Spatial Pattern Analysis of Epidemics of Citrus Bacterial Spot in Florida Citrus Nurseries

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ABSTRACT

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The spatial pattern of citrus bacterial spot caused by *Xanthomonas campestris* pv. *citrumelo* was studied in four nurseries in central and southwest Florida. When discrete frequency distributions were fitted to disease incidence data from each nursery, results were often inconclusive. Indices of dispersion suggested aggregation of disease in all nurseries. Ordinary runs analysis indicated within-row and across-row aggregation in all nurseries. Spatial lag correlation analyses further suggested a high degree of within-row autocorrelation of diseased levels in all nurseries, corresponding to apparent mechanical spread of the pathogen down nursery rows. The analysis also indicated some significant across-row autocorrelation of disease level, probably associated with natural spread across rows or perhaps mechanical spread across rows by handling of infected plants. In one nursery, high disease incidence on rootstock plants was directly related to high disease incidence on new scion shoots in the same area. Two nurseries had discrete areas of high disease incidence that were interpreted as apparent disease foci. Disease gradients in one of these nurseries were shallowest within rows to the north, corresponding to mechanical pruning in that direction. Disease gradients from the apparent focus in a second nursery, where little maintenance was performed, were shallowest diagonally across north-south oriented rows, indicating that the predominance of natural spread emanated from a focus both to the northeast and southeast directions. The presence or absence of natural disease spread versus mechanical spread alone was directly related to the aggressiveness of the bacterial strains, presence or absence of copper bactericides, and cultural management.

Additional keywords: citrus canker, disease eradication, isopath, *Xanthomonas*.

Citrus bacterial spot (CBS), caused by *Xanthomonas campestris* pv. *citrumelo* pv. nov. (Syn = *Xanthomonas campestris* pv. *citri* Strain E (5) is characterized by flat, necrotic lesions on foliage and young stems of susceptible citrus cultivars (7,9,11,15,18,19). Foliar lesions usually exhibit water-soaked margins and chlorotic halos when young, but water-soaking and chlorosis may decrease with a lesion's age. Older lesions, especially those caused by aggressive strains of the pathogen, often become brittle, crack, and fall out of the leaf blade, resulting in a shot-hole appearance of leaves (9). Stem lesions on some highly susceptible cultivars can be raised and usually lack chlorotic halos. The disease was originally believed to be a novel form of citrus canker caused by *X. citri* (Syn = *X. campestris* pv. *citri* Strain A) (2,5,14). However, recent studies comparing pathogenicity, physiology, genetics, and serological associations have clearly demonstrated the uniqueness of the nursery strains of the CBS pathogen (1,4,5,14).

An eradication campaign for this new disease was initiated by the Florida Department of Agriculture, Division of Plant Industry, in conjunction with the USDA Animal and Plant Health Inspection Service, in 1984. As a result, more than 23 million nursery and young reset citrus trees were destroyed and interstate commerce of citrus restricted, severely impacting the Florida citrus industry (7,18,19).

The spread of epiphytic populations of *X. c. citrumelo* and eventual disease development were studied previously in simulated epidemics, but these epidemics were hampered severely by unfavorable meteorological constraints (7). Spatial analysis of citrus canker epidemics in both replant and nursery situations has previously been reported (8,10). Spatial analyses of a bacterial spot epidemic including disease gradient estimations have been reported for only a single nursery (9). Directional disease spread can be examined by comparing slopes of disease gradients calculated in different directions from a disease focus (13). A new disease gradient calculation method has recently been used to examine the directionality of disease dispersion of CBS from proposed foci of disease in a regular lattice of plants (9).

Several statistical procedures exist for the quantitative analysis of spatial patterns of disease at a single point in time. Various indices of dispersion have been used to assess the degree of spatial randomness or aggregation of disease (6,21). Ordinary runs analysis has been demonstrated as the preferred method of unidirectional analysis to assess the presence or absence of aggregation of diseased individuals along or across rows in a population of diseased plants (16), and spatial autocorrelation analysis offers a unique opportunity to examine the relatedness of diseased individuals located in two-dimensional patterns of spatial proximity to one another (3,17).

Epidemics of CBS are usually both short lived and not spatially extensive because of the eradication program presently in place. Therefore, the opportunity to examine extensive outbreaks of CBS is unique and future opportunities uncertain. The purpose of this study was to characterize spatial patterns of disease from these rare but natural outbreaks of CBS in Florida and to further characterize the spatial patterns of disease during epidemics of CBS in citrus nursery situations in relation to cultural practices. Isopath contour maps were examined to identify presumed foci of disease and disease gradient analysis used to examine directionality of spread in response to cultural practices. Where possible, disease was assessed following alternative disease-control measures to eradication.

MATERIALS AND METHODS

The four most extensive outbreaks of CBS that occurred from September 1987 to February 1988 were studied soon after discovery and before eradication measures commenced. Eventual eradication/disease-containment measures ranged from destruction of the entire nursery to attempted control through defoliation and application of bactericide without destruction, depending on the perceived risk of contaminating other nurseries and citrus orchards. The outbreaks were in four different nurseries in central and southwest Florida and were designated in this study as Frostproof, Venice, Ocoee, and Lake Wales. These nurseries were not examined by the authors until disease outbreaks were identified. Postidentification cultural practices were dictated by the Florida Department of Agriculture and USDA/APHIS in compliance with the dictates of the current eradication program. Thus, cultural practices employed in these nurseries were beyond our control and replication was not possible.

The Frostproof nursery in Polk County, FL, consisted of ~5.7 ha of citrus of mixed cultivars and rootstocks of different ages. Citrus bacterial spot was restricted to a single nursery bed in the southwest corner of the nursery consisting of ~16,800 plants. Cultivars in the bed were: Ruby Red grapefruit and Henderson Red grapefruit (*Citrus paradisi* Macf.) and Parson Brown orange (*C. sinensis* (L.) Osbeck). All were budded onto sour orange rootstock (*C. aurantium* L.) planted in seven north-south double rows ~183 m in length and ~1.4 m apart. The six westernmost rows (~0.25–0.50 m in height), and the one easternmost row (~1.0 m in height), were 15 and 27 mo after budding, respectively.

