

# Co-Diffusion of Serologically Distinct Isolates of Citrus Tristeza Virus Vected by *Toxoptera citricida* in Northern Costa Rica

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**ABSTRACT.** *Toxoptera citricida* was introduced in Costa Rica in 1989, and is now the predominant aphid species in citrus. This efficient vector of citrus tristeza virus (CTV) is rapidly spreading CTV throughout the country. To monitor temporal interactions between two groups of isolates, three plots were established in commercial citrus plantings in Guanacaste, Costa Rica in October of 1992, each consisting of 400 trees of Valencia orange on carrizo and grapefruit rootstocks. All trees in each plot were sampled and assayed for CTV infection twice per year. All samples were assayed first by ELISA DAS-I with a monoclonal antibody mixture to detect all isolates. Positive sources were assayed further with the monoclonal antibody MCA13 which detects most decline or stem-pitting CTV isolates. In two plots, mild (designated mild because of non-reactivity to MCA13) and MCA13-positive isolates existed initially. In the third plot, mild isolates predominated and MCA13-positive isolates appeared and increased over time. Biocharacterization tests of MCA13-positive isolates indicated that five out of seven isolates caused a mild decline reaction of sweet orange on sour orange and two of these caused mild stem pitting in grapefruit. Competition between MCA13-positive and MCA13-negative isolates was examined by calculating the probability of infection of previously infected vs. uninfected trees. Rates of infection for the two isolates were roughly the same within each plot. The MCA13-positive isolates infected trees previously infected with mild isolates and uninfected trees without preference. Therefore, if isolate competition was occurring it was quite weak. Naturally occurring MCA13-positive and mild-CTV isolates in these plots apparently co-migrated independently without detectable competition or interference.

*Toxoptera citricida* (Kirkaldy), the brown citrus aphid, is the most efficient vector of citrus tristeza virus (CTV) worldwide. Since introduction of the brown citrus aphid into Argentina in the 1930's from South Africa, it has progressed steadily through the South American continent and since the 1990's, into Central America (10, 11, 18, 21). Simultaneously, it has spread northward through the Caribbean islands and, in October 1995, was found in southeast Florida (Halbert, S. E., Florida Department of Agriculture and Consumer Services, unpublished data). Reports on rate of CTV increase from Asia, as well as observations in several South American countries where *T. citricida* is

present, indicate that CTV epidemics progress rapidly in its presence. Where the brown citrus aphid is absent and the melon aphid, *Aphis gossypii*, is the predominant vector, CTV epidemics progress much slower (5, 8, 9, 12, 13).

With the exception of cross-protection experiments, little attention has been given to the interaction of CTV isolates under field or greenhouse conditions (16, 19). Although not all CTV isolates induce cross protection, when it occurs there is an interaction among CTV isolates characterized by interference or competition of one or more of the isolates present (16, 19). However, the mechanism of this interaction is unknown and probably complex. The spatial spread of CTV isolates which exhibit less intense or no competition has not been examined previously.

With ELISA, selective monoclonal antibodies can be used to differentiate CTV isolates into two major groups: 1) those that cause

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decline on sour orange rootstock and stem pitting of scions, and 2) those that are mild and cause little or no recognizable symptoms in commercial citrus (1, 3, 4, 6, 15, 20). The selective monoclonal MCA13 can be used to differentiate the majority of severe stem-pitting and sour orange decline isolates from the mild isolates (15). A few isolates exist that cause stem pitting and/or decline which cannot be differentiated with MCA13. However, characterization tests on Costa Rica isolates indicated that no such isolates are known to exist in northern Costa Rica (S. Garnsey, unpublished data).

The purpose of this study was 1) to determine rates of diffusion of naturally occurring mild and MCA13-positive populations of CTV, which exist in commercial citrus plantations in northern Costa Rica, and 2) to determine if there is a competitive interaction between these populations relative to the spread of MCA13-positive CTV isolates or if MCA13-negative and MCA13-positive isolates co-diffuse independently with no interference.

