History, Present Incidence, and Spatial Distribution of *Citrus tristeza virus* in the California Central Valley

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ABSTRACT. *Citrus tristeza virus* (CTV) has been a primary concern in the San Joaquin Valley of California since its detection in 1956. Virus eradication was deemed necessary and was undertaken by the California Department of Food and Agriculture. Since 1963 the eradication program has been managed by the Central California Tristeza Eradication Agency (CCTEA) and funded by citrus growers. The replacement of biological indexing on lime by ELISA in 1990 revolutionized detection, and allowed the first systematic survey of all commercial acreage to be conducted from 1990 to 1995. It estimated a disease incidence of 0.367%. An international workshop held in 1995 recommended a 3-yr Operational Plan to remove over 50% of the CTV-infected trees annually to keep ahead of disease increase. In 1998 the aggressive tree removal plan was deemed a success and was replaced by a Maintenance Plan that is still in effect today. All commercial acreage is surveyed every 5 yr using the Hierarchical Subsampling (HS) Method, which more accurately estimates low levels of disease incidence than previous methods used. Groves testing positive are prioritized from highest to lowest infection levels, and surveyed in preparation for tree removal as resources allow. Currently, the estimated incidence in areas practicing eradication is 0.120%. The HS method is based on the spatial distribution of CTV-positive trees and simulation modeling and was developed by the statistical examination of plot maps of 36 orchards at various times. The spatial patterns of the CTV-infected orchards were examined by Beta-binomial and spatial autocorrelation analyses. Since the brown citrus aphid (*Toxoptera citricida*) does not exist in California at this time, the spatial patterns of CTV spread are believed to be predominantly associated with the melon aphid (*Aphis gossypii*) or the use of infected propagation material. Further, these analyses indicated that spatial patterns associated with the Central California CTV/*A. gossypii* pathosystem are difficult to distinguish from random patterns, both on an individual tree basis and on larger spatial scales of groups of trees over distance. This is in contrast to situations where the CTV/*T. citricida* pathosystem has been similarly examined.

Index words. Surveys, epidemiology, vector transmission, dissemination.

Citrus tristeza virus (CTV) is generally recognized as the most economically important virus disease of commercial citrus. Symptoms of tristeza vary according to the rootstock/scion combination and the strain of the infecting virus. These can be mild causing no economic impact or severe including collapse of sweet orange varieties grafted onto sour orange rootstock, stem pitting of sweet orange and grapefruit, decrease of the productive life of a tree, stunting, and small fruit size. Recent molecular characterization of the virus indicates less than 60% homology between some strains. Further, disease symptoms have not been correlated to specific nucleotides or sequences of the CTV genome.

Present distribution of CTV in California. There are approximately 275,200 acres of commercial citrus grown within the state of California (3), distributed in three distinct growing regions (Fig. 1), each with different levels of disease pressure. Less than 1% is grown in northern California in the vicinity of Oroville; little is known of the incidence of CTV in this area as no survey work has been conducted. Another 18% is grown in southern California along the coast from Ventura to San Diego and in the desert region of the Coachella Valley. With the exception of the Coachella Valley and some portions of Riverside

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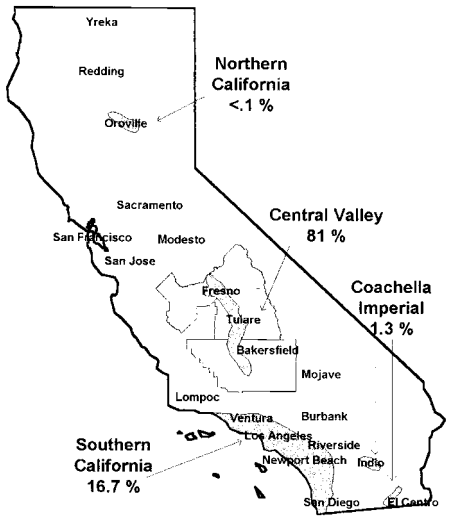

Fig. 1. Distribution of commercial citrus in California. There are approximately 275,200 acres of citrus commercially grown in the state of California. In the northern, colder region, mostly mandarin varieties are grown. The majority of citrus in the southern region is varieties of lemons grown along the coast. The Coachella Valley and Imperial County are desert climes where grapefruit and lemons dominate. The Central (or San Joaquin) Valley favor navel and Valencia oranges but many exotic citrus varieties are produced.

County, CTV is endemic to this area. The majority of the industry (81%) is found in the central San Joaquin Valley, in the four counties of Fresno, Kern, Madera, and Tulare. Here, the majority of citrus is high-quality table fruit including many exotic varieties grown for the fresh fruit and export markets. The San Joaquin Valley is unique because the industry has maintained a low level of CTV incidence. This is partially attributed to its geographical isolation by mountains: the Sierra Nevada to the east, the Tehachapis to the south and the Coast Range to the west. These barriers impede the natural entry of exotic pests and diseases into the Valley. Programs implemented by man including a budwood and nursery registration program, a statewide quarantine enforced by the California Department of Agriculture (CDFA) (Fig. 2) and County Agricultural Commissioners, and an active suppression program also contribute to controlling the spread of CTV. Despite these programs, CTV is found in this region.