Citrus bacterial spot in the Venice nursery in Sarasota County, FL, was restricted to the two easternmost of five beds of Swingle citrumelo (*C. paradisi* × *Poncirus trifoliata* (L.) Raf.) rootstock seedlings, 0.15–0.45 m in height. Each bed consisted of 14 north-south rows, 243 m in length, 0.9 m apart, and 0.15 m between plants within row and ~23,000 plants/bed.

The Ocoee nursery in Orange County, FL, consisted of 95,000 trees in 0.9 ha in 58 north-south rows. Rows averaged 75 m in length with 0.75 m between rows and 0.1 m between trees within rows. Swingle seedlings to be used as rootstocks were planted on three different occasions in contiguous sections of a single bed within the nursery. Disease was restricted to the easternmost section of the nursery in which Swingle seedlings had been budded ~2 mo before disease assessment with red grapefruit, Hamlin, and Pineapple orange. In most cases, the new scions were 0.05–0.20 m in length and the rootstock tops had not yet been removed at the time of disease assessment.

The Lake Wales nursery in Polk County, FL, consisted of ~520,000 trees, primarily of Swingle seedlings, in 28 nursery beds, of which six beds were infected with CBS. Five of the infected beds were located at the northern end of the nursery and the sixth bed on the southeastern edge of the nursery. All nursery beds were planted with 10 rows each, 1.2 m between rows, and 0.15 m between plants within rows and varied from 149 to 170 m in length.

Disease assessments. In the Venice and Lake Wales nurseries, individual nursery beds were divided into ~3-m quadrats oriented along rows. The proportion of symptomatic plants, i.e., disease incidence, was recorded within each quadrat. Because the Frostproof and Ocoee nurseries had fewer diseased plants, disease was assessed as disease severity, number of diseased leaves/total number of leaves per plant for each tree, and the location of individual infected trees was recorded. The data for these two nurseries were later reduced to quadrats by averaging individual tree assessments over 3-m sections of each row to be consistent with the Venice and Lake Wales nurseries. Disease severity data was, however, used for disease gradient analysis discussed below.

After disease assessment in the Frostproof nursery, trees from a 6-m section of the nursery bed with the most severe disease were removed and transported to a disease containment field site at the University of Florida, Agricultural Research and Education Center in Hastings, FL. This section of the plot was reestablished with plants in the same spatial orientation and location as in the original nursery and nine more disease assessments were made during the next 461 days. The plot was protected from freezing weather during the winter by constructing a temporary polyethylene house over it. The covering was removed in early spring and the plot again exposed to ambient meteorological conditions.

Before disease eradication in the Ocoee nursery, 21 symptomatic and 21 asymptomatic-exposed trees were removed from the nursery and transported to the Hastings research site. These trees, consisting of Ruby Red grapefruit and Hamlin sweet orange, were planted in a 4.6- × 6.1-m grove planting pattern on raised beds to study endurance of the disease when removed from a nursery and established in a new grove environment. Eight more disease assessments were made over the following 380 days.

To study alternative disease control measures to host destruction, the plants in the two infected nursery beds of the Venice nursery were pruned of disease, sprayed with copper bactericide, stripped of all foliage and bud-grafted to citrus scion material. Disease assessments were made by the Florida Department of Agriculture and Consumer Services, Division of Plant Industry, monthly over the next 18 mo.

Pathogen confirmations. To confirm the presence of *X. c. citrumelo*, a total of 66, 61, 26, and 41 single-colony, bacterial cultures were obtained from foliar lesions from the Frostproof, Ocoee, Lake Wales, and Venice nurseries, respectively. Each culture was tested for pathogenicity by inoculations of detached leaves (9,12) and characterized serologically with monoclonal and polyclonal antibodies or antisera reactions, and by restriction

length fragment polymorphisms (Gottwald and Graham; and Gottwald, Alvarez and Hartung, *unpublished data*).

Spatial pattern analysis. The mean and variance of disease incidence in each 3-m-row quadrat from each nursery or individual nursery beds were calculated. Quadrat count data were arranged in frequency categories and a FORTRAN program was used to estimate parameters of discrete frequency distributions and calcuate a chi-square statistic for goodness-of-fit to Poisson and negative binomial distributions to the data (6,20). Data in the tail classes were combined when fitting freqency distributions. For the Poisson distribution, a nonsignificant chi-square, goodness-of-fit statistic ($P \geq 0.05$) indicates randomness. For the negative binomial distribution, a significant ($P < 0.05$) chi-square statistic indicates a random distribution or lack of aggregation of diseased plants.

The variance-to-mean ratio and Lloyd's-index-of-patchiness were used as indices of dispersion for disease-quadrat data (21). Aggregation of diseased plants was also tested by ordinary runs analysis for each nursery or nursery bed within and across rows (16). A nonrandom distribution (i.e., aggregation) of disease was concluded if the observed number of runs were less than the expected values at $P = 0.05$.

Spatial lag correlation analysis was performed on each nursery or nursery bed to assess the autocorrelation among disease incidence values among quadrats as previously described (3,17). Correlation matrices were generated in which each quadrat was compared to the values of all proximal quadrats. Cluster size and shape were estimated by interpreting the correlation matrices (3).

Aggregation of CBS-diseased plants in the Frostproof and Ocoee nurseries, and CBS-diseased quadrats in the Lake Wales and Venice nurseries, was also examined visually by the use of isopath maps. Maps were generated by a data contouring program (Surfer, Golden Software, Golden, CO) for each nursery or nursery bed. Isopath lines were generated corresponding to 0.01 increments of disease incidence. The areas within each isopath level were estimated by redigitizing the contour maps and estimating the areas using AUTOCAD (Autodesk, Inc., Sausalito, CA). The distribution of isopath bounded areas were graphed as area (m^2) by isopath level. Response surface graphs were generated from the same data to visualize disease intensity.

