Analysis of Foci of Asiatic Citrus Canker in a Florida Citrus Orchard

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ABSTRACT

Gottwald, T. R., Graham, J. H., and Egel, D. S. 1992. Analysis of foci of Asiatic citrus canker in a Florida citrus orchard. Plant Dis. 76:389-396.

In October 1990, the occurrence of Asiatic citrus canker in an orchard in south Florida was apparently related to spread of *Xanthomonas campestris* pv. *citri* from dooryard trees 230 m away on an adjacent property. The establishment of apparent initial foci of disease in the orchard coincided temporally with a major rainstorm with high winds during mid-August 1989. Infection of the dooryard trees on the adjacent property was related by regulatory officials to an introduction of inoculum from an outbreak of Asiatic citrus canker on the west coast of Florida in 1986. Restriction endonuclease digest patterns of DNA taken from the pathogens during the 1986 and 1990 outbreaks were identical. There were three extensive and several minor areas of diseased trees in the orchard. The three most extensive areas of disease each had trees near the center of the cluster with stem lesions that predated all other foliar lesions in the cluster. From isopath maps of these areas a main focus of diseased trees was found, surrounded by what appeared to be secondary foci. A greater within-row than across-row aggregation for each area was detected by ordinary runs analyses. A predominant direction of disease spread among the areas of diseased trees was not found in analysis of disease gradients. A slightly stronger association of diseased trees within than across rows was found in spatial lag autocorrelation analyses, but noncontiguous groups of diseased trees also occurred that coincided with secondary foci at oblique angles to the oldest diseased trees. If natural spread within the orchard did occur, it may have been confounded by mechanical spread of *X. c. citri* caused by orchard management practices, such as pesticide applications.

Citrus canker, caused by *Xanthomonas campestris* pv. *citri* (Hasse) Dye, causes erumpent lesions on fruit, foliage, and young stems of susceptible cultivars of citrus (14). In Asia and South America, when the disease is severe, defoliation and dieback can occur, and diseased fruit are less valuable or are unmarketable (14,15). The disease can cause nominal to significant damage during seasons when spring and summer rains are combined with wind speeds in excess of 8 m/sec (19,20,21). In an attempt to prevent the introduction of the pathogen, many citrus-growing areas restrict the importation of citrus from areas or countries known to be infested.

Accepted for publication 4 November 1991 (submitted for electronic processing).

The occurrence of citrus canker in Florida has been rare, and it has been restricted to counties on the West Coast adjacent to major citrus production areas in central and southern Florida. Some researchers believe that citrus canker poses a significant disease threat to the industry (12), whereas others contend that it would not cause significant damage under environmental conditions prevalent in Florida (24).

In citrus nurseries with citrus canker, dissemination of *X. c. citri* is primarily by splash dispersal (11,20,21). The result is the development of numerous secondary foci that eventually coalesce into larger, irregularly shaped areas of disease, which makes the description and quantification of disease gradients difficult and of limited value. Slopes of disease gradients associated with citrus canker in nurseries fluctuate over time because of disease-induced defoliation on severely diseased nursery plants and infection of newly emerging foliage (11). Lloyd's index of patchiness (23) has previously been used to demonstrate highly significant aggregation of disease associated with splash dispersal. This aggregation decreased as the secondary foci coalesced (11). Ordinary runs analysis (16) was also utilized to demonstrate a slightly higher within- than across-nursery row aggregation, although aggregation was demonstrable in all nurseries studied throughout the epidemics irrespective of within- vs. across-row direction (11). Spatial lag autocorrelation analysis has been used previously (1,17,18) to examine the spatial relatedness of trees diseased with both Asiatic citrus canker and citrus bacterial spot in Argentina and the United States, respectively (7,11).

In simulations of epidemics in citrus groves in Argentina, slopes of disease gradients also fluctuated in response to disease-induced defoliation. However, unlike citrus nurseries, gradient slopes were directly related to the direction of windblown rain, and gradients were shallowest downwind and steepest upwind from the foci of infection (7). Rates of disease progress estimated with the linear form of the Gompertz model were also significantly greater in the downwind direction (4). Aggregation of diseased trees was indicated by ordinary runs and doublet analysis throughout the epidemics (7).

Asiatic citrus canker rarely occurs in Florida, and the disease is under strict quarantine. All grove trees in Florida are under continual inspection by repeated surveys by the Florida Department of Agriculture and Consumer Services, and all affected trees are destroyed immediately upon confirmation of the disease. Thus, temporal studies of the citrus canker pathosystem in Florida are impossible, and studies are restricted to gleaning deducible information from spatial data collectible prior to tree destruction. This study represents a spatial analysis of an isolated epidemic of citrus canker in south Florida in 1990 and an attempt to reconstruct conditions that most probably gave rise to the

spatial arrangement of disease that existed. In a continual attempt to eradicate the disease, all diseased and numerous trees surrounding the studied diseased trees in the orchard were destroyed soon after confirmation. In 1991, a few additional infected trees were found near the original infection sites in this orchard and lead to the destruction of the entire orchard in further attempts to contain the disease. No further occurrences have been found near this site at the time of this writing. The purpose of this study was to examine the intensity, spatial relationships, and disease gradients of foci of disease of Asiatic citrus canker in a rare orchard epidemic in Florida and to relate these parameters to biological factors and citriculture practices that may have contributed to the occurrence and development of the epidemic.