An abbreviated history of CTV in California is presented in Table 1. In 1963 the Central California Citrus Pest Control Agency assumed responsibility for locating and removing CTV-infected trees and was later renamed as the Central California Tristeza Eradication Agency (CCTEA). The CCTEA was formed when five citrus pest control districts in the counties of Fresno, Tulare and Kern entered into an Agreement for Joint Operations of the Tristeza Eradication Program (Joint Powers Agreement, or JPA). Currently the JPA is composed of three pest control districts; two of the original five districts withdrew in 1995 and 1996 (Fig. 3). The program is grower-funded through a special assessment on growers' citrus acreage.

The CCTEA's stated purpose is twofold: 1) To identify and eradicate CTV in a timely, orderly and cost-effective manner; and 2) To encourage and support appropriate research programs that pursue methods to eliminate the threat of the virus. Information and data acquired during program operations are made available to collaborative researchers. The intention is to ensure that all trees planted in "Suppressive Areas" remain virus-free by locating any CTV reservoirs that may not have been previously detected and by utilizing new technologies, as they become available.

The CCTEA implemented a new Operational Plan in July 1998 (Table 1). Rather than aggressive tree removal, it was based on maintenance components including the weekly monitoring of virus titer in commercial trees, a new Systematic Subsampling Survey using the Hierarchical Subsampling Method, and

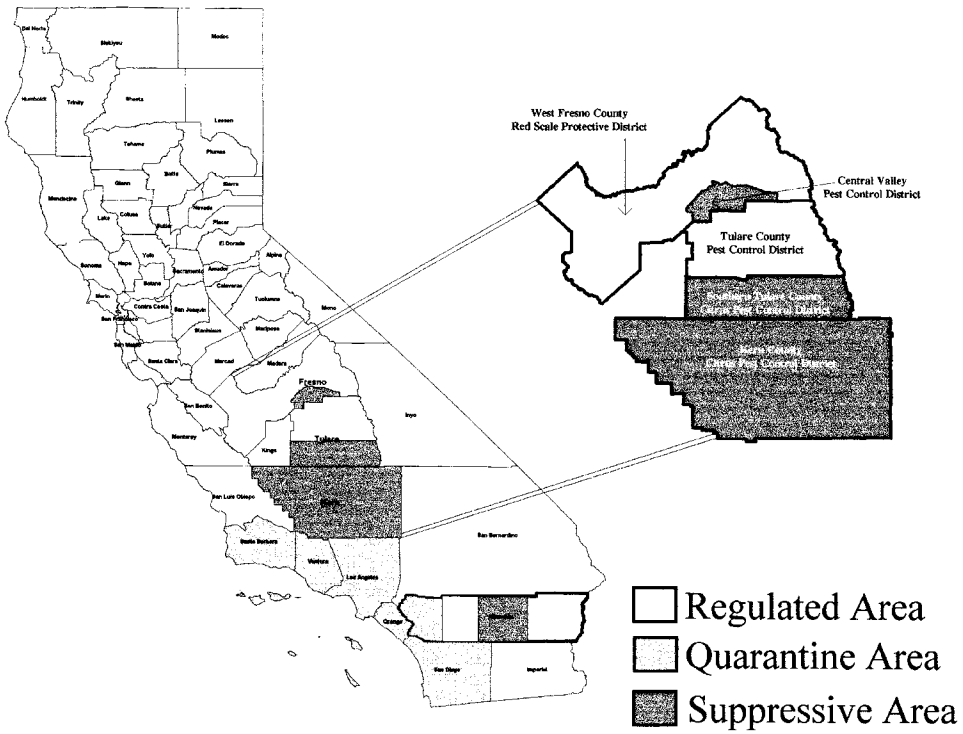

Fig. 2. The Citrus Tristeza Virus Interior Quarantine designated areas as revised in 1998. The Tulare County Pest Control District withdrew from the mandatory eradication program in 1996. There has been no monitoring of CTV incidence there since 1998. The West Fresno County Red Scale Protective District withdrew from mandatory eradication in 1995 but continues to survey on a small scale. No infection has been found in this district in the last 4 yr. Riverside County contains all three designations.

Singles Survey prompted by a regularly updated Collection Priority List. From 1998 through 2002, all commercial acreage was on a 4-yr sampling cycle. In July 2002, the duration was extended to a 5-yr cycle.

The eradication program has traveled some rocky roads in recent years. In 1997 the Agency and the Kern County Citrus Pest Control District filed an abatement action against four growers who refused to remove over 7,000 CTV-infected trees. After the Kern County Superior Court ruled in favor of tree removal, the growers took the case to the State of California Court of Appeals. Early in 2000, the three-member panel of judges unanimously found that, under the state's Citrus Pest District Control Law, maintenance of citrus trees infected with CTV constitutes a public nuisance and such trees must be removed. The case was sent back to the Kern County Superior Court for enforcement and finally, on July 7, 2000 removal of these trees began. Five citrus groves encompassing 80 acres and containing almost 5,400 infected trees were bulldozed. Unfortunately, this did not occur before the tristeza virus had the opportunity to spread into groves adjacent to this highly-infected, 80-acre parcel.