Disease gradient assays for the Frostproof and Venice nurseries was used to examine gradients from apparent foci. Gradient analysis was accomplished by subjecting disease severity data to the GRADCALC program (9). The program calculated the distance from a proposed focus of infection to every other point in the spatial matrix. The average disease severity was then calculated for those plants falling within concentric 3-m bands around the focus. Subsets of these data consisting of those points falling within two vector lines emanating from the focus were tested to determine the lowest slope (b) of the gradients. Disease severity was related to distance using $\ln(y)$ vs. $\ln(x)$, Y vs. $\ln(x)$, Y vs. X, Logit(Y) vs. X, Probit(Y) vs. X, Y vs. Probit(X) vs. Probit(Y) vs. Probit(X) gradient models, where Y = disease severity and X = distance from focus (9,13). The gradients from transformed data were analyzed by linear regression to obtain the slope (b) of disease severity regressed on the distance from the proposed focus.

RESULTS

Indices of dispersion and probability distribution. The number of plants in citrus nurseries is usually large; however, the proportion of CBS-infected plants in the four nurseries studied was generally quite low (Table 1). Variance-to-mean ratio and Lloyd's-index-of-patchiness were greater than one for all nurseries and individual nursery beds, indicating spatial aggregation of disease in all situations examined. The magnitude of each of these indices of dispersion further indicated moderate to considerable aggregation in most cases. Fitting various probability distributions to the data from each nursery situation gave inconsistent results. In the case of the Venice nursery and three of the individual beds of the Lake Wales nursery, the Poisson and negative binomial distributions fit equally well (Table 1). Due to the inconsistent fit of Poisson and negative binomial probability distributions to CBS disease data, additional analyses were used to better characterize the aggregation of diseased plants within the individual nurseries.

Ordinary runs analysis indicated aggregation of diseased individuals both within and across rows of the Frostproof and Venice nurseries (Table 2). Lack of indicated aggregation in most rows or across row quadrats was due to a total lack of disease in these areas. Aggregation was also indicated for the Ocoee nursery for disease incidence data on scions, rootstocks, and scion and rootstock combined, within and across rows. Only a few rows in each nursery bed of the Lake Wales nursery had diseased plants. Within-row aggregation was generally indicated for those rows within each nursery bed of the Lake Wales nursery which contained diseased plants. When trees in more than one row within

TABLE 1. Indices of dispersion and goodness of fit to probability distributions for incidence of citrus bacterial spot in citrus nurseries in Florida

Block[a]	Symptomatic plants (%)	Total plants ($\times 10^3$)	Variance[b] to mean ratio	k[c]	Lloyd's[b] index of patchiness	No. of[d] classes	Class[e] size	Probability distribution[f] (Probability of a greater X^2)	
								Poisson	Negative binomial
Frostproof	0.014	16.8	106.03	0.119	51.44	10	25	0.001	0.737
Venice	0.009	67.2	117.06	1.055	8.23	9	50	1.000	0.999
Ocoee-S	0.007	55.0	95.95	0.023	55.62	9	25	0.000	0.552
Ocoee-R	0.002	55.0	58.72	0.182	24.31	8	25	0.000	0.586
Ocoee-SR	0.008	55.0	62.83	0.259	31.21	8	25	0.001	0.205
Lake Wales									
Bed 1	0.005	9.8	263.03	0.018	6,136.32	5	10	0.000	0.388
Bed 2	0.0002	14.0	...	...	...			...	...
Bed 3	0.029	14.0	46.51	0.082	12.94	10	10	1.000	0.950
Bed 4	0.033	14.0	64.57	0.028	22.11	12	10	0.000	1.000
Bed 6	0.110	12.7	83.75	0.436	3.86	9	25	1.000	0.972
Bed 15	0.023	14.6	65.46	0.064	11.69	13	10	1.000	0.881

[a]In the Ocoee nursery S = scions, R = Swingle rootstock, SR = both scion and rootstock, combined in analysis. Count data utilized for all analysis in all nurseries were quadrats consisting of the number of diseased trees in each 3-m section of individual nursery rows. Lake Wales nursery divided into individual beds for analysis.

[b]Value >1 are indicative of aggregation of diseased plants.

[c]k = Index of dispersion of negative binomial.

[d]Number of classes (0,1,2, etc.) used to test probability distributions.

[e]Class size chosen for analysis was based on the ability to subdivide the data into at least 5 or more frequency classes.

[f]X^2 goodness-of-fit probability (P) of a greater X^2 for Poisson and negative binomial. If $P \geq 0.05$, one fails to reject null hypothesis that observed distribution = theoretical distribution. Thus, $P < 0.05$ for Poisson indicates aggregation, while $P \geq 0.05$ for the negative binomial indicates aggregation.

a bed were infected (Lake Wales beds 3, 6, and 15), across-row aggregation was usually demonstrated. In the Lake Wales nursery bed 6, five of the 10 rows had diseased plants. Ordinary runs analysis did not demonstrate any within-row aggregation but did demonstrate a high level of across-row aggregation of diseased plants in bed 6 of the Lake Wales nursery (Table 2).

Spatial autocorrelation correlograms were examined for each nursery/nursery-bed. An example of a correlogram of the Frostproof nursery is given in which there was significant autocorrelation for the first three spatial lags within rows, for the first two spatial lags across rows, and the first two lags diagonally (Table 3). In addition, the disease level in a second group of quadrats, seven to eight spatial lags (21.4–24.4 m) further away within rows and in rows immediately adjacent, were also significantly autocorrelated. The two-dimensional proximity pattern for this and the remaining correlograms were best seen graphically (Fig. 1). Estimations of the cluster size and orientation were derived from the graphic representation of proximity patterns for each nursery/nursery-bed. Although cluster size varied, a relationship between clusters separated by 21–24 m (cluster center to cluster center considering 3.05 m quadrat size) was indicated for both the Frostproof and Venice nurseries. A strong and extensive within-row and moderate across-row relationship of correlated disease levels was observed for the Ocoee nursery when scion infection alone was considered. A scattered proximity pattern of predominantly individual outlying quadrats was demonstrated for the Ocoee nursery when rootstock infections were considered individually or in combination with scion infections. Within the five diseased beds of the Lake Wales nursery, disease incidence of quadrats was correlated predominantly within rows with some across-row autocorrelation in Beds 6 and 15 (Fig. 1).

