The Effect of Aphid Vector Population Composition on Local and Background Components of Citrus Tristeza Virus Spread

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ABSTRACT. Composition of aphid vector populations has been shown to affect the evolution of spatial patterns of citrus tristeza virus (CTV) by affecting transmission and spread of the virus. However, the spatial processes associated with various vector populations are not well described. In this study, the spatio-temporal dynamics of CTV were examined using research plots representing two diverse pathosystems: i) where the melon or cotton aphid, *Aphis gossypii*, was the predominant species and the brown citrus aphid, *Toxoptera citricida,* was absent, and ii) where *T. citricida* was the predominant vector. Data were analyzed using a spatio-temporal stochastic model for disease spread that was fitted using Markov-Chain Monte Carlo stochastic integration methods. For the CTV/*Aphis gossypii* pathosystem, the model parameter likelihood values supported the theory that CTV was spread through a combination of random background transmission (transmission originating from outside the plot) and a local interaction (transmission from sources within the plot) that operated over short distances. Conversely, for the CTV/*Toxoptera citricida* pathosystem, results often suggested a local short range interaction that was not restricted to nearest-neighbor interactions, and that the presence of background infection was not necessary to explain the observed spread.

Recent publications have demonstrated that CTV pathosystems can be separated into two general categories (8, 9, 10). These two categories are characterized by the species composition of aphid vectors that exert the major influence on spread of CTV. Historically, the most common pathosystem in the Western Hemisphere has been the CTV/*Aphis gossypii* pathosystem. Although other aphid species are often present and at times more numerous than *A. gossypii*, the aphid species assumed to most influence spread of CTV in this pathosystem is *A. gossypii*. This is due to its greater ability to transmit CTV as compared to other species (2, 12, 13, 15). Citrus is not a primary host for *A. gossypii* and is generally not heavily colonized by this aphid. Apparently, CTV is often vectored by *A. gossypii* as migrants of this aphid species move through the orchards from surrounding areas or crops (10, 15).

Historically, the pathosystem most prevalent throughout Asia and the Far East has been the CTV/*Toxoptera citricida* pathosystem. This pathosystem is dominated by *T. citricida,* but other aphid species, including *A. gossypii*, are still present although overshadowed in epidemiological significance (11). Citrus is the primary host for *T. citricida*, which can form large colonies on citrus under favorable conditions (1, 12, 14). In contrast to *A. gossypii*, citrus is the sole host for *T. citricida*. The transmission efficiency of *T. citricida,* combined with the large populations it establishes in commercial citrus in relationship to other aphid species, results in changes in the spatial and temporal dynamics of CTV once the virus is established. Following the introduction of *T. citricida* into Argentina in the 1930s, the aphid subsequently spread throughout South America, the Caribbean, and into Florida (9, 14). As *T. citricida* became a component of the pathosystem, CTV increase and spread were elevated. In South America this resulted in CTV-related tree and crop losses (9, 11, 14).

Increase of virus infection is strongly affected by pathosystem composition. Epidemics of CTV

decline-inducing isolates progressed from low to high CTV incidence (0.5 to 95%) in 8 to 15 yr depending upon scion cultivar, etc. in the presence of the CTV/*A. gossypii* pathosystem, whereas, the CTV/*T. citricida* pathosystem resulted in the same increase in 2 to 6 yr (8, 9, 10). Spatial spread of CTV is also affected by pathosystem. In the presence of the CTV/*A. gossypii* pathosystem, spread has been demonstrated to be either random (8) or a combination of local and background interaction (5). In contrast, CTV spread associated with the CTV/*T. citricida* pathosystem has been demonstrated to result from primarily local interactions within a defined area of about four to nine trees (9).

Gibson has recently demonstrated the use of a spatio-temporal stochastic model for disease spread that was fitted using Markov-Chain Monte Carlo integration methods (5, 6, 7). In the present study, this same methodology was applied to compare multiple assessments through time. The purpose of this study was to examine and compare the underlying mechanisms that affect CTV spread in relationship to the two aforementioned pathosystems. Specifically, we wanted to determine the contribution of local transmission (i.e., acquisition of the virus from CTV-positive individuals in a defined host population followed by transmission to other individuals within that host population) versus the contribution of background transmission (i.e., virus transmission from vectors originating outside the host population being studied) on the spatial patterns of CTV.