Spatial analyses: Data generated by ELISA methods is here considered binary, that is, the virus is taken to be either present or absent. Several methods exist to perform a quantitative analysis of spatial patterns of disease at a single point in time. When looking to see if disease incidence is aggregated *within* quad-

TABLE 1
HISTORY OF *CITRUS TRISTEZA VIRUS* IN CALIFORNIA

Late 1800s	Tristeza was introduced to California when over a million satsuma mandarins grafted on trifoliate orange stock and budwood of various scion varieties were imported from Far East countries.
1908	Meyer lemons infected with seedling yellows strains were imported into the United States and distributed throughout California.
1939	Tristeza was recognized as a new problem in southern California when sweet orange trees grafted on sour orange rootstock began to collapse—quick decline.
1945	Gil Stout headed a survey by the California Department of Food and Agriculture of 7,656,041 citrus trees on 113,557 acres. CTV-infected trees were found on 62, 25, 12, and 2 properties in Los Angeles, Orange, San Bernardino, and Riverside Counties, respectively, but none in Ventura or San Diego Counties or in central California.
1945-1960	Nearly all trees in Los Angeles, Orange, Ventura, San Bernardino, and Riverside Counties became infected with CTV. An interior quarantine was enacted by the State of California to prevent spread into CTV-free areas which prohibited the sale of citrus trees propagated within designated quarantine areas.
1955	Survey results showed nearly all Meyer lemons in the state infected with CTV.
1955-1963	5,600 Meyer lemon trees removed from over 3,800 properties within the San Joaquin and Coachella Valleys. “Improved virus-free Meyer Lemon” variety released.
1956 & 1959	Surveys conducted by the California Department of Food and Agriculture discovered CTV-infected trees in Tulare County located within the San Joaquin Valley.
1958-1960	Probable distribution of >12,000 infected nursery trees to growers in Tulare County.
1963	The Central California Citrus Pest Control Agency, later renamed as the Central California Tristeza Eradication Agency (CCTEA), took on the responsibility for locating and removing tristeza infected trees in Fresno, Tulare, and Kern Counties. Five citrus pest control districts in these counties joined together under a joint powers agreement to: identify and eradicate CTV in a timely, orderly, and cost-effective manner; and encourage and support appropriate research programs that assist to eliminate the threat of the virus. The CCTEA is grower-funded by a special assessment on citrus acreage.
1990	Several groves in Tulare County were found highly infected with CTV. ELISA replaced Mexican lime indexing as the approved diagnostic procedure. This allowed for an expanded survey program.
1990-1995	The first systematic subsampling survey of all known citrus groves (about 191,000 acres) in the three counties was conducted. It revealed an estimated infection rate of 0.367%. Based on these results, a Collection Priority List (CPL) was developed of all groves with suspected infected trees, sorted from highest to lowest infection.
1995	International CTV workshop and panel of experts called to focus on the CTV situation in the San Joaquin Valley. Participants concluded that effective eradication would be possible in the tri-county area if an aggressive effort was initiated as soon as possible.
1995	Three-year Aggressive Removal Operational Plan implemented at significantly increased assessment rates. Primary objective: to annually remove greater than 50% of trees infected with CTV. Efficacy of this plan was evaluated by subsampling 10% of citrus acreage, randomly selected, each year.
1995	The least-affected West Fresno County Red Scale Protective District withdraws from eradication program due to cost issues.
1996	Tulare County Pest Control District withdraws from program on grower objection to tree removal. The Central Valley Pest Control District, Southern Tulare County Citrus Pest Control District, and Kern County Citrus Pest Control District representing approximately 105,000 citrus acres, continue the CCTEA in a Joint Powers Agreement.
1996-1998	Grower assessments (per hundred-tree acre) for Agency operations reduced and customized by district.

TABLE 1 (CONTINUED)
HISTORY OF *CITRUS TRISTEZA VIRUS* IN CALIFORNIA

1998	CCTEA Operational Plan evaluated by both the CCTEA Board of Commissioners and the Technical Advisory Committee for the eradication program. It was determined that the removal of CTV-infected trees had been effective based on survey results: 1992-95 and 1997-98 surveys estimate 0.316% and 0.167% infection, respectively. The committee recommended that eradication should continue but an aggressive removal program is no longer necessary.
March 1997-2000	Kern County Pest Control District and the CCTEA filed suit against a group of growers who refused to remove CTV-infected trees; 3 groves were > 70% infected. Both the Kern County Superior Court and the 5th District Court of Appeals ruled in favor of tree removal. Known infected trees were removed immediately and groves were re-surveyed.
July 1998-2002	The Agency implemented a new Operational Plan based on maintenance components that included a new Systematic Subsampling Survey of 25% of citrus acreage per year using the Hierarchical Survey Method; removing as many CTV-infected trees as resources allowed; and the weekly monitoring of virus titer in commercial trees. The overall average infection level for 1998-2002 was 0.120%.
July 2002-current	Agency Operational Plan revised to decrease the annual systematic subsampling acreage to 20% annually (5-yr rotation). During 2002, a greater emphasis will be placed on the Collection Priority List to keep incidence in a single grove below 1.0%.

rats (groupings of field trees) of various sizes, the most appropriate method is the Beta-binomial discrete distribution (13, 15). Spatial autocorrelation analysis (SAA) can be used with both binary and quantitative data to analyze the spatial pattern of disease incidence *between* quadrats (1, 7). Madden and Hughes (16) suggested that for analysis of disease incidence data, the simultaneous study of correlations within quadrats (as provided by the beta-binomial distribution analysis) and between quadrats (as provided by SAA) presents new and interesting possibilities for quantifying plant disease epidemics.