Three-dimensional response surface and isopath analysis. The response surface of the Frostproof nursery graphically illustrated the clustering of high disease incidence along the center of the eastern perimeter rows of the nursery bed (Fig. 2A). The disease isopath map indicated a "comet"-shaped isopath contour line of high disease incidence (Fig. 2C). An expanded three-dimensional response surface across the nursery bed in the area of the "comet head" of highest disease incidence coincided with the 6-m section of the bed, which was transplanted to the Hastings containment field plot (Fig. 2B). Examination of the trees after transplanting revealed a grapefruit tree with a stem lesion characteristic of CBS that was older than any seen in the nursery previously. The location of the tree with the stem lesion corresponded to one of the four peaks of foliar disease incidence approaching 0.5 (50% of the foliage infected). Isopath mapping of the "comet-head" further identified two small areas of highest disease incidence bounded by a 0.15 disease isopath line, the southernmost of which cor-

TABLE 2. Results of ordinary runs analysis of aggregation of citrus bacterial spot in four nursery outbreaks in Florida

Plot[a]	Direction of rows	Disease[b] proportion, nursery	Disease[c] proportion, quadrat	Proportion significantly[d] aggregated	
				Within row	Across row
Frostproof	NS	239/16,800 (0.014)	27/280	2/14	6/20
Venice	NS	619/67,200 (0.009)	111/280	6/14	6/20
Ocoee-S	NS	117/55,000 (0.002)	12/340	2/17	3/20
Ocoee-R	NS	389/55,000 (0.007)	40/340	4/17	2/20
Ocoee-SR	NS	449/55,000 (0.008)	40/340	4/17	2/20
Lake Wales					
Bed 1	EW	46/9,828 (0.005)	4/180	1/10	0/19
Bed 3	EW	410/14,040 (0.029)	25/180	2/10	3/18
Bed 4	EW	462/14,040 (0.033)	10/180	1/10	0/18
Bed 6	EW	1,406/12,740 (0.110)	78/180	0/10	8/19
Lake Wales					
Bed 15	NS	337/14,560 (0.023)	27/190	1/10	7/19
Bed 2	EW	3/14,040 (0.0002)	1/180	...	...

[a]In the Ocoee nursery S = scions, R = Swingle rootstock, SR = both scion and rootstock considered in analysis. Lake Wales nursery divided into individual beds for analysis.
[b]Number of diseased plants/total number of plants in nursery, and proportion diseased plants.
[c]Number of quadrats in which disease occurred/total number of quadrats per plot.
[d]Number of rows in which disease was aggregated/total number of rows; and number of across-row nursery bed sections in which disease was aggregared/total number of sections.

TABLE 3. Example of correlogram of spatial lag correlations of incidence of citrus bacterial spot on citrus nursery plants in the Frostproof nursery

Spatial lags across row (E-W)	Spatial lags within rows (N-S)										
	0	1	2	3	4	5	6	7	8	9	10
0	−1.000	0.516*[a]	0.369*	0.242*	−0.023	0.050	−0.015	0.220*	0.291*	−0.014	−0.030
1	0.351*	0.149*	0.083	0.015	−0.016	0.094	−0.058	0.205*	0.451*	0.217	−0.012
2	0.151*	0.104	0.276*	0.091	−0.024	−0.008	−0.016	0.011	0.094	0.035	−0.033
3	0.061	0.042	0.002	−0.004	−0.012	−0.006	−0.003	−0.045	−0.054	−0.021	−0.034
4	0.034	0.028	−0.008	−0.016	−0.028	−0.030	−0.025	−0.031	−0.016	−0.002	−0.036
5	−0.002	−0.002	−0.016	−0.024	−0.027	−0.028	−0.025	−0.030	−0.018	−0.006	−0.036

[a]* = Correlation significantly different from zero at $P = 0.05$. Quadrats consisted of ~ 3-m sections of individual rows.

responds to the location of the grapefruit tree with the old stem lesion (Fig. 2D).

Similar analysis of the Venice nursery also revealed an edge effect with the highest disease incidence located in the central area of the western perimeter of the nursery (Fig. 3A). Isopath analysis indicated a large central cluster of disease trailing off to the southeast with secondary clusters predominantly in the southern half of the nursery (Fig. 3B).

In the Ocoee nursery, both scion shoots and rootstock sprouts existed on the same plants. Therefore, response surfaces and isopath maps of the easternmost section (which contained all the diseased plants) of the nursery were generated to examine the relationship of disease intensity of scions and rootstocks individually and in combination (Fig. 4A and B). Scion infections were restricted predominantly to the northwestern corner of the nursery section where trees had been bud grafted, whereas rootstock infections were more generally distributed (Fig. 4A and B). Highest disease incidence in the rootstocks corresponded to the highest disease incidence of the new scion shoots, although disease incidence on the rootstock was generally lower than on the scion of the same plant. Average disease incidence from the combined scion and rootstock evaluations reflected the individual components of the disease in Fig. 4C. Isopath maps further illustrated the aggregation of scion disease in the northwestern corner of the nursery section and the rootstock disease more generally distributed (Fig. 4D–F).

Only two of the Lake Wales nursery beds contained sufficient disease to justify response surface and isopath mapping. In both cases, citrus bacterial spot was distributed predominantly down individual rows with some adjacent rows also showing disease (Fig. 5). Although disease was distributed nearly the entire length of the nursery beds, areas of high disease intensity, i.e., aggregations, can be discerned by examination of disease contour lines of the respective isopath maps (Fig. 5C and D). These areas extended across five and three rows in beds 6 and 15, respectively.

Isopath contour area analysis. The area circumscribed by isopath contour lines was inversely proportional to disease intensity. Thus, as expected, the area occupied by higher disease contours is very small and lower disease contours much more extensive (Fig. 6A–D). The areas circumscribed by disease contour levels can generally be represented by a common decay curve. The magnitude of the area of the individual disease contour levels depended on the total area of the nursery or nursery section studied and the extensiveness of the disease infection. Disease in the Venice nursery was restricted to the smallest area, whereas in the Ocoee nursery, disease was more widespread.

Disease gradient analysis. The most severe disease in the Frostproof nursery occurred in the easternmost row of 14-mo-old grapefruit trees about 120 m from the southern end of the bed (Fig. 2A and B). This point was used as a presumed focus for gradient calculations and gradients were calculated to the north and south along the row (Table 4). The slope was shallowest to the north, indicating a possible predominance of spread in that direction. Directional analysis to the west across rows was not warranted, because individual rows contained different cultivars and presumably different susceptibilities to CBS.