MATERIALS AND METHODS

In October 1990 the Florida Department of Agriculture and Consumer Services, Division of Plant Industry, reported a new occurrence of Asiatic citrus canker in a 2-yr-old, 162-ha citrus planting located near Lake Placid, Highlands County, Florida. The planting was surrounded predominantly by pasture, with the exception of the eastern edge, which had a few widely scattered rural homesites. It consisted of seven major blocks of nearly equal area, with Hamlin and Valencia orange (*Citrus sinensis* (L.) Osbeck) planted in rows; trees were 3.0 m apart within rows and 7.8 m between rows. The plantation was well maintained, and its trees were large and vigorous for their age. Herbicide was routinely applied by an applicator that dragged large pads soaked with herbicide over weeds and occasionally over low-lying branches. Foliar sprays of pesticides were applied by air-blast sprayer.

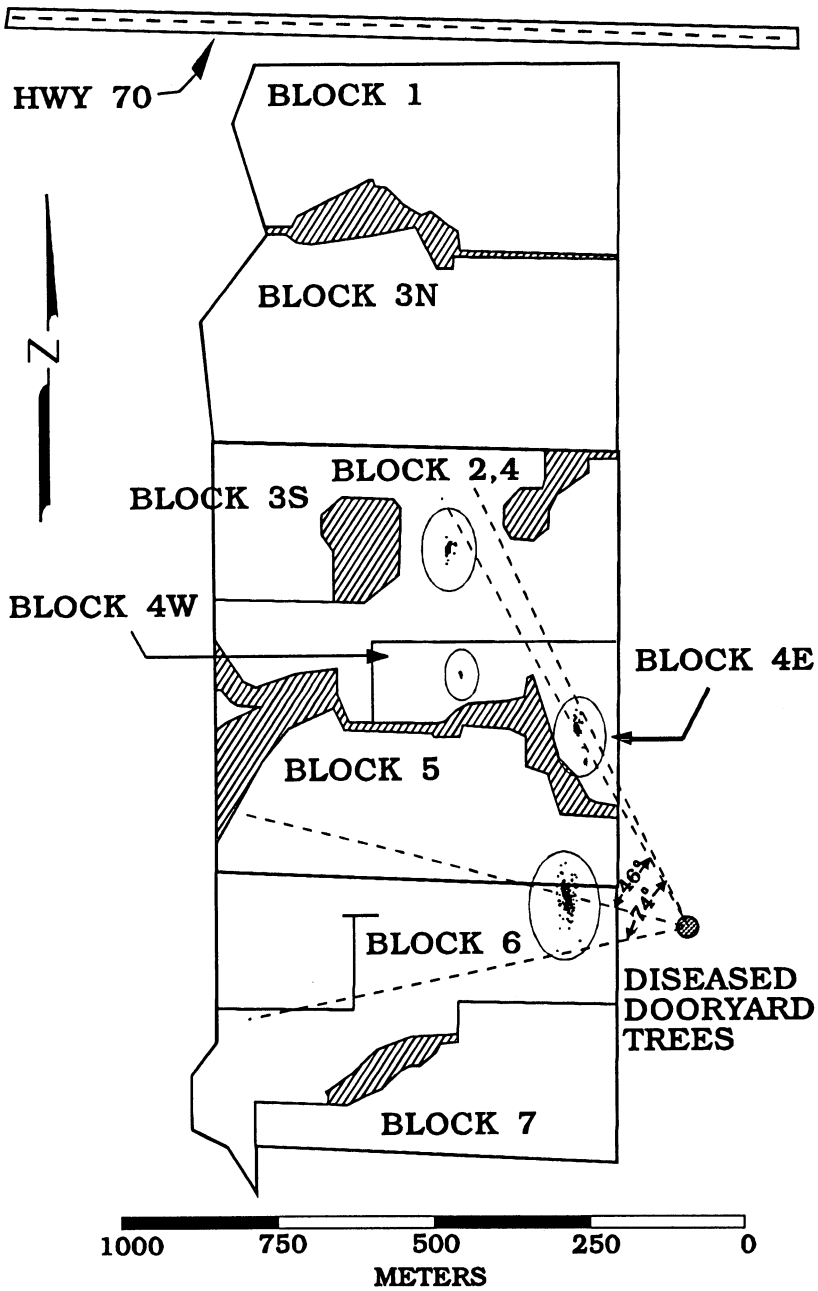

Fig. 1. Map of citrus orchard in Highlands County, Florida, affected with Asiatic citrus canker. The orchard was divided into large blocks of trees separated by drainage canals and ponds (striped areas). The ellipses circumscribe the major foci of disease by *Xanthomonas campestris* pv. *citri*. The small hatched circle to the east of the orchard denotes the position of 5- to 10-yr-old dooryard trees with citrus canker on an adjacent property, which are presumed to be the source of infection for the orchard. The dashed lines at 46° angles indicate the presumed first dispersal of the pathogen to the orchard estimated by the position of the oldest infections in the foci of disease in the orchard. The dashed lines at 74° angles are inclusive lines that indicate the extent of secondary spread at the time of disease assessment. Tree rows run north-south.