## MATERIALS AND METHODS

**Test plots and sampling.** The three plots used in this study were located in large commercial plantings in northwest Costa Rica in the province of Guanacaste. Each plot consisted of 20 rows each with 20 trees per row in a rectangular planting pattern and consisted of Valencia sweet orange on either Carrizo citrange or a local grapefruit rootstock and ranged from 1 to 5 years old at the beginning of the study. Plots were sampled twice per year in spring and fall and every tree was sampled and tested independently. Samples consisted of four leaf petioles from young but fully expanded flush taken from the periphery of each tree. The petioles were placed in separate number-coded envelopes and 20 individual envelopes corre-

sponding to one row of trees were placed in sealable plastic bags to which was added ca. 50 g of indicating silica gel. The silica gel was changed as needed until the specimens were completely dried. The dry samples were then transported to the USDA-ARS laboratory in Orlando for processing (7).

**ELISA processing.** Each sample of four leaf petioles was placed in 5 ml of PBS-Tween buffer and pulverized for 30 sec in a Kleco tissue pulverizer. Extracts were assayed for presence of CTV via double sandwich indirect (DAS-I) ELISA described in an accompanying paper (7). Isolates of CTV were differentiated into two groups, designated here as *mild*, i.e. non-decline-inducing, and *severe*, i.e. potentially decline- or stem-pitting-inducing, isolates. These designations were 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 CTV and are able to detect all CTV isolates (4, and Garnsey et al., unpublished data). The second probe consisted of the single monoclonal antibody MCA13, which reacts to the majority of decline-inducing and stem-pitting isolates of CTV but does not react to mild isolates causing neither symptoms (15). The ability to discriminate between decline-inducing 'severe' and non-decline-inducing 'mild' CTV isolates was confirmed for those isolates existing in the northern Costa Rican study area (S. Garnsey, unpublished data). Thus a sample reacting to the first probe but not the second was designated as *mild*, whereas a sample that reacted to both probes was designated as *severe*.

**Analysis of temporal and spatial spread.** The incidence and spatial location of mild- and severe-CTV infected trees were mapped for each plot by assessment date and the temporal change in incidence calcu-

lated. Temporal data were fitted to exponential and Gompertz models by linear regression (2, 14). The appropriateness of the models was determined by examination of residual plots and correlation of observed vs. predicted values (8, 9, 14).

The change in the number and proportion of mild, severe, and uninfected trees was examined between sets of two consecutive assessment dates. Assessment periods were selected for each of the plots based on the presence of sufficient num-

bers of both mild- and severe-CTV infected trees to perform the statistical calculations (Fig. 1). The proportion of new CTV severe-positive trees, i.e. *hits*, that occurred in trees previously tested positive for mild-CTV and trees previously tested negative for CTV on the prior assessment period were calculated, such that:

$$\Gamma_m = s/m \quad \text{and} \quad \Gamma_u = s/u,$$

where  $s$  is the number of new severe-CTV infected trees that occurred on

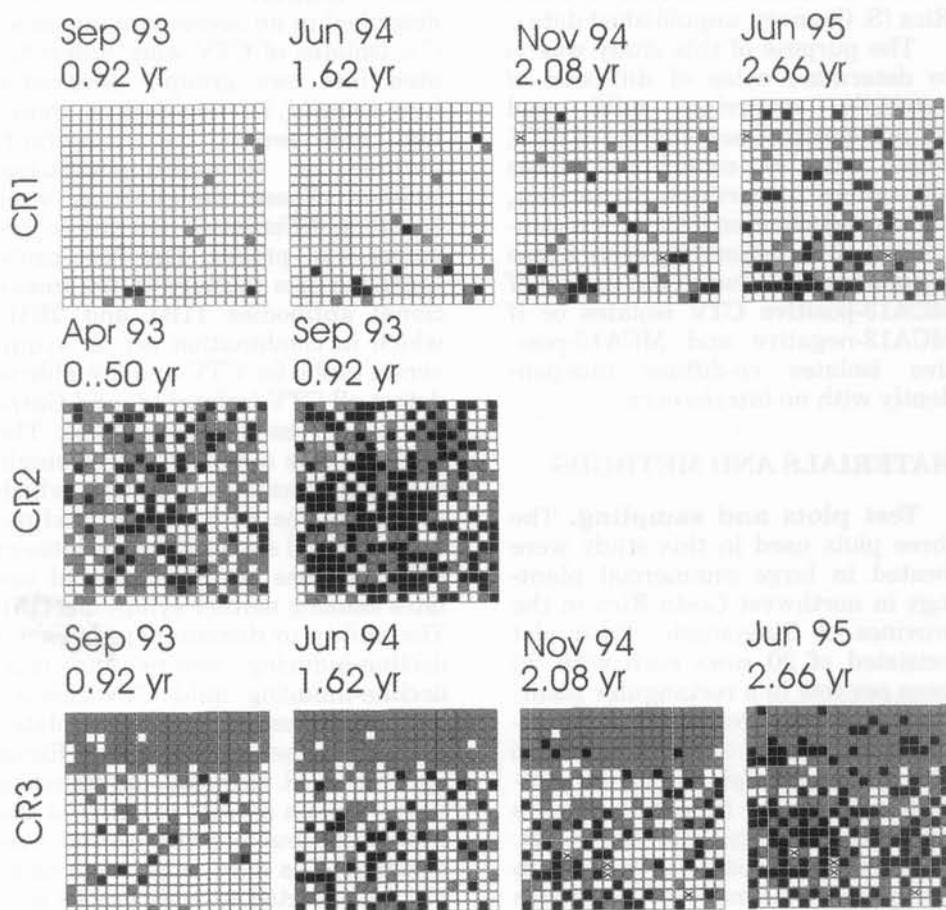


Fig. 1. Plot maps for the CTV experimental plots for northern Costa Rica. Each plot consists of 20 trees per row and twenty rows. Plot 1, plot 2, and plot 3 incidence maps are displayed on the first, second, and third row of the figure, respectively. White, gray and black squares indicate the relative location of uninfected, mild CTV-infected, and severe CTV-infected trees, respectively. Only the assessment periods used for comparisons are shown. For plot CR2 after September 1993, the number of severe CTV positive trees was too large for a sufficient number of statistical comparisons to be made. CTV incidence in plot CR4 was too low to use in this study.

previously mild-CTV infected,  $m$ , and previously uninfected,  $u$ , trees, respectively. Because the number of previously uninfected and previously mild-CTV infected trees was not equivalent for each of the comparisons being made, a weighting factor,  $w$ , was used such that:

$$w = 100/(\Gamma_m + \Gamma_u).$$

Thus, the relative probability of a new severe hit on a previously mild-CTV infected tree,  $P_m$ , and the probability of a new severe hit on a previously uninfected tree,  $P_u$ , were calculated as:

$$P_m = w * \Gamma_m \quad \text{and} \quad P_u = w * \Gamma_u,$$

respectively.

## RESULTS

**Analysis of temporal disease progress.** Fig. 1 shows the temporal

increase and spatial spread of MCA13-positive and MCA13-negative isolates in the three experimental plots selected. Both exponential and Gompertz models fit the CTV temporal data well (Table 1). In the majority of cases, the CTV progress curves did not reach an asymptote (Fig. 2). Therefore, the Gompertz model was not fully utilized because only the exponential function portion of the model equation was employed. Because the exponential model is simpler, fully utilized, and a better estimator of CTV progress in most cases, it was the general model chosen to describe CTV progress over time. For all three plots studied, total CTV progress was very rapid. Model predictions based on the Costa Rican plot data indicated that CTV increase from ca. 5 to 95% incidence would require only 4-6 years,

TABLE 1  
LINEAR REGRESSION ANALYSIS OF THE INCIDENCE OF CITRUS TRISTEZA VIRUS IN GROVES IN NORTHERN COSTA RICA OVER TIME DETECTED BY DIFFERENTIAL ELISA