Anecdotal accounts of CTV spread and increase in California are varied. The objectives of the present study include: 1) to provide an accurate history and background of citrus tristeza dynamics in California to date; 2) to document spatial patterns of CTV using recent data in individual plantings; and 3) to compare these spatial patterns with those recently analyzed in Florida, Spain, Dominican Republic and Costa Rica.

MATERIALS AND METHODS

CTV detection. Indirect DAS-ELISA using a polyclonal antibody developed in the laboratory of Dr. D. J. Gumpf, University of California, Riverside (Gumpf Rabbit anti-CTV) was used to detect CTV (19).

Determination of virus titer. Titer is the relative level of virus detected in a tree using ELISA as measured by optical density (OD). Sampling test trees on a weekly basis determined 1) the correct times to start and stop field collection of tissue; 2) which survey method was appropriate; and 3) the most suitable type of tissue to collect. Leaf tissue including the petiole was collected from known positive sources. Wherever possible, a minimum of five trees per site were sampled. Five sites, representative of the entire San Joaquin Valley, were selected. However, with the continuous removal of infected trees within the JPA Districts, it was sometimes difficult to locate groves with a sufficient number of infected trees to monitor on a weekly basis for a long term. Test trees were sometimes therefore limited to locations not practicing eradication (e.g., the Tulare County Pest Control District or the West Fresno County Red Scale Protective District) (Fig. 3). It was sometimes necessary to re-examine the level at

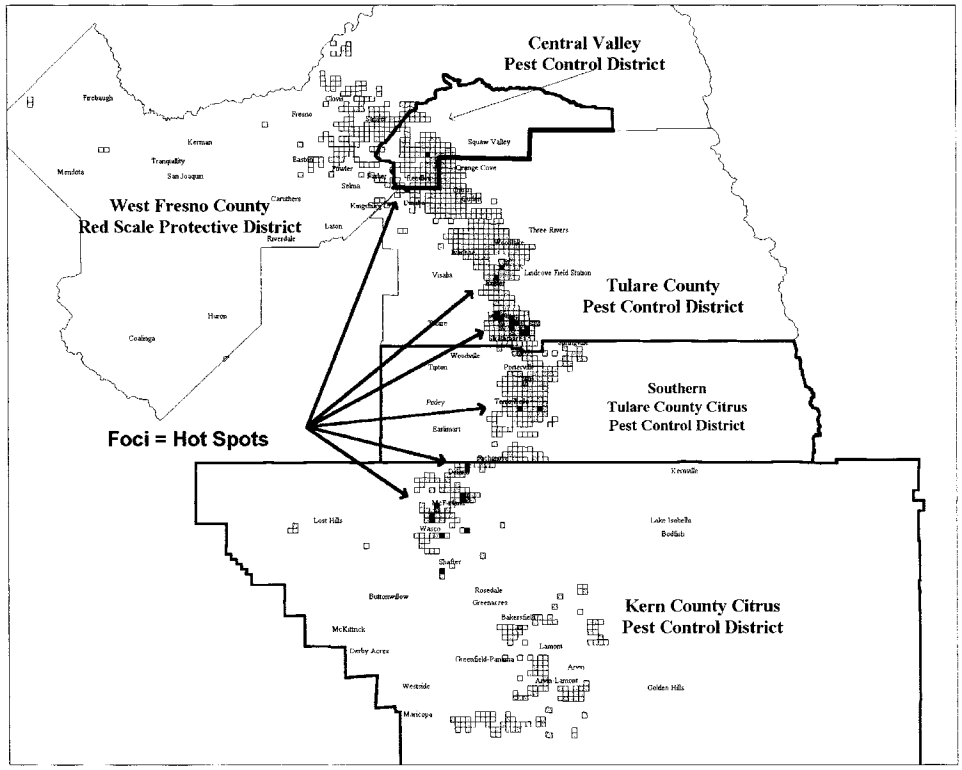

Fig. 3. Disease foci or "hot spots" identified in the Central (or San Joaquin) Valley. Infected trees within the Central Valley Pest Control District, Southern Tulare County Citrus Pest Control District, and Kern County Citrus Pest Control District (bolded) are removed upon diagnosis. The West Fresno County Red Scale Protective District and the Tulare County Pest Control District withdrew from the mandatory eradication program in 1995 and 1996, respectively. Black squares represent a 1 mi² section in which one or more groves had a tree removal rate greater than 10%; medium gray squares, a rate of 5 to 10%; and light gray, less than 5%. The majority of squares are white and represent sections where no significant CTV incidence was found.

which test trees were sampled; that is, the five trees could be sampled from a one-mile square section as opposed to one individual grove.