Spatial analysis. On 19 October 1990, after brief examination of the afflicted areas, a disease-rating scheme was devised to assess all the trees in and around individual foci of disease. Each tree was divided into four quadrants—northeast, southeast, southwest, and northwest. Trees were rated 0, 0.25, 0.5, 0.75, or 1.0, depending on how many quadrants had diseased foliage. For each focus of disease, several trees beyond the last diseased tree in a row were examined to ensure inclusion of noncontiguous diseased trees in the analysis. Trees were also examined for the presence of stem lesions on wood of the previous season's (1989) growth. Trees were assessed only once, since destruction of diseased and surrounding nondiseased trees was to commence within a few days in an attempt to eradicate the citrus canker from the area. An adjacent noncommercial property, which had three dooryard trees with symptoms of citrus canker, was examined on the same day.

Data from individual foci of disease were corrected for the 3.0- × 7.8-m rectangular planting pattern, and the resulting data matrix was used to generate two-dimensional isopath (line of similar disease incidence) maps of each focus to visualize the spatial arrangement of disease incidence. The maps were prepared using a data-contouring software package (Surfer, version 4, Golden Software, Golden, CO), through which individual isopath lines were generated that corresponded to 0.10 increments of disease incidence. Graphic representation of the entire planting was prepared with AUTOCAD (version 11, Autodesk, Inc., Sausalito, CA).

Analysis of disease gradients from apparent foci was accomplished by subjecting disease incidence data to the GRADCALC program, which calculated the distance from a proposed focus of disease to every other point in the spatial matrix (8,9). The average disease incidence was then calculated for those trees within concentric 5-m annuli around the focus. Subsets of these data,

consisting of those points falling within two vector lines emanating from the focus at an angle of 90° from each other, were tested to determine the lowest slope (b) of the gradient to investigate directionality. Since no temporal data were available to determine the most appropriate temporal transformation for disease, the logit(y) vs. ln(x) gradient model was used to relate disease incidence to distance, where y = proportion of disease incidence, logit(y) = ln[y/(1−y)], and x = distance (m) from the focus. The individual gradients were occasionally discontinuous (i.e., values of zero disease incidence existed at given distances from a focus followed by several more valid data points further from the focus). To avoid the problem of taking the logit of zero, a small number equivalent to the lowest reading for disease incidence was added to each data point of all gradient data sets prior to transformation. The transformed gradient data were analyzed by linear regression to obtain the slope (b) of disease incidence regressed on the distance from the proposed focus.

Because disease was found in discrete foci, aggregation of diseased trees was assumed. Ordinary runs analyses were performed on data from each focus to determine if aggregation was more prevalent within or across orchard rows (16). Spatial lag autocorrelation analysis was performed by the LCOR2 program on each focus of disease within the orchard to assess the autocorrelation among diseased trees (10). Correlation matrices were generated in which each tree was compared to all proximal trees. Cluster size and shape were estimated by interpreting the correlation matrices and by generating graphic representations of their associated two-dimension proximity patterns via Corel Draw (Corel Systems Corp., Ottawa, Ontario, Canada).

Meteorological relationships. Meteorological events were examined from radar films of the National Weather Service corresponding to dates during which heavy rains were recorded in the orchard at approximately the time when lesions were thought to have been initiated. The radar scans were superimposed on a map of the area in which the orchard was located. The times during which major storms occurred, their directional movement, and comments from the National Weather Service for these rainstorm events were examined to aid in interpretation of pathogen dispersal.

DNA restriction endonuclease analysis. Restriction endonuclease analyses of DNA extracted from newly isolated *X. c. citri* strains and those *X. c. citri* strains from other Asiatic citrus canker outbreaks in Florida were compared to determine the relationship among strains. Methods for restriction endonuclease analysis were the same as those previously reported (2,3).

RESULTS

Disease within the orchard was limited, consisting of 250 diseased trees out of 69,401 total trees in 162 ha. Some trees had moderate disease severity, and a small amount of defoliation was associated with severe disease on one branch of one tree. All orchard trees appeared vigorous. Secondary spread of the pathogen appeared to have been augmented by orchard management practices (i.e., via herbicide applicator and air-blast sprayer). In the focus in block 6, new lesions occurred on newly matured foliage apparently as a result of these practices.

On an adjoining property to the southeast of the orchard, three moderately to heavily diseased trees were found with Asiatic citrus canker about 200 m from the eastern edge of the orchard and 230 m from the center of the nearest focus of disease in block 6. (Fig. 1). Two of these trees were lemon, and the third was a trifoliate type, probably Swingle citrumelo. The trees were chlorotic and in poor condition, possibly because of poor nutrition and freeze damage. The size of the trunks and water sprouts was indicative of 5- to 10-yr-old trees. An abandoned bird's nest from the 1989 season was found in one of the most heavily infected dooryard lemon trees.

Spatial analysis. Diseased orchard trees were concentrated in three main clusters and a few small secondary clusters (Fig. 1). The largest cluster was