CTV Population	Plot	Model <sup>1</sup>	Rate ( $b$ )	Std. Err.	y-intercept	Std. Err.	Coef. of determ. ( $r^2$ ) <sup>2</sup>	Corr. Coef. ( $R^{*2}$ ) <sup>3</sup>
Total CTV	1	E	0.972	0.087	-4.158	0.146	0.969	0.867
		G	0.396	0.068	-1.516	0.114	0.894	0.868
	2	E	0.355	0.057	-0.840	0.095	0.969	0.972
		G	1.806	0.168	-0.503	0.280	0.967	0.972
	3	E	0.404	0.006	-1.329	0.011	0.999	0.992
		G	0.627	0.070	-1.454	0.117	0.952	0.992
Severe CTV	1	E	2.132	0.009	-7.780	0.021	1.000	0.823
		G	0.756	0.076	-2.745	0.173	0.990	0.883
	2	E	0.665	0.114	-1.670	0.190	0.895	0.971
		G	1.252	0.117	-0.877	0.194	0.967	0.970
	3	E	1.268	0.134	-4.714	0.223	0.957	0.880
		G	0.510	0.083	-1.686	0.138	0.905	0.880
Mild CTV	1	E	0.688	0.068	-4.010	0.113	0.963	0.915
		G	0.237	0.032	-1.422	0.053	0.934	0.915
	2	E	-0.514	0.149	-1.165	0.248	0.748	0.801
		G	-0.271	0.090	-0.210	0.150	0.694	0.801
	3	E	0.230	0.051	-1.277	0.085	0.835	0.915
		G	0.233	0.052	-0.252	0.086	0.835	0.915

<sup>1</sup>Exponential (E) and Gompertz (G) models respectively.

<sup>2</sup>Coefficients of determination ( $r^2$ ) and rates ( $b$ ) were estimated by linear regression of transformed disease incidence over time. Disease incidence values were transformed by  $y$ ,  $\ln(y)$ , and  $-\ln(-\ln(y))$  for exponential, and Gompertz transformations, respectively.

<sup>3</sup>Correlation coefficients ( $R^{*2}$ ) of predicted values against observed values and the presence or absence of patterns in residual plots were examined to test appropriateness of models.

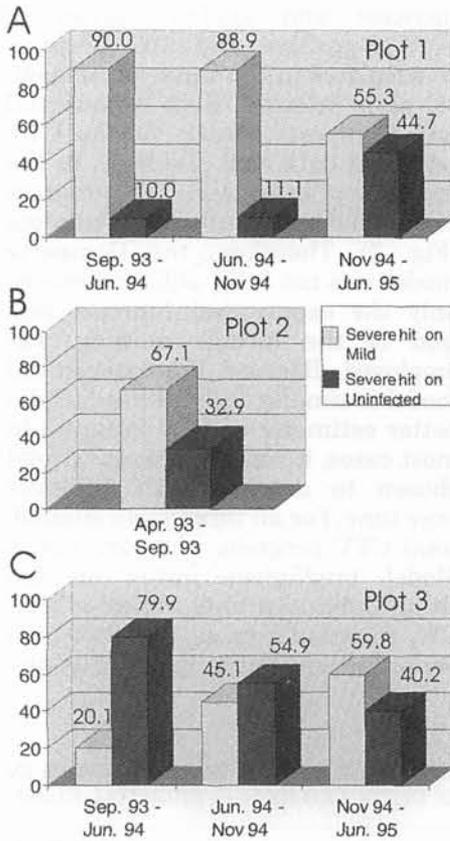


Fig. 2. CTV incidence over time in experimental plots in the Guanacaste province of northern Costa Rica. Note the rapid increase of virus infection during the ca. three years of the test. Decreases in mild CTV incidence are due to masking by severe hits on previous mild trees.

which is much faster than rates calculated for previous CTV epidemics where *A. gossypii* was the most efficient vector species present (5, 8, 9, 12, 13). In two cases, the incidence curves for mild CTV-infected trees decreased over a portion of the time examined (Fig. 2). This decrease was related to masking of previous mild-CTV infected trees by severe, MCA13-positive isolates. The mild CTV isolates were undoubtedly still present, but could not be differentiated once the trees became co-infected with MCA13-positive CTV isolates. Thus, the rates of mild CTV isolate increase were underestimated and this underestimation

became worse as MCA13-positive infections increased.