The isopathic contour map of the Venice nursery indicated a potential focus of disease on the westernmost row of Swingle trees in the infected nursery bed. This indicated focus was used in directional disease gradient analysis to determine probable direction of disease movement. The "Y vs. $\ln(X)$" and "Probit(Y) vs. Probit(X)" models best described directional disease gradients in the Venice nursery, although none of the models tested fit all the gradients particularly well (Table 5). Disease gradients were steepest in directions more approximating across rows (i.e., to the east) and shallowest in directions more approximating down

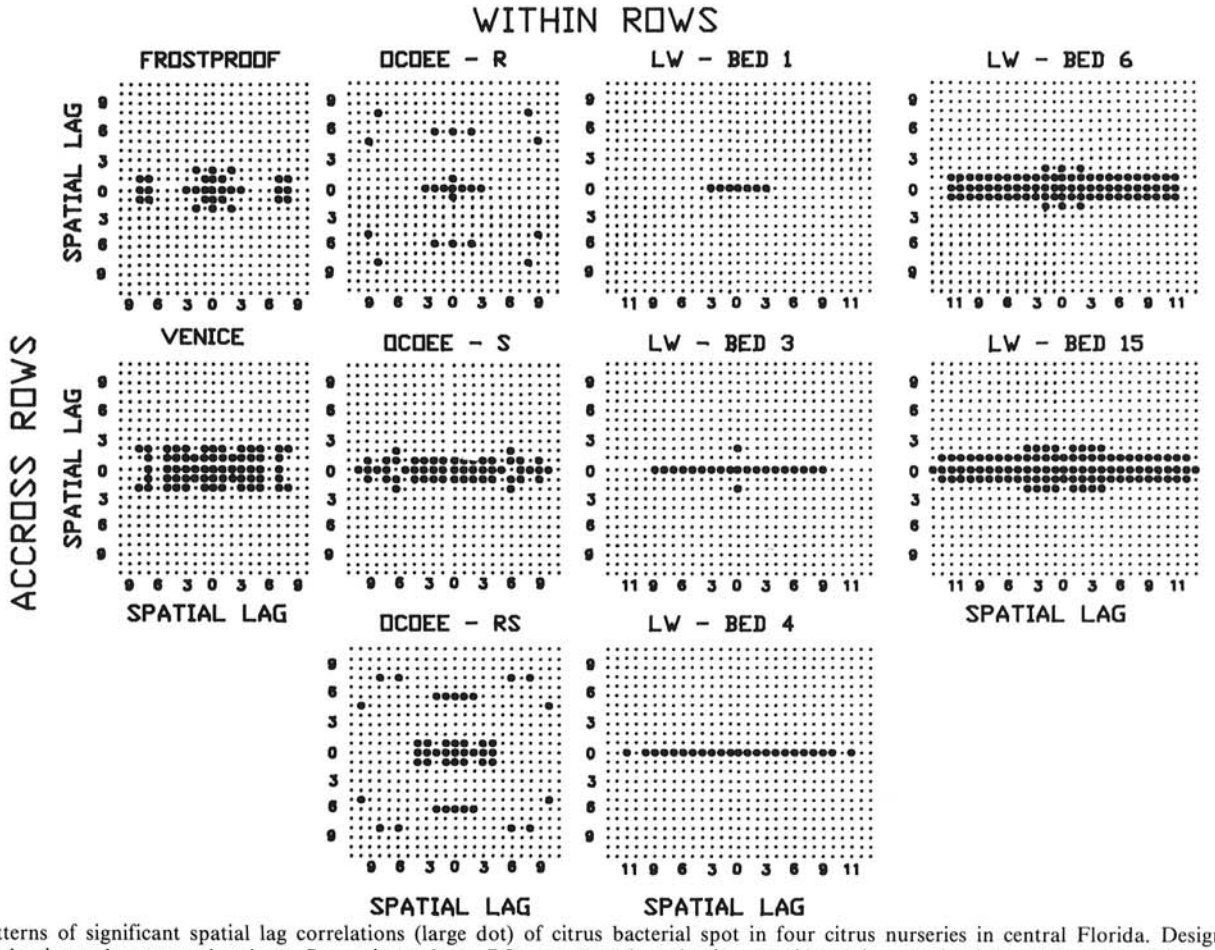

Fig. 1. Patterns of significant spatial lag correlations (large dot) of citrus bacterial spot in four citrus nurseries in central Florida. Designations: R = Swingle citrumelo rootstocks alone, S = scions alone, RS = rootstock and scion considered in combination, LW = Lake Wales nursery divided into individual nursery beds.