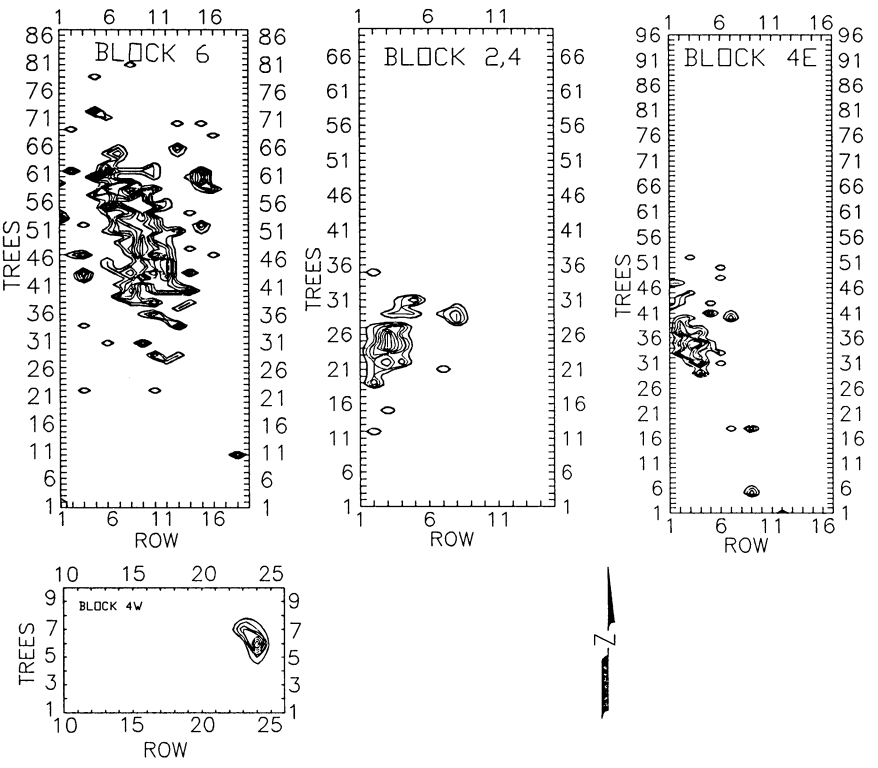

Fig. 2. Maps of computer-generated isopaths (lines of similar disease incidence) of foci of Asiatic citrus canker in a citrus orchard in Highlands County, Florida. Lines are indicative of 0.1 increments of disease incidence. Note abundance of secondary foci of infection.

Table 1. Linear regression analysis of directional incidence of citrus canker on distance for three foci of disease using the logit(y) vs. ln(x) gradient model

Focus block	Direction	Slope (b) + error	Intercept	r^2
4E	All	−2.55 + 0.24	0.77	0.91
	N	−1.93 + 0.51	1.34	0.57
	S	−2.00 + 0.23	1.59	0.95
	E	...	...	...
	W	−2.04 + 0.39	1.45	0.93
6	All	−2.16 + 0.43	0.58	0.65
	N	−2.18 + 0.26	2.22	0.84
	S	−2.45 + 0.29	2.93	0.91
	E	−1.36 + 0.16	1.07	0.95
	W	−1.62 + 0.34	1.44	0.85
2,4	All	−2.09 + 0.27	−0.44	0.95
	N	−2.28 + 0.15	1.77	0.99
	S	−2.40 + 0.07	1.89	0.99
	E	−1.75 + 1.00	0.85	0.50
	W	...	...	...

located on the eastern dividing line of blocks 5 and 6 about 230 m from the diseased dooryard trees; it consisted of 151 diseased trees. Two somewhat smaller clusters were located in block 4E (48 trees) and block 2,4 (35 trees), about 410 and 810 m, respectively, from the diseased dooryard trees. A small cluster also occurred in block 4W (four trees), 613 m from the infected dooryard trees. A few small groups of one to three trees with very few lesions were seen in blocks 5 and 6 and were not considered for analysis. All areas of disease in the orchard lay within a 74° arc, whose hypothetical vertex was the diseased dooryard trees, whereas all trees with older stem lesions (presumably established during the initial influx of inoculum) lay within a 46° arc (Fig. 1).

The isopaths were visually examined, and the spatial position of stem lesions was located (Fig. 2). In all cases, the stem lesions occurred in the areas of most severe disease, and all trees with stem lesions had the highest disease rating (1.0). Because of their central location, trees with stem lesions were presumed to be the center of each focus of disease, and gradients were calculated from these central trees outward.

The logit(y) vs. ln(x) model for disease gradients fit the data well in most cases (Table 1). Only three of the foci examined had sufficient numbers of diseased trees to calculate accurate gradients. For the focus in block 4E, incidence of disease decreased too rapidly over distance to the east to calculate a gradient. The gradients in the other three directions were nearly equivalent, and the slopes were not significantly different (Table 2 and Fig. 3). For the focus in block 6, gradients to the north and south of the focus of disease were steeper than the slopes to the east and west (Table 1 and Fig. 3), and the slope of the gradients to the north and south were significantly different compared with the slope of the gradient to the east (Table 2). For the focus in block 2,4, disease decreased too rapidly over distance to calculate a gradient (Table 2 and Fig. 3), and the slopes of the remaining gradients were not significantly different (Table 2).

Based on ordinary runs analysis, there was more within-row than across-row aggregation for blocks 4E, 2,4, and 6. There was no aggregation in either direction in block 4W, which had a very low incidence of diseased trees (Table 3).

Spatial autocorrelation correlograms were examined for each focus of disease in the orchard. An example of a correlogram is given for the focus in block 2,4, in which there was significant autocorrelation for the first eight lags (trees) within orchard rows, for the first two lags across orchard rows, and for an oblique diagonal seven lags within row by two lags across row (Table 4). There was also a second noncontiguous group of significantly autocorrelated lags extending from lags 4–6 across rows and from lags 0–7 within rows (Table 4). The two-dimensional proximity patterns for this and the remaining correlograms are best seen graphically (Fig. 4). In general, the cluster size was more extensive within than across orchard rows. However, noncontiguous clusters of autocorrelated diseased trees that existed for foci in blocks 4E, 2,4, and 6 were located at oblique diagonals across rows. The presence and position of these noncontiguous clusters indicated that pathogen dispersal over longer distances occurred in orientations other than directly within (north-south) or across (east-west) orchard rows (Fig. 4). The presence of significantly autocorrelated noncontiguous portions of the proximity patterns was directly related to the presence and prevalence of secondary foci seen in the isopath contour maps for each focus (compare Figs. 2 and 4).