Sampling method and survey types. All commercial citrus acreage within the three participating pest control districts was surveyed during 1998-2002, targeting approximately 25% of the acreage annually. The selection process for the 4-yr cycle was completed prior to the fall, 1998 collection season. One-mile-square sections within a Township/Range (TRS) were randomly selected until 25% of the total acreage was accounted for. This process was repeated three times. In this way, sections are selected only once, so that in year five, the acreage surveyed would be the same as that sampled in year one (new plantings added). At the start of the 2002 fall collection season, this plan was extended over a 5-yr cycle instead of four. Sections were kept in chronological order except those containing a zero or low disease incidence which were put off until the following year.

The Hierarchical Sampling (HS) Method (10, 12) was utilized for the subsampling survey. In this method, tissue is collected from 25% of the trees in a grove by combining three leaves from each of four trees into one sample. Every fourth group of four trees is sampled. Field trials have indicated that this survey

method is currently the most accurate available, especially in groves with low virus incidence.

Removal of CTV-infected trees was based on a Collection Priority List (CPL). The CPL compiles all sources of infection information such as prior removals, systematic subsampling results, etc. It is: 1) based on the most recent survey results; 2) sorted by percent disease incidence, from highest to lowest, regardless of survey type; 3) updated semi-annually; and 4) addressed starting with the highest estimates of infection as resources allow. Currently, groves with an estimated incidence of 0.80% or greater are targeted for a singles survey. As a means of cost savings, composite sampling is conducted. Initially, two trees are collected as one sample, six leaves per tree. When a positive sample is identified, each tree of the two is tested individually. Before a tree is officially diagnosed as infected with CTV, two independent tissue collections and assays must be conducted, both must have positive results.

Typically, the Hierarchical Subsampling and Composite Singles Methods are used only when the overall average titer is consistently above 0.75 (OD reading) and/or in groves with adequate flush. Tissue collection is not recommended when titer falls below 0.6 except under special circumstances which include: presence of healthy flush in a grove, collection of four samples per tree—one from each directional quadrant (north, south, east, west), feather-shoots, young woody stems, or fruit peduncles.

Spatial analysis. Spatial analysis of the distribution of CTV-infected trees was undertaken to determine if patterns of CTV distribution were similar to those reported from Spain and Florida where similar aphid vector species exist. Twenty-five citrus blocks were selected based on the following criteria: the availability of detailed maps, a sufficiently large number of trees, and a CTV-incidence ranging from 0.004 to 0.19%. In 11 of these blocks, two years of CTV-incidence data were collected, resulting in 36 block-by-assessment year combinations. Data for the second year in these blocks represented new finds of CTV-infected trees as efforts to suppress the virus continued by tree removal.

To examine the data for aggregation of the virus at different spatial scales, the disease incidence data from each block were partitioned into 2 × 2 tree quadrats. Aggregation *within* quadrats was assessed via Beta-binomial analysis, whereas aggregation *between* quadrats was assessed by spatial autocorrelation analysis. The index of dispersion (I_β) associated with the beta-binomial distribution was used to test for the presence of aggregation (10, 13, 15, 16, 17). A large I_β (>1) combined with a small P (< 0.05) suggests aggregation of diseased trees (16). Within each citrus block quadrats containing infected trees were evaluated for the strength and directionality or orientation of disease aggregation. Spatial autocorrelation analysis was performed using the LCOR2 software program because of its ability to handle quantitative values for each tree (2, 9). Input data included the [x,y] spatial location and disease incidence of trees for each 2 × 2 quadrat on each assessment date in the individual citrus blocks. The program determined proximity patterns of positive quadrats (lag positions (SL+)) and calculated the size and shape of core and reflected clusters of SL+, strength of aggregation, and within-row, across-row, and edge effects.

RESULTS

Spatial analysis. Only 10 of the 36 possible block-by-assessment year combinations indicated via Beta-binomial analysis that CTV was aggregated within the 2 × 2 quadrats via Beta-binomial analysis (Table 2). Of these the majority occurred in

TABLE 2
BETA-BINOMIAL INDEX OF DISPERSION (I_β) FOR THE INCIDENCE OF *CITRUS TRISTEZA VIRUS* IN CITRUS PLANTATIONS IN CENTRAL CALIFORNIA