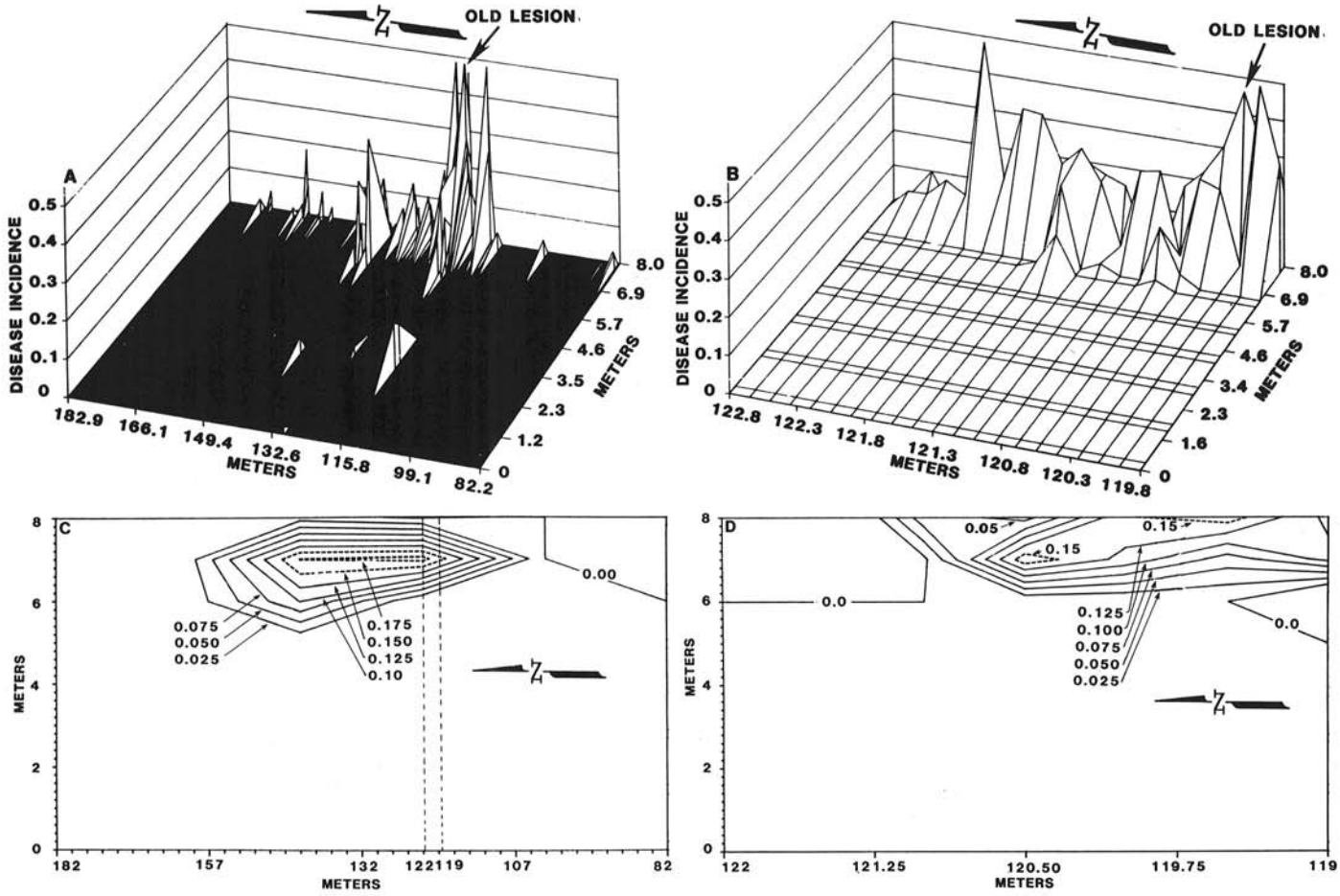

Fig. 2. Maps of disease severity of citrus bacterial spot in Frostproof citrus nursery. **A,** Three-dimensional response surface; highest disease incidence (arrow) was associated with an old stem lesion, the oldest lesion seen in the nursery. **B,** Expanded response surface (119–123 m area) across nursery bed in area of highest disease intensity. **C,** Isopath contour map of the estimated position of similar disease incidence levels of the entire nursery bed. **D,** Expanded isopath contour map of area of highest disease intensity (119–122 m section represented by dotted lines in C). The 0.15 disease incidence contains lines in upper right corner that correspond to proximity of old lesion.

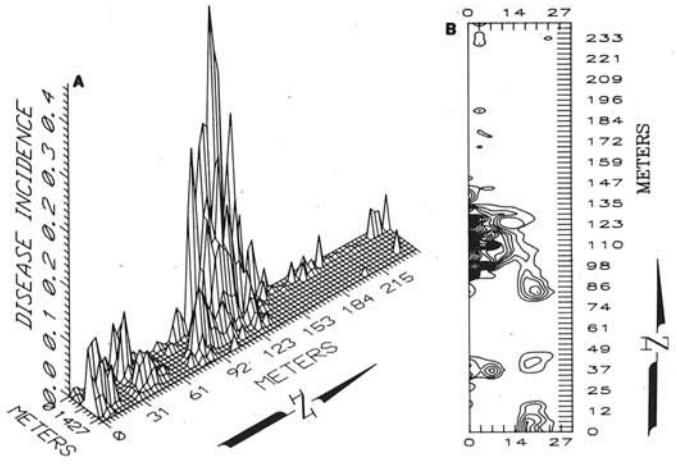

Fig. 3. Map of incidence of citrus bacterial spot in the Venice citrus nursery. **A,** Three-dimensional response surface and, **B,** isopath contour map of the Venice citrus nursery. Note the concentration of highest disease incidence on western edge of nursery and presumed natural spread to east and south evidenced by lower disease incidence contour lines.

rows from the proposed focus. The shallowest disease gradient slopes were in the more southern directions (i.e., E/SE, SE, and S/SE) and the northern directions (i.e., N/NE and NE), indicating a predominance of disease spread from the proposed focus generally toward the southeast and northeast along rows (Table 5), which is consistent with isopath maps of disease incidence (Fig. 3).

Disease progression over time was examined in the section of the Frostproof nursery that had been transplanted to the Hastings research site. Disease incidence and severity (previously defined) increased slightly after transplanting (Fig. 6E). This increase was likely the result of the further development of subclinical infections that took place in the original nursery. After this initial increase, the disease gradually decreased over the next 200 days. Infected foliage abscised slowly during this period and very little new infection took place. Thus, the plants grew well and essentially recovered from the disease without treatment.

Similarly, disease progression of plants removed from the Ocoee nursery outbreak and planted in a grove configuration at the Hastings research site was monitored (Fig. 6F). Disease increased on infected plants and additional lesions became evident on exposed plants immediately after they were potted and placed in a greenhouse to overwinter. These new lesions were probably due to the further development of subclinical infections. Disease on infected and exposed plants decreased to nondetectable levels after planting in the field due to the eventual loss of diseased foliage and lack of new infections under field conditions.