Meteorological relationships. Weather records from the orchard, provided by the orchard production manager, indicated that a major rain event had occurred on 14 August 1989, during which the orchard received 10.2 cm of rain. Although local winds can vary greatly during thunderstorms, radar scans from the National Weather Service for the Tampa Bay radar station indicated severe thunderstorm activity with 15-knot (5.14 m/sec) winds in the vicinity of the Highlands County orchard between 12:30 and 2:30 a.m. on 15 August. The storm was moving north by northeast.

DNA restriction endonuclease analysis. This analysis was used to help determine the possible source of the *X. c. citri* strain found in the Highlands County orchard. The restriction endonuclease patterns of the strains of *X. c. citri* from the Highlands County orchard and the dooryard trees of the adjacent property were identical to patterns of strain 9771 from the 1986 Manatee County orchard when restricted with *Spe* I (Fig. 5). There was a polymorphism at 571 kb, however, for strain T1 from an unrelated and isolated outbreak of citrus canker in North Florida as compared with all other strains (Fig. 5, arrow). Restriction endonuclease patterns of the strains from the orchard in Highlands County and the adjacent property

Table 2. Comparison of slopes for logit disease vs. ln distance gradient model with t tests for GRADCALC-generated disease incidence vs. distance gradients in four directions

Block	Direction	Direction[a] South	East	West
4E	North	0.124, df = 15	···	0.172, df = 13
	South	···	···	0.001, df = 5
	East	···	···	···
6	North	0.698, df = 20	2.663*, df = 17	1.279, df = 17
	South	···	3.309**, df = 11	1.841, df = 11
	East	···	···	0.696, df = 8
2,4	North	0.754, df = 5	0.529, df = 6	···
	South	···	0.655, df = 5	···
	East	···	···	···

[a]Values in each column indicate t test statistic for the comparison and the degrees of freedom associated with that test; disease incidence = proportion of tree quarters that were diseased; single and double asterisks represent values that are significantly different at $P = 0.05$ and 0.01, respectively; H_0: $b_1 = b_2$, H_A: $b_1 \neq b_2$, where b_n is the slope value for the logit disease vs. distance gradient model. Dashes indicate that data for at least one of the directions was insufficient to calculate a disease gradient. Thus, no t test was possible for that combination of gradients. Blanks represent redundant reciprocal comparisons.

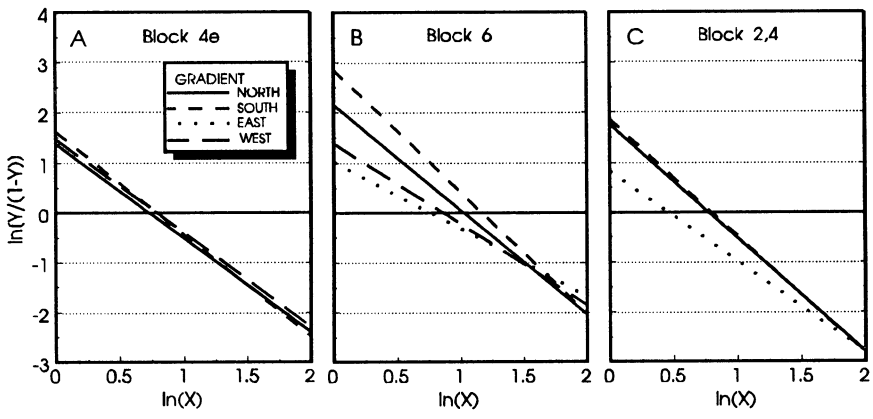

Fig. 3. Logit(y) vs. ln(x) transformed disease gradients in the four major cardinal directions from presumed foci of Asiatic citrus canker in three blocks of trees in a citrus orchard in Highlands County, Florida. Note that slope of the directional gradients does not vary greatly among directions. Slopes are compared statistically (see Tables 1 and 2).

were also identical to patterns of strain 9771 when restricted with *Xba* I. As with *Spe* I–generated restriction endonuclease patterns, strain T1 had one polymorphism difference to the aforementioned strains when restricted with *Xba* I (*data not shown*).

DISCUSSION

The Florida Department of Agriculture and Consumer Services, Division of Plant Industry, did a thorough survey of all orchards and dooryard plantings of citrus within an 8-km (1-mi) radius of the diseased orchard. Nursery sources of trees used to establish the orchard were also checked. No other infestation of Asiatic citrus canker was found. Because of this and the spatial proximity of the diseased dooryard trees to the foci of disease in the orchard in Highlands County, the dooryard trees were most probably the source of bacterial inoculum. Previous studies of citrus canker (4,8,11) indicate that the inclusive angle of inoculum dispersal (whose vertex was arbitrarily designated as the position of diseased dooryard trees) appears to be plausible for the dispersal pattern that developed. In addition, the foci with the greatest number of diseased trees were those closest to the dooryard source.