MATERIALS AND METHODS

The experimental design was based on separating the research plots into two major groups according to the predominant aphid vector species present in the plots. The first group consists of plots where *A. gossypii* was the predominant vector species = CTV/*A. gossypii* pathosystem. In this group data were collected annually over a 7-yr period from 1989 to 1995 in nine plots established within large commercial plantings of the U.S. Sugar Corporation in south Florida. All plots consisted of Rhode Red Valencia orange grafted on sour orange rootstock planted in 1987. Each plot consisted of approximately 476 trees arranged in 14 north-south oriented rows each with 34 trees per row in a rectangular pattern. All data were collected prior to the 1996 introduction of *T. citricida* into Florida. For this group, CTV incidence was low at the beginning of the study in all cases.

The second group consisted of plots where *T. citricida* was the predominant vector species = CTV/*T. citricida* pathosystem. In this case data were collected from four plots, each established within commercial plantations in northwest Costa Rica. All plots consisted of approximately 20 rows of trees, each with 20 trees per row in a rectangular planting pattern within larger commercial plantings that ranged from 1- to 5-yr-old at the beginning of the study. Plots were designated: CR1 = a pineapple sweet orange planting on Cleopatra mandarin, CR2 = Valencia sweet orange planting on Cleopatra mandarin, CR3 = a Valencia sweet orange planting on local grapefruit, and CR4 = a pineapple sweet orange planting on Carrizo rootstock. No aphid control procedures were applied in any of the plots. *T. citricida* was present in all locations when the experiments were started. For this group CTV incidence varied from low to moderate at the beginning of the study.

Sample collection and ELISA processing. The two pathosystems differed in rates of virus increase and thus different sampling intervals were used. The CTV/*A. gossypii*

pathosystem plots (in which CTV incidence progressed slowly) were sampled once per year in the spring, whereas the CTV/*T. citricida* pathosystem plots (where CTV incidence increased more rapidly) were sampled in the spring and fall of each year. Samples consisted of four leaf petioles from young, nearly fully expanded leaves taken from the periphery of each tree. Every tree was tested independently. For the Costa Rica plots, 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 of a moisture-indicating silica gel. The silica gel was changed as needed and the dry samples were then transported to the USDA-ARS laboratory in Orlando. For the Florida plots, fresh samples were transported to the U.S. Sugar Corporation research labs in Clewiston, Florida, for immediate processing. 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 presence of CTV via double sandwich indirect (DAS-I) ELISA as previously reported (3, 4).

Spatio-temporal analysis. Data for the CTV epidemics were analyzed using the spatio- temporal stochastic model for disease spread which was fitted using Markov-Chain Monte Carlo (MCMC) stochastic integration methods. For a thorough description of the MCMC model, its application, and interpretation of results, refer to Gibson (6). The results of the spatio-temporal analysis can be viewed graphically in a two-dimensional parameter space representing a series of 'likelihood' contours of parameter densities. The two parameters represent local (a_2) versus background (b) interactions. The parameter b quantifies the rate at which a susceptible individual acquires the disease due to primary infection from sources outside the host population, whereas a_2 quantifies the manner in which the infective challenge presented to a susceptible individual by a diseased individual in the population decreases with the distance between them. As a_2 increases the secondary transmissions occur over shorter ranges and, so long as b is not so large that primary infections dominate, disease maps generated by the model exhibit aggregation.

RESULTS AND DISCUSSION

As previously reported, the temporal increase of CTV differed dramatically depending on the pathosystem, i.e., CTV increase is much more rapid for the CTV/*T. citricida* versus the CTV/*A. gossypii* pathosystem (8, 9, 10). In the present study we will examine the spatio-temporal dynamics of each pathosystem individually. Using the MCMC model, there was a remarkable similarity among the likelihood contours associated with each pathosystem. Therefore only a representative surface is shown for each pathosystem (Fig. 1a,b). For the CTV/*A. gossypii* pathosystem, the highest likelihood values corresponded to cases where a_2 was positioned towards the maximum of its range and b was nonzero, usually in the range [0.25, 1.0] (Fig. 1a).

In contrast, inspection of the analogous likelihood surfaces for the cases where *T. citricida* is the main vector, revealed a quite different story (Fig. 1b). In some cases, disease incidence was too high during the first assessment, or did not change sufficiently over the duration of the epidemic for the data to be of value. Therefore, plots were selected only if they represented epidemics with a wide range of disease incidence over several assessments. With this caveat understood, the likelihood was negligible, except