**Analysis of CTV isolate interaction.** The probability or likelihood of severe-CTV isolate infection of previously uninfected and mild-CTV isolate infected trees is shown in Fig. 3. For Plot 1, there was a disproportionately high number of severe isolate hits on previously mild isolate infected trees. This is because these calculated proportions represent only 2, 3, and 4 severe hits on previous mild isolates, respectively, and can be attributed to chance. Plot 2 also demonstrated a higher probability of severe isolate hits on previously mild isolate infected trees. For Plot 3 the results were mixed. For the first two comparisons it was more likely that a severe isolate would hit an uninfected tree. This

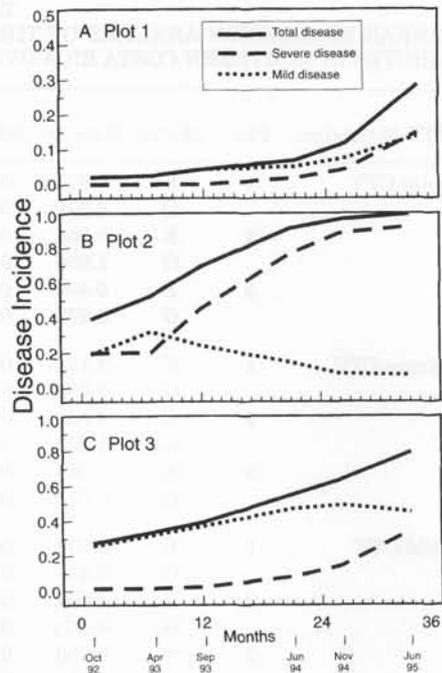


Fig. 3. Relative probabilities that a severe CTV isolate would infect previously uninfected trees and trees previously infected with a mild CTV isolate. Bars indicate the calculated probabilities for each plot for comparison of the two assessment periods indicated.

trend was reversed for the third comparison.

**CTV biocharacterization.** To date, seven CTV isolates from the study plots in northern Costa Rica have been incorporated into the worldwide CTV collection and tested against a host range for symptom expression at the USDA, ARS laboratory in Beltsville, Maryland, USA. Two of the seven isolates were MCA13-negative and did not cause stem pitting of either grapefruit or sweet orange. Five of the seven isolates were MCA13-positive caused a mild decline reaction and two of these also caused mild stem pitting in grapefruit.

## DISCUSSION AND CONCLUSIONS

Temporal increase of CTV was very rapid for all plots examined in northern Costa Rica. Models developed from this data indicated that only 4-6 years are required to reach asymptotic (i.e. >95%) levels of CTV. In contrast, 8-15 yrs are required to reach an asymptote of CTV infection where *T. citricida* was absent (8, 9). Other factors could be involved but the major difference is presumably due to the prevalence of *T. citricida*. Results presented are consistent with those found in Reunion, Dominican Republic, and Puerto Rico where *T. citricida* is also the predominant vector (12, 13).

Primary infections in commercial plantations where the study plots were established almost certainly arose from propagation of some infected bud sources collected elsewhere in Costa Rica. However, at least some CTV-infected budwood sources can be traced to Florida, which is known to have a significant proportion, 4 to 76%, of nursery trees infected with severe-CTV isolates (17). Therefore, some of these isolates were imported into northern Costa Rica with the importation of propagating material from Florida.

At least two serologically distinct CTV isolates were present in the study areas. However, it was only practical to discriminate the field CTV isolates detected into broad groups, i.e., *mild* and *severe* based on their reaction to MCA13. Therefore, we studied the interaction of the population of mild-CTV isolates with the population of severe-CTV isolates. The interaction among individual mild isolates and among severe isolates was not examined.

Mild- and severe-CTV sources existed both within, and in the immediately surrounding areas outside of the study plots. Therefore, the origin of new infections in the study plots is unknown but presumed to have been vectored from infected trees both inside and outside the plots. It is unlikely that severe isolates preferentially infected trees previously infected with mild isolates. This would presume that there is a synergistic compatibility for coinfection between mild-CTV and severe-CTV isolates which has never been reported. The more plausible explanation is that this is a chance occurrence and that the mild- and severe CTV isolates that exist in the commercial plantings studied co-diffused and disseminated through the plots with no discernible competitive interference. A similar occurrence for the diffusion of severe CTV isolates into trees previously infected with mild CTV isolates in Florida has been previously reported (16). Therefore, we conclude that the co-diffusion of mild-CTV and severe CTV isolate populations was generally independent. This further indicates that the population of naturally-occurring mild CTV isolates in the area afforded little or no cross-protection against the population of severe isolates. Similar lack of cross-protection has been previously observed (16, 19).

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