No stem lesions could be found on the dooryard trees on the adjacent property that predated those in the orchard; however, the severity of disease on such poorly growing, frost-damaged trees led us to believe that they had been diseased for at least two or more seasons. Thus, the lesions on the dooryard trees appeared to predate those in the orchard and were believed to be the source of the outbreak. This conclusion stimulated personnel from the Division of Plant Industry to investigate the origin of the infections on the homeowner property. The investigation led to the discovery that the resident had been employed by the state as an inspector with the citrus canker eradication program on the west coast of Florida in 1986 and had worked at the site of the Asiatic citrus canker outbreak in Manatee County. At that time, the inspector had direct contact with trees infected with the Asiatic citrus canker pathogen. The inspector was deceased and could not be questioned, and thus no conclusive tie could be made between the current outbreak and the previous outbreak on Florida's west coast. (The preceding is from the transcript of public testimony by R. Gaskella during a joint state-federal Citrus Canker Technical Advisory Committee meeting on 26 October 1990 at the Citrus Education and Research Center, University of Florida, Lake Alfred.)

The potential connection of the present outbreak of citrus canker to the previous western Florida outbreak prompted the examination of restriction endonuclease digests of isolates of *X. c. citri* from both outbreaks. Although isolates of *X. c. citri* from various locations around the world do not show a great deal of genetic heterogeneity by RFLP analysis (6,13), some small differences between strains can be detected by restriction endonuclease digest patterns (5). Identical restriction endonuclease patterns for strains from the outbreaks in Highlands and Manatee counties are consistent with the conclusion that the two outbreaks had a common source of bacterial strain. This contention is further strengthened by the polymorphism for the strain taken from the unrelated outbreak in north Florida in 1985. The 1986 outbreak in Manatee County seems the most likely source of infection for the dooryard trees, which gave rise to the 1989–1990 outbreak in the orchard in Highlands County.

Stem lesions occurred on the central trees in the three most extensive foci of disease in blocks 4E, 2,4, and 6. Initially, patterns of incidence of citrus canker in the orchard were probably the result of three contemporaneous infections during the middle of the 1989 season, as indicated by the age of the wood on which the stem lesions occurred. Secondary spread from these three disease foci resulted in the pattern seen in October 1990. The initial contemporaneous infection of the orchard most likely occurred in mid-1989.

The major rainstorm during mid-August 1989 was the only severe storm in the vicinity of the orchard that occurred during that time of the year. It fit the temporal estimate for initial dissemination of the pathogen from the dooryard trees to the newly planted orchard. Although the prevailing wind direction during the storm was to the north, wind direction of local cells is often quite variable. Therefore, it is likely

Table 3. Results of ordinary runs analysis of aggregation of citrus canker in four foci of disease in an orchard in Highlands County, Florida

Block	Incidence of diseased trees[a]	Proportion significantly aggregated[b]	
		Within rows	Across rows
4E	36/320 (0.11)	4/14 (0.29)	6/51 (0.12)
4W	4/100 (0.04)	0/10 (0)	0/10 (0)
2,4	48/700 (0.07)	4/8 (0.50)	0/41 (0)
6	152/1394 (0.11)	8/17 (0.47)	6/82 (0.07)

[a]Number of diseased trees/total number of trees in the focus; the incidence of diseased trees is in parentheses.
[b]Number of rows in which disease was aggregated/total number of rows; number of across-row tests in which disease was aggregated/total number of tests is in parentheses.

Table 4. Sample correlogram of spatial lag correlations of incidence of trees with citrus canker in a focus of disease in block 2,4 of an orchard in Highlands County, Florida

Spatial lags within row (north-south)	Spatial lags across (east-west)[a]							
	0	1	2	3	4	5	6	7
0	1.00000**	0.44502**	0.12524**	0.02369	0.10301**	0.07109*	−0.01778	−0.01582
1	0.68204**	0.39928**	0.09991**	0.03921	0.10279**	0.11442**	0.01106	−0.01606
2	0.53651**	0.36261**	0.11960**	0.04680	0.13865**	0.23035**	0.03991	−0.01630
3	0.36358**	0.26668**	0.10424**	0.03851	0.19858**	0.28828**	0.05421	−0.01655
4	0.23598**	0.22079**	0.10389**	0.04609	0.18634**	0.30266**	0.08309*	−0.01681
5	0.19015**	0.17484**	0.11355**	0.02983	0.17409**	0.22985**	0.12655**	−0.017070
6	0.08966**	0.13340**	0.08311**	0.03739	0.12570**	0.12790**	0.12631**	−0.017350
7	0.06192**	0.10103**	0.05764*	0.02904	0.04111	0.04040	0.09688**	−0.01763
8	0.06148**	0.04123	0.04217	0.02067	0.02877	0.02558	0.05283	−0.01792
9	0.04279	0.04073	−0.00847	0.00433	−0.01977	0.02530	0.08172*	−0.01823
10	0.02407	0.01279	0.03123	0.01183	−0.02010	0.01044	0.05221	−0.01854
11	0.04181	−0.00148	0.00060	−0.01253	−0.02045	−0.01902	0.00804	−0.01887
12	0.02371	0.00315	−0.00470	0.00312	−0.02067	−0.01921	−0.02141	−0.01906

[a]Single and double asterisks represent correlations significantly different from zero at $P = 0.05$ and 0.01, respectively. Comparisons between adjacent trees are considered first-order lag comparisons (lag 1 comparisons), whereas comparisons of trees separated by one, two, or three trees would be considered second-, third-, and fourth-order lag comparisons, respectively.