Block	Year	df	I_β	P	Aggregation
Exeter 03	1993	479	1.07	0.150	n
Exeter 21	1992	467	1.02	0.386	n
Exeter 22	1992	527	1.11	0.046	a
	1993	527	1.09	0.076	n
Exeter 28	1992	510	1.08	0.097	n
	1993	510	1.03	0.326	n
Exeter 29	1992	305	1.00	0.500	n
	1993	305	0.99	0.558	n
Exeter 29a	1992	209	1.15	0.068	n
	1993	209	1.07	0.228	n
Exeter 38	1992	224	1.11	0.123	n
	1993	224	1.21	0.018	a
Lindsay 04s	1992	503	1.27	0.000	a
Lindsay 32	1992	288	0.98	0.583	n
Lindsay 49a	1992	319	0.93	0.811	n
	1993	319	1.08	0.150	n
Lindsay 49b	1992	220	1.03	0.351	n
	1993	220	0.91	0.815	n
Lindsay 50a	1992	185	0.95	0.676	n
	1993	185	1.05	0.305	n
Lindsay 50b	1992	237	0.93	0.764	n
	1993	237	0.84	0.983	n
Lindsay 51	1992	780	1.02	0.322	n
	1993	780	1.09	0.048	a
Lindsay 94	1992	488	1.13	0.023	a
Strathmore 1	1992	576	1.32	0.000	a
Strathmore 2	1992	130	0.95	0.638	n
Strathmore 26	1992	323	1.18	0.016	a
Strathmore 32a	1992	449	1.08	0.121	n
Strathmore 32b	1992	424	1.14	0.023	a
Strathmore 33a	1992	591	0.95	0.785	n
Strathmore 33b	1992	591	0.99	0.545	n
Strathmore 34	1992	377	1.11	0.076	n
Strathmore 36	1992	399	1.62	0.000	a
Strathmore 52	1992	161	1.49	0.000	a
	1993	161	1.36	0.002	a

[a]Index of dispersion (I_β) and associated probability (P) values for the indicated quadrat sizes by year for CTV-infected citrus plots in Central California. Values presented for each assessment date are I_β (= observed variance/binomial variance) and P (= significance probability). P-values were calculated by comparison of df X 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) I_β and a small P (< = 0.05) suggest rejection of H_0: random pattern, in favor of H_1: aggregated pattern of diseased trees (a = aggregation; n = non-aggregation).
For all blocks and assessment years, incidence of diseased trees was determined by polyclonal ELISA test for CTV.

blocks in the center of the Tulare County Pest Control District. These blocks are associated with the main focus of disease in the Central Valley. For the remaining 26 blocks, the spatial pattern of CTV incidence could not be distinguished from random.

At the next level of spatial hierarchy, i.e., between quadrats, 19 of 36 possible block-by-assessment year combinations demonstrated aggregation among adjacent quadrats (Table 3). In the majority of these cases the number of significantly positive spa-

TABLE 3
SPATIAL AUTOCORRELATION ANALYSIS FOR *CITRUS TRISTEZA VIRUS* IN CITRUS BLOCKS IN CENTRAL CALIFORNIA

Plot	Year	Disease incidence[a]	Significant lags[b]		Strength of aggregation[c]	Core cluster size[d]	Reflected cluster size[e]	Total no. of clusters[f]	Effects[g]		
			SL+	SL-					Within row	Across row	Edge
Exeter 03	1993	0.019	9	0	0.11	2	1	10	4	1	ns
Exeter 21	1992	0.027	7	0	0.14	2	1,2	6	0	3	ns
Exeter 22	1992	0.067	25	0	0.56	15	1,2,3	8	5	2	ns
	1993	0.108	26	0	0.62	17	1,7	5	5	5	ns
Exeter 28	1992	0.022	5	0	0.20	2	1	5	1	0	ns
	1993	0.057	7	0	0.14	2	1,2	6	2	3	ns
Exeter 29	1992	0.078	3	1	0.33	2	1	3	1	1	ns
	1993	0.177	1	0	1.00	2	0	1	0	1	ns
Exeter 29a	1992	0.096	1	1	0.00	0	1	2	0	1	ns
	1993	0.192	4	3	1.00	4	1	2	0	3	S
Exeter 38	1992	0.071	3	0	0.00	0	1	4	1	0	ns
	1993	0.162	2	1	1.00	3	0	1	1	0	ns
Lindsay 04s	1992	0.014	19	0	0.16	4	1,2,3	12	3	0	ns
Lindsay 32	1992	0.016	2	0	0.00	0	1	2	0	0	ns
Lindsay 49a	1992	0.019	2	0	0.00	0	1	2	1	0	ns
	1993	0.035	4	0	0.00	0	1,2	3	1	0	ns
Lindsay 49b	1992	0.024	1	0	0.00	0	1	1	0	1	ns
	1993	0.056	7	0	0.14	2	1	7	2	1	ns

[a]Disease incidence = number of CTV-infected trees/total number of trees in each citrus block.
[b]Number of [X,Y] lags significantly greater (SL+) or less (SL-) than expected by chance at $\alpha = 0.05$.
[c]Strength of aggregation = an estimate of the density of the core cluster that gives a relative indication of aggregation among adjacent quadrats.
[d]Core cluster size = the number of significant lag distance positions contiguous with the origin [0,0] of the autocorrelation proximity pattern that form a discrete group.
[e]Reflected cluster size = the number of contiguous SL+ in various clusters not contiguous with the core cluster. Indicates aggregation over distance or nonadjacent quadrats.
[f]Total number of clusters = the number of contiguous clusters of SL+ in the proximity pattern. A value >2 m indicates there is aggregation in nonadjacent quadrats.
[g]Row and column effects = the number of SL+ within-row and within-column of the row and column defined by the [0,0] lag. Edge effects are significant (S) or non-significant (ns) if (the number of SL+ at the distal edges of the proximity pattern/total number of SL+) is ≥5% and <5%, respectively. Indicates relative aggregation for the core cluster or among adjacent quadrats.