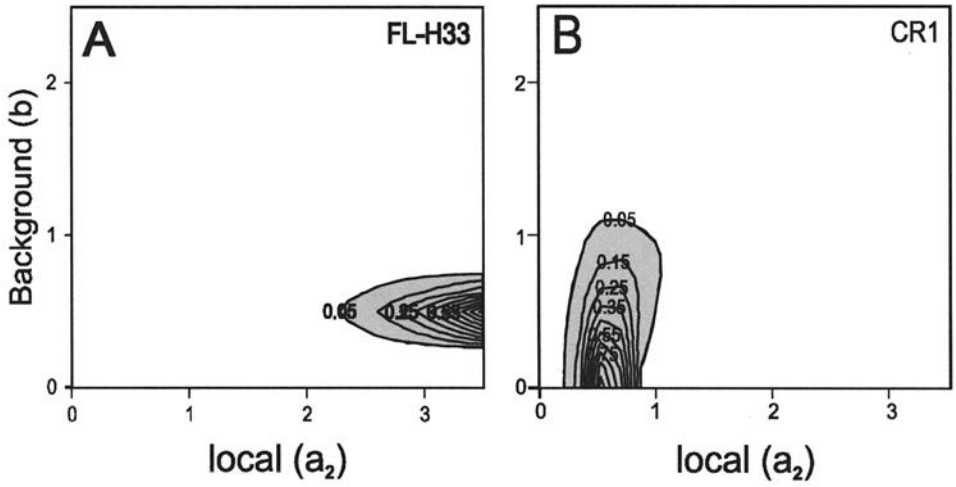

Fig. 1. Spatio-temporal comparisons of Markov-Chain Monte Carlo model fits to two CTV pathosystems. A) CTV/*A. gossypii* pathosystem: Contour plots of parameter densities for a representative sweet orange plot in south central Florida sampled over seven assessments. Graphic obtained by estimating the value of densities over a 14 × 50 grid of parameter values with contours estimated by interpolation. B) CTV/*T. citricida* pathosystem: Contour plots of parameter densities for a representative plot in Costa Rica sampled over eight assessments. Graphic obtained by estimating the value of densities over a 20 × 20 grid of parameter values with contours estimated by interpolation.

over a region of parameter space in which b is typically less than 1 and a_2 assumes values towards the lower end of its range. Importantly, the likelihood value is large for cases where $b = 0$ (Fig. 1b).

Interpretation of these spatio-temporal stochastic model results led to strikingly different conclusions concerning CTV spread depending upon the CTV pathosystem investigated. For the CTV/*A. gossypii* pathosystem, the model suggested that CTV spread through a combination of random background transmission (from inoculum sources outside the plots) and a local transmission that operated over short distances and was primarily nearest neighbor. In the case of the South Florida plots, a change was seen in CTV incidence as the canopies and root systems of individual trees grew together within row. At this point, within row transmission was apparent and was thus consistent with the nearest-neighbor transmission effect suggested by the stochastic model. The model parameters also indicated that the patterns were highly unlikely to have arisen as a result of purely secondary transmission, at least for this particular model. Previous analyses of the CTV/*A. gossypii* pathosystem investigated in Spain were incapable of distinguishing the spatial patterns of CTV-infected trees from a random pattern (8). When the stochastic model was applied to a portion of the Spanish data, the same conclusions were arrived at as for the CTV/*A. gossypii* data sets used in the present study (5).

In contrast, when the model was applied to the CTV/*T. citricida* pathosystem for which *T. citricida* was the predominant vector but *A. gossypii* was also present, the results suggested a short-range local infection mechanism which was not restricted to nearest-neighbor interactions. Results also suggested that transmission may have been purely local and that the presence of background infection from sources outside the plot was not necessary to explain the observed virus spread.

As discussed in recent studies, the two CTV pathosystems differ significantly due in part to the dispersal behavior of the predominant vector species, that is, *T. citricida* = citrus colonizer versus *A. gossypii* = migrator from surrounding crops through citrus (8, 9, 10). These studies lead to the not unexpected conclusion that the diverse aphid vector biology in relationship to citrus influence virus transmission and dispersal. These interactions are reflected in sharp differences in the spatio-temporal dynamics of the virus. The stochastic MCMC model analyses provide additional support for these conclusions, but other interpretations are possible. The stochastic model chosen was very simple and represents the background and local components of the infection process in an elementary way while the biology of the CTV system is extremely complex and disease spread is affected by many processes operating at diverse scales. Nevertheless, we believe the analyses presented here are a valuable way of summarizing the CTV spatio-temporal data, suggesting the quantitative and qualitative effect of the transmission process on spatio-temporal dynamics. In the case of the two pathosystems investigated in this study, the model clearly differentiated the spatio-temporal dynamics between the two pathosystems in a way consistent with the current understanding of CTV virus/aphid vector dynamics.

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