that the rainstorm could have transported inoculum from the infected dooryard citrus to the orchard. The presence of the abandoned bird's nest in the most heavily infected dooryard lemon tree on the adjacent property suggests the possibility of bird dissemination of initial inoculum. Single-tree infection foci are consistent with bird dissemination. However, this mode of dissemination seems less likely than windblown-rain dissemination of the bacteria to have initiated three contemporaneous disease foci. Nevertheless, the distance of approximately 230 m from

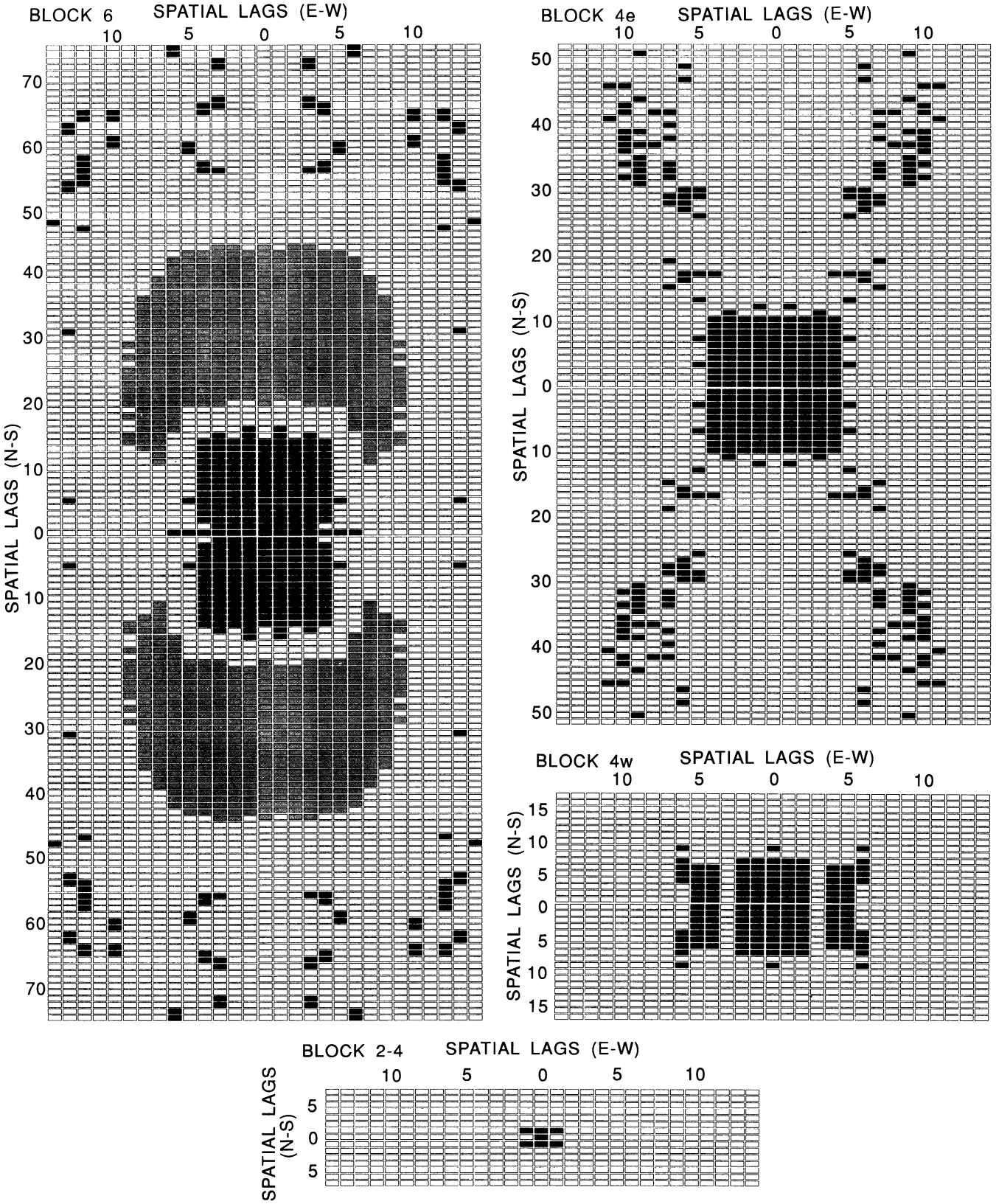

Fig. 4. Spatial proximity patterns of significant autocorrelations of Asiatic citrus canker in four foci of disease in a citrus orchard in Highlands County. Graphic representations of the proximity patterns were derived from correlograms of spatial lag correlations such as those presented in Table 4. Black rectangles denote significant positive autocorrelations, and gray rectangles denote significant negative autocorrelations ($P \leq 0.05$) at the indicated spatial lag positions. Note significant positive autocorrelations at oblique angles to center of proximity pattern and noncontiguous groups of autocorrelations at the periphery of the patterns.

the dooryard trees to the nearest focus of disease in the orchard is much greater than has been previously recorded for windblown-rain dissemination of citrus canker bacteria (22). Dissemination by windblown rain and epidemic development of Asiatic citrus canker under Florida conditions are in direct conflict with that previously predicted (24).

Disease gradient analyses—considering each entire focus without directional preference and directional gradients for each focus—were fit reasonably well by the logit(y) vs. ln(x) gradient model. The individual foci of disease were irregular in shape, although they were somewhat more elongate in the north-south than on the east-west axis. However, no predominant directionality of secondary spread (from the oldest lesions in each focus) was demonstrable among the disease foci. The largest cluster of diseased trees in block 6 had a distinct northwest-to-southeast axis, although only the gradient from the focus to the west was significantly different (flatter) than the other slopes. Analyses of disease gradients were confounded, as they often are, with citrus canker due to the occurrence of numerous secondary foci (9,11). The presence of noncontiguous secondary foci makes calculation of gradients difficult. If both diseased and disease-free trees are taken into account, the calculated slopes have large standard errors, making it less likely to detect differences between the slopes of different gradients.