TABLE 3 (CONTINUED)
SPATIAL AUTOCORRELATION ANALYSIS FOR *CITRUS TRISTEZA VIRUS* IN CITRUS BLOCKS IN CENTRAL CALIFORNIA

Plot	Year	Disease incidence[a]	Significant lags[b]		Strength of aggregation[c]	Core cluster size[d]	Reflected cluster size[e]	Total no. of clusters[f]	Effects[g]		
			SL+	SL-					Within row	Across row	Edge
Lindsay 50a	1992	0.011	2	0	0.00	0	1	2	0	0	S
	1993	0.025	1	0	0.00	0	1	1	1	0	ns
Lindsay 50b	1992	0.020	2	0	0.00	0	1	2	2	0	ns
	1993	0.043	2	2	0.00	0	1	2	0	0	ns
Lindsay 51	1992	0.017	13	2	0.00	0	1,2	13	0	1	S
	1993	0.028	9	1	0.11	2	1	9	1	0	S
Lindsay 9_4	1992	0.061	8	1	0.00	0	1	8	1	1	S
Strathmore 1	1992	0.011	17	0	0.00	0	1,4	14	5	1	ns
Strathmore 2	1992	0.004	1	0	0.00	0	1	1	0	0	ns
Strathmore 26	1992	0.014	7	0	0.00	0	1	7	2	1	ns
Strathmore 32a	1992	0.049	5	0	0.20	2	1,2	4	1	1	ns
Strathmore 32b	1992	0.017	13	0	0.15	3	1,2	11	3	2	S
Strathmore 33a	1992	0.006	12	0	0.00	0	1,2	11	2	4	S
Strathmore 33b	1992	0.012	4	0	0.25	2	1	4	2	1	S
Strathmore 34	1992	0.041	3	0	0.00	0	1,2	2	0	0	ns
Strathmore 36	1992	0.019	21	0	0.24	6	1,2,4	10	2	2	S
Strathmore 52	1992	0.088	3	0	0.33	2	1	3	2	0	S
	1993	0.112	3	0	0.67	3	1	2	2	1	S

[a]Disease incidence = number of CTV-infected trees/total number of trees in each citrus block.
[b]Number of [X,Y] lags significantly greater (SL+) or less (SL-) than expected by chance at $\alpha = 0.05$.
[c]Strength of aggregation = an estimate of the density of the core cluster that gives a relative indication of aggregation among adjacent quadrats.
[d]Core cluster size = the number of significant lag distance positions contiguous with the origin [0,0] of the autocorrelation proximity pattern that form a discrete group.
[e]Reflected cluster size = the number of contiguous SL+ in various clusters not contiguous with the core cluster. Indicates aggregation over distance or nonadjacent quadrats.
[f]Total number of clusters = the number of contiguous clusters of SL+ in the proximity pattern. A value >2 m indicates there is aggregation in nonadjacent quadrats.
[g]Row and column effects = the number of SL+ within-row and within-column of the row and column defined by the [0,0] lag. Edge effects are significant (S) or non-significant (ns) if (the number of SL+ at the distal edges of the proximity pattern/total number of SL+) is ≥5% and <5%, respectively. Indicates relative aggregation for the core cluster or among adjacent quadrats.

tial lags (one spatial lag = one 2 × 2 tree quadrat) in the core cluster was 2 to 6, indicating that aggregation was not extensive. The two cases for which the number of significantly positive spatial lags associated with the core cluster was more extensive, i.e., 15 and 17 positive lags, were both associated with the Exeter 22 block. This was not observed in Exeter 21, the adjacent block to the east, however. Also in Exeter 22, newly detected CTV-positive trees in 1993 were often adjacent or near CTV-positive trees which had been removed the previous year. However, this trend was not noted in any of the other 10 blocks for which two consecutive years of data existed. For the majority of possible block-by-assessment year combinations for which core clusters existed, there was no clear indication of directionality of aggregation, within-row, or across-row effects. Significant edge effects existed for 11 out of 36 possible block-by-assessment year combinations.

DISCUSSION

Spatial analysis. Aggregation within 2 × 2 quadrats, that is among immediately adjacent trees, was found in only 28% (10 of 36) of the blocks examined. In the majority of cases, the spatial pattern of CTV-infected trees indicated a random pattern. However, when the data were examined on a larger spatial scale as between adjacent quadrats, 53% (19 of 36) of the blocks examined demonstrated some aggregation. This indicates that spatial aggregation of CTV does exist within the majority of the citrus blocks examined, however this aggregation is more intense at a greater spatial scale than merely adjacent trees.

This finding is consistent with that found for spatial patterns of CTV in Spain and Florida where similar aphid vector species exist (7, 8). Gibson recently re-examined the CTV data from Spanish plots in light of a new spatio-temporal stochastic model (4, 5, 6, 11). He likewise found that spread of CTV was due to both local and long-distance interactions and supported the idea that both inter-and intra-plot transmission of CTV was implicated. The presence of significant edge effects associated with spatial autocorrelation analysis in this study for some of the blocks examined indicates the possibility of spatial spread into the blocks from outside sources, and further corroborates Gibson's findings for CTV. This is not unexpected, because of the similarity of aphid vector species in California and Spain, and the possibility of spread of CTV into the plots from external sources is comparable.

