, showed limited virus increase which was significantly different from grapefruit, Plot 7. The two grapefruit plantings, Plots 5 and 7, located in the two different study areas, expressed significantly different virus increase. Virus increase for the two lemon plots was not significantly different (Table 6).

Rates of virus increase were also compared for CTV-decline (MCA13-positive) isolates via *t*-test (Table 6). In the Boca de Arenal area, virus increase was not significantly different for Plot 5, grapefruit compared to Plot 6 orange. Although MCA13-positive isolates existed in the Península de Nicoya area, they did not increase appreciably in the grapefruit or lemon plots.

## DISCUSSION

Long-distance spread of CTV was common and reoccurred numerous times over the 11 assessments out to a distance of 4.61 km (2.86 mi). Interestingly, there did not seem to be a prevalence of CTV spread to trap trees nearer to infection sources.

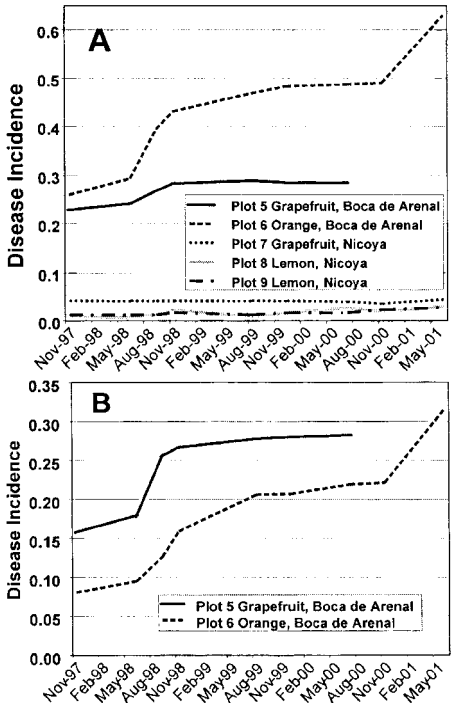


**Fig. 5. *Citrus tristeza virus* increase for different host species in various areas of Costa Rica.**

In fact, spread to the most distant trap trees was just as likely as to those trees located nearest the virus source. This helps to explain the rapid spread of CTV where *T. citricida* is present. Spatial patterns of CTV spread in the presence of *T. citricida* have been examined previously (8, 11, 13). Where *T. citricida* is present, spread of CTV appears to be predominantly by this vector, both within plots and over long distances, contributing to more rapid local and regional virus dissemination.

Spatial associations over short distances were present but not prevalent. Associations among adjacent trees did occur in orange and grapefruit plots, however, associations within groups of trees occurred only in relatively few instances. These findings indicated virus transmission on a local level by *T. citricida* and other vectors in combination and were consistent with previous studies (11, 13).

Rates of virus increase determined in this study were as expected and as experienced elsewhere. That is, orange plantings had a significant rate of virus increase, whereas, virus increase in grapefruit was very low. Similar results have been reported for grapefruit in Spain, Florida, Costa Rica, and the Dominican Republic (10, 11, 13). In contrast, anecdotal evidence from Australia and South Africa suggests that stem-pitting isolates increase rapidly in grapefruit (2, 19, 20, 23). Comparative

TABLE 5
NONLINEAR LOGISTIC REGRESSION ANALYSIS OF THE INCIDENCE OF *CITRUS TRISTEZA VIRUS* ISOLATES DETECTED BY ELISA IN PLOTS IN COSTA RICA

| Assay | Plot | Parameter estimate r | Asymptotic standard error | Asymptotic 95% confidence limits | | r | Residual pattern |
|---|---|---|---|---|---|---|---|
| | | | | Lower | Upper | | |
| All CTV | 5 | 0.00663 | 0.000943 | 0.00433 | 0.00894 | 0.48266 | - |
| | 6 | 0.00673 | 0.000818 | 0.00485 | 0.00862 | 0.79270 | - |
| | 7 | 0.00315 | 0.000389 | 0.00223 | 0.00406 | 0.17078 | + |
| | 8 | 0.00273 | 0.000207 | 0.00224 | 0.00322 | 0.74973 | - |
| | 9 | 0.00268 | 0.000193 | 0.00223 | 0.00314 | 0.95392 | - |
| MCA13 only | 5 | 0.00660 | 0.000838 | 0.00455 | 0.00865 | 0.51506 | - |
| | 6 | 0.00496 | 0.000349 | 0.00415 | 0.00576 | 0.84816 | - |

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 ($\rho$) and the presence/absence of patterns in residual plots were examined for the appropriateness of the model.

TABLE 6
PAIRED T-TEST COMPARISON OF LINEAR LOGISTIC RATE PARAMETERS OF *CITRUS TRISTEZA VIRUS* IN COSTA RICA

| Assay | Plot | Parameter estimate | Standard error | t-values | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | df | Plot 6 | Plot 7 | Plot 8 | Plot 9 |
| All CTV | 5 | 0.0003 | 0.0001 | 6 | 3.7717** | 2.9835** | 1.6546 | 1.4926 |
| | 6 | 0.0010 | 0.0002 | 8 | | 6.1261** | 0.5000 | 2.0582** |
| | 7 | 0.0000 | 0.0001 | 7 | | | 2.7622** | 3.8064** |
| | 8 | 0.0008 | 0.0003 | 7 | | | | 0.8012 |
| | 9 | 0.0006 | 0.0001 | 7 | | | | |
| MCA13+ | 5 | 0.00075 | 0.00026 | 6 | 1.5358 | | | |
| | 6 | 0.00116 | 0.00059 | 8 | | | | |

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 5 vs. Plot 7 is 6 + 7 - 2 = 11. * and ** indicate differences detected by *t*-test for $P = 0.05$ and 0.01, respectively.

epidemiology studies need to be conducted in Australia and/or South Africa to compare rates of virus increase in grapefruit versus sweet orange to more clearly define virus increase and spread of stem pitting isolates there.

One new finding from this study was that decline isolates had a slightly faster rate of increase than non-decline isolates in the same plantings. Rates of CTV increase has not previously been examined in commercial lemon plantings. In this study, lemons had the lowest, nearly undetectable, rate of virus increase. This substantiates vector-transmission tests which indicate that lemon is one of the commercial citrus species least susceptible to vector transmission of CTV (14).

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