Ordinary runs analysis showed that a greater within-row than across-row aggregation of diseased trees was found in blocks 4E, 2,4, and 6. We interpreted this as secondary spread of the pathogen, which occurred more readily within orchard rows than across rows. However, across-row aggregation was detected for block 6.

With spatial lag autocorrelation analyses, clusters of significantly autocorrelated spatial lags were more elongate in the within-row orientation (north-south) than across rows (east-west). Significant autocorrelations were also present for spatial lags at oblique across-row angles and for noncontiguous clusters of spatial lags, both directly across rows (block 2,4) and at oblique across-row angles (blocks 4E, 2,4, and 6). The significantly autocorrelated spatial lags at oblique angles appear to be related to the presence of secondary foci whose centers were located at oblique angles several trees down and across rows from the primary focus.

The orchard management practices in the Highlands County orchard probably contributed to secondary spread and confounded any spread by windblown rain beyond recognition. Numerous lower limbs, especially on the Valencia trees, appear to have been infected when wet pads of the herbicide applicator were dragged over diseased branches and inoculum was moved up and down orchard rows. The lower limbs of numerous trees showed evidence of mechanical damage and disease lesions associated with these wounds. Movement of inoculum up into the canopy and from tree to tree within and across rows was likely accomplished or significantly augmented by an air-blast sprayer. Orchard trees were still less that 2 m high, and the spray from an air-blast sprayer blew over active lesions and was likely carried across orchard rows. If winds picked up the inoculum-laden overspray, it could easily have traveled across orchard rows, and if the wind was not blowing directly east-west across rows, the inoculum would reach trees at the oblique angles indicated by isopath maps and spatial lag autocorrelation analysis. Thus, the spatial patterns of secondary spread from foci of disease potentially could be accounted for by the orchard management practices.

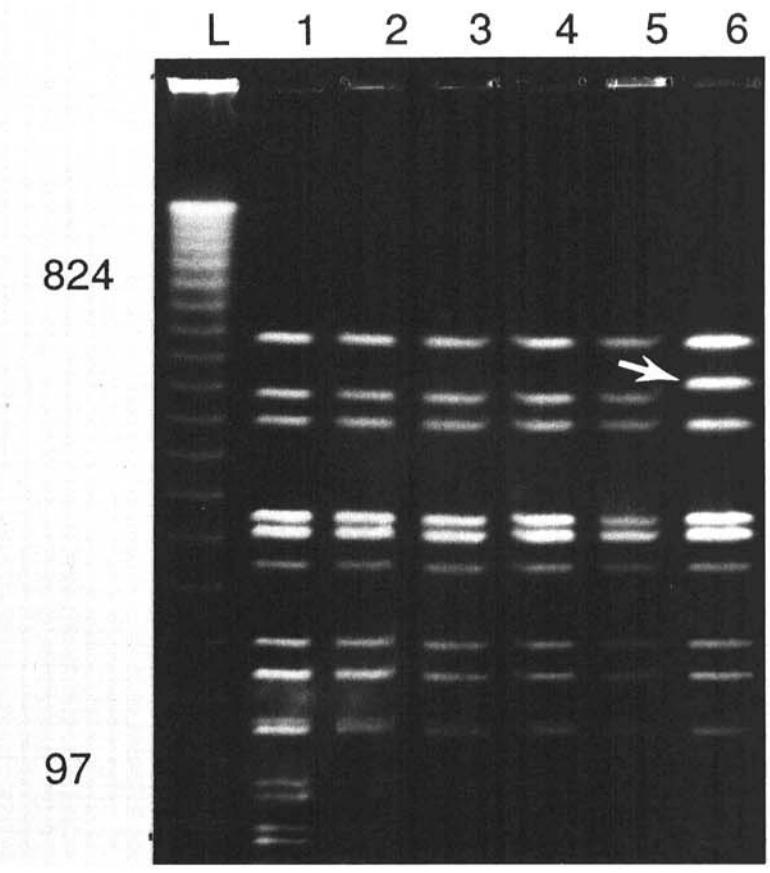

Fig. 5. Restriction endonuclease patterns of total DNA from strains of *Xanthomonas campestris* pv. *citri* digested with *Spe* I. Electrophoresis was by pulsed field electrophoresis for 22 hr with a pulse time increasing linearly from 4 to 50 sec. Lane 1, phage concatemers; lanes 1, 2, and 3, strains 7321, 7218, and 7225, respectively, from stem lesions in each disease focus from an orchard in Highlands County ; lane 4, strain 7405 from residence adjacent to Highlands County orchard; lane 5, strain 9771 from an outbreak in Manatee County; lane 6, T1 from an isolated outbreak in Alachua County in north Florida. Molecular sizes are given in kilobases. Arrow indicates polymorphism of T1 strain at 571 kb.

ACKNOWLEDGMENTS

We thank T. Riley, J. Bittle, A. Dow, P. Bell, and L. Wysocki for technical assistance and the Florida Department of Agriculture and Consumer Services, Division of Plant Industry, and orchard owners for allowing us access to quarantine areas.

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