The data presented are a subset of those previously examined by Beta-binomial distribution analysis in order to develop the Hierarchical Subsampling method (12). Subsequently this method was extended for sampling in situations where *Toxoptera citricida* is the predominant vector. Contrary to *A. gossypii*, the *T. citricida*/CTV pathosystem results in disease spatial patterns that are highly aggregated (13). Further evidence for its utility in conducting large scale virus surveys, the HS method was adopted for national surveys in both the United States and Canada for *Plum pox virus* (PPV), a potyvirus of stone fruits recently introduced in North America. (14).

The California citrus industry continues to fear the introduction of the brown citrus aphid; it is not a matter of "if", but "when" it will arrive in this state. A Brown Citrus Aphid Task Force was formed in 1993 and continues to meet periodically (1). An action plan exists that will be implemented immediately upon the first knowledge of introduction. Initial actions include: the contact of appropriate industry officials; conduct an intensive delimitation survey(s) to determine distribution; and commence local chemical control

if feasible. Research continues on searching for suitable biological control agents and the development of CTV-resistant varieties. The threat of this efficient vector is one of the prime reasons growers in the San Joaquin Valley continue to support the eradication program.

LITERATURE CITED

1. Bezark, L. G., J. Ghazanfari, R. Penrose, J. Sorensen, D. Woods, and D. Zadig
   1994. Brown citrus aphid/Citrus tristeza virus: Situation assessment and pest response guide. Calif. Dept. Food and Agric., Div. Plant Ind., Sacramento, CA. 70 pp.
2. Campbell, C. L. and J. P. Noe
   1985. The spatial analysis of soilborne pathogens and root diseases. Annu. Rev. Phytopathol. 23: 129-148.
3. California Department of Food and Agriculture Resource Directory
   2000. A.G. Izumi, ed. 176 pp.
4. Gibson, G. J.
   1997. Investigating mechanisms of spatiotemporal epidemic spread using stochastic models. Phytopathology 87: 139-146.
5. Gibson, G. J.
   1997. Markov chain Monte Carlo methods for fitting spatiotemporal epidemic stochastic models in plant pathology. Appl. Stat. 46: 215-233.
6. Gibson, G. J. and E. J. Austin
   1996. Fitting and testing spatiotemporal stochastic models with applications in plant pathology. Plant Pathol. 45: 172-184.
7. Gottwald, T. R., M. Cambra, P. Moreno, E. Camarasa, and J. Piquer
   1996. Spatial and temporal analysis of citrus tristeza virus in eastern Spain. Phytopathology 86: 45-55.
8. Gottwald, T. R., S. M. Garnsey, and J. Borbón
   1998. Increase and patterns of spread of citrus tristeza virus infections in Costa Rica and the Dominican Republic in the presence of the brown citrus aphid, *Toxoptera citricida*. Phytopathology 88: 621-636.
9. Gottwald, T. R., S. M. Richie, and C. L. Campbell
   1992. LCOR2-Spatial correlation analysis software for the personal computer. Plant Dis. 76: 213-215.
10. Gottwald, T. R., and G. Hughes
    2000. A new survey method for citrus tristeza virus assessment. In: *Proc. 14th Conf. IOCV*, 77-87. IOCV, Riverside, CA.
11. Gottwald, T. R., G. Gibson, S. M. Garnsey, and M. Irey
    2000. The effect of aphid vector population on local and background components of CTV spread. In: *Proc. 14th Conf. IOCV*, 88-93. IOCV, Riverside, CA.
12. Hughes, G. and T. R. Gottwald
    1998. Survey methods for citrus tristeza virus assessment. Phytopathology 88: 715-723.
13. Hughes, G. and T. R. Gottwald
    1999. Survey methods for citrus tristeza virus incidence when *Toxoptera citricida* is the predominant vector. Phytopathology 89: 487-494.
14. Hughes, G., T. R. Gottwald, and L. Levy
    2002. The use of hierarchical sampling in the surveillance program for *Plum pox virus* in the United States. Plant Dis. 82: 259-263.
15. Hughes, G. and L. V. Madden
    1993. Using the beta-binomial distribution to describe aggregated patterns of disease incidence. Phytopathology 83: 759-763.
16. Hughes, G., N. McRoberts, L. V. Madden, and T. R. Gottwald
    1997. Relationships between disease incidence at two levels in a spatial hierarchy. Phytopathology 87: 542-550.
17. Madden, L. V. and G. Hughes
    1994. BBD—Computer software for fitting the beta-binomial distribution to disease incidence data. Plant Dis. 78: 536-540.
18. Madden, L. V. and G. Hughes
    1995. Plant disease incidence: Distributions, heterogeneity, and temporal analysis. Annu. Rev. Phytopathol. 33: 529-564.
19. Nikolaeva, O. V., A. V. Karasev, D. J. Gumpf, R. F. Lee, and S. M. Garnsey
    1995. Production of polyclonal antisera to the coat protein of citrus tristeza virus expressed in *Escherichia coli*: Application for immunodiagnosis. Phytopathology 85: 691-694.