DISCUSSION

Probability distributions are traditionally fit to disease data in an attempt to evaluate if a population of diseased plants is aggregated (i.e., clustered) or randomly distributed (6,20). In the case of some of those citrus nurseries examined in this study in which large numbers of observations were made, significant chi-square statistics were observed for both the Poisson and

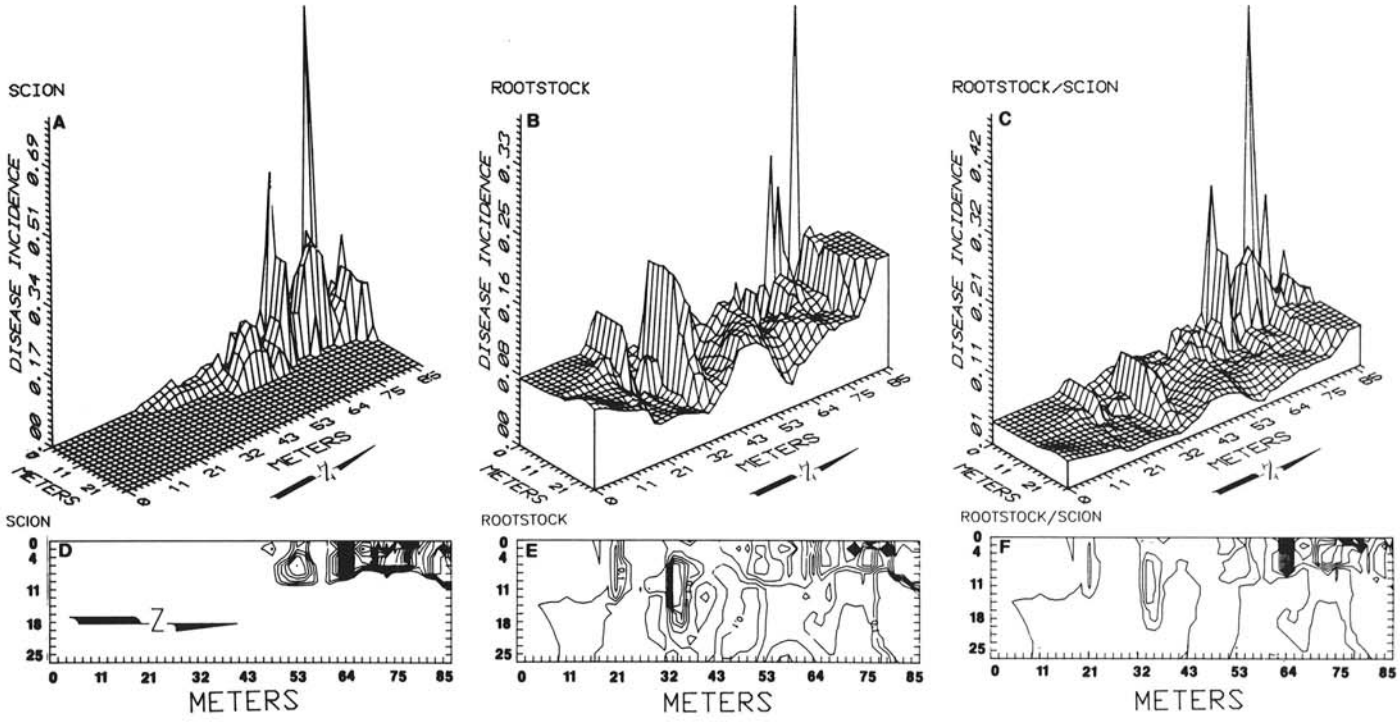

Fig. 4. Three-dimensional response surfaces and isopath contour maps of disease incidence of citrus bacterial spot in the Ocoee nursery for scion, rootstock disease, and rootstock and scion disease combined. NOTE: General dispersion of disease associated with rootstocks and restricted scion disease predominantly to northwest corner of nursery.

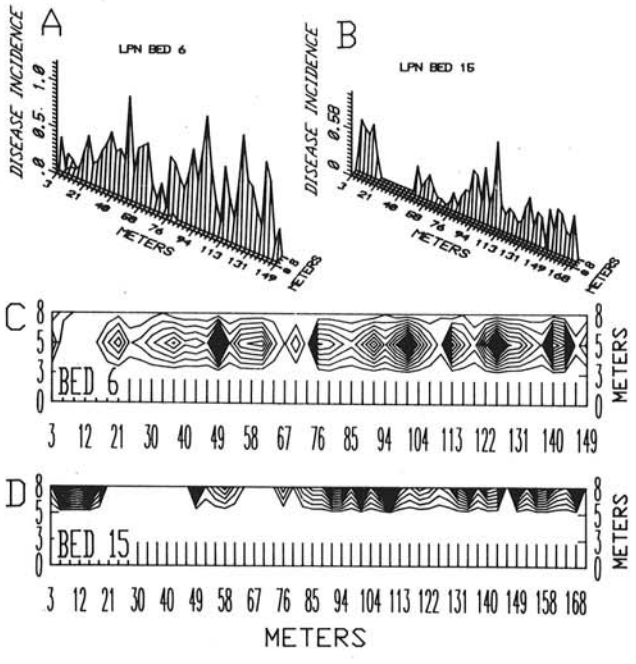

Fig. 5. Three-dimensional response surface and isopath contour maps of disease incidence of citrus bacterial spot in two nursery beds of the Lake Wales nursery. Note the high degree of aggregation associated within nursery rows (east-west) with some limited lateral movement (north-south) that was indicative of mechanical spread of the disease down nursery rows.

negative binomial distributions, indicating randomness and aggregation of diseased plants, respectively. This inconsistency is most likely due to the large number of O-class observations in those data sets resulting from low proportions of diseased plants in these instances. The low proportion of diseased plants caused a heavy weighting near the origin due to numerous O-class observations combined with large numbers of observations in the first few frequency classes proximal to the O-class. Slight changes in the range designations of these first few frequency classes of such data sets can result in significant changes in the chi-square statistics and alter the indication of randomness or aggregation. The instability of the fit of the Poisson and negative binomial distributions to these large data sets with very low proportions of disease suggests that the probability distributions might not be the most appropriate indicators of randomness or aggregation for these data. Thus, other indicators of randomness or aggregation such as the variance-to-mean ratio and Lloyd's-index-of-patchiness were examined for more definitive analysis (21).

Once the presence of aggregation of CBS disease in nurseries was indicated, ordinary runs analysis was found to be very useful to test for directionality of aggregation within and across rows. However, ordinary runs analysis only indicates the presence or absence of aggregation of disease within or across rows, but does not indicate either the cluster size or proximity of clusters to each other. Thus, once such aggregations down and across rows were indicated, size and spatial correlations between clusters were examined by spatial autocorrelation analysis (17). This analysis is particularly useful to determine average cluster size as well as orientation, approximate distance, and correlation with adjacent clusters. The intensity of disease and its spatial location can be visualized with three-dimensional response surfaces. Isopath maps were very useful to visualize aggregations of disease, size, and orientation of clusters, and the presence of foci of infection. When foci of infection were identified, as in the Frostproof and Venice nurseries, disease gradient analysis was used to determine if there was a predominance of directionality to pathogen spread (9).

Of the disease gradient models tested for data from the Venice nursery, those with the highest r^2 of linear regression included Probit(Y) vs. Probit(X), further substantiating the use of this model as a useful description of directional disease gradients in CBS epidemics (9).

The four nurseries studied represent four different disease situations with regard to citrus bacterial spot. In the Frostproof nursery, maintenance personnel used mechanical shears to hedge the taller and older row of grapefruit on the eastern edge of

the infected block. A few lesions were evident on older foliage, especially in the area where the older stem lesion was noted near the presumed focus. Disease occurred predominantly on the cut and tattered foliage resulting from hedging and on the new, posthedging flush of foliage. Disease gradients were flattest in the northern direction, which corresponded to the direction of hedging. Little across-row spread was recorded. This suggested that disease was introduced in very few locations within the nursery bed and spread almost entirely by mechanical means. The highly susceptible grapefruit tissue, wounding caused by hedging, and closely spaced interlocking grapefruit trees, provided an advantageous situation for within-row spread. The distance between rows, the presence of less susceptible cultivars in adjacent rows, and the lack of mechanical hedging in adjacent rows of younger plants, probably inhibited across-row spread. Two different bacterial strains of *X. c. citrumelo* were identified by serological assay and restriction fragment length polymorphism assay, corresponding to a large northern and small southern cluster of diseased plants (Gottwald, Alvarez and Hartung, *unpublished data*). Thus, more than one introduction of inoculum may have occurred in order to account for the occurrence of two different strains resulting in two foci of disease separated by some distance.

The spatial pattern of disease in the Ocoee nursery indicated a method of pathogen dissemination different from the Frostproof nursery. Swingle rootstock seedlings were purchased on three different occasions and used to establish the nursery. Only plants resulting from the last planting became diseased. Some lesions occurred on the very lowest leaves on the rootstock plants indicating that the disease probably entered the nursery on contaminated planting material. The disease on rootstock seedlings was relatively low and widely scattered throughout the third planting, although some plants with higher disease incidence were located in the northwestern corner of this section.

TABLE 4. Linear regression analysis of disease severity on distance from a presumed focus in the Frostproof citrus nursery

Direction[a] of spread tested	Gradient model[b]					
	ln (*Y*) vs ln (*X*)		(*Y*) vs ln (*X*)		Probit (*Y*) vs Probit (*X*)	
	Slope (*b*)	r^2	Slope (*b*)	r^2	Slope (*b*)	r^2
South	−2.242	0.475	−0.030	0.340	−0.106	0.086
North	−2.093	0.322	−0.020	0.400	−0.093	0.158

[a] Proposed focus is on the eastern edge of nursery bed 120 m from southern edge corresponding to area of highest disease severity. Across-row gradients could not be calculated as different cultivars were located in individual rows.

[b] X = Distance from the proposed focus, Y = disease severity, measured as the number of leaves infected/total number of leaves per plant, b = slope of the transformed gradient, and r^2 = the coefficient of regression.

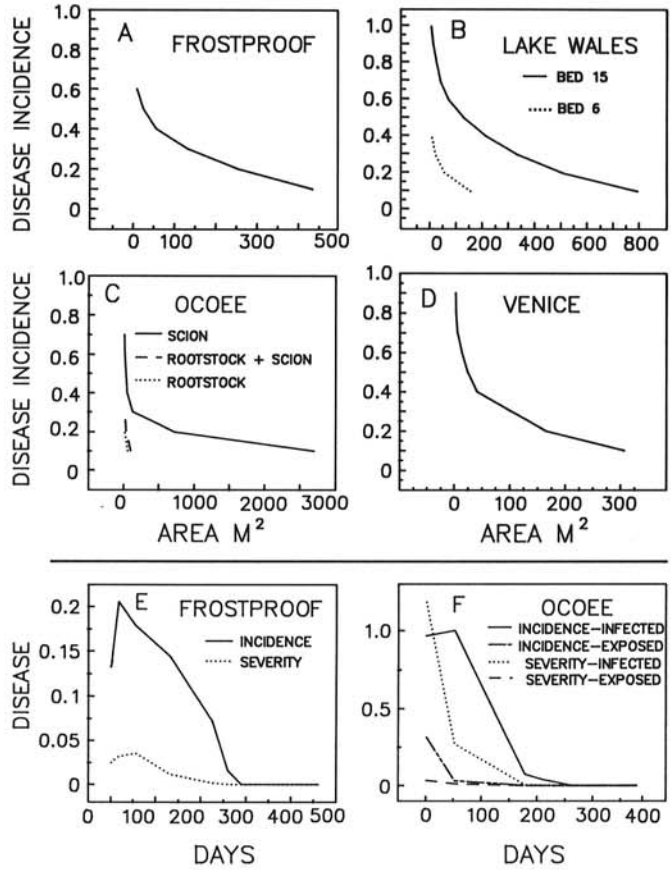

Fig. 6. A–D, Area within each nursery circumscribed by isopath contour lines of similar disease incidence. **E–F,** Disease progress curves for both disease incidence (proportion of diseased plants and disease severity (proportion of leaves infected per plant) over time of plants removed from the indicated nursery and reestablished at the Hastings, FL, disease containment research site.

TABLE 5. Linear regression analysis of directional disease severity on distance from a proposed focus in the Venice citrus nursery

Direction of spread tested and magnetic heading in degrees[a]		Angle[b] of arc in degrees	Gradient model[c]					
			ln(*Y*) vs ln(*X*)		(*Y*) vs ln(*X*)		Probit(*Y*) vs Probit(*X*)	
			Slope (*b*)	r^2	Slope (*b*)	r^2	Slope (*b*)	r^2
E	270	180	−1.167	0.235	−0.36	0.575	−1.293	0.912
NE	315	90	−2.179	0.397	−0.039	0.629	−1.324	0.936
E	270	90	−3.007	0.958	−0.100	0.895	−1.591	0.988
SE	225	90	−1.093	0.113	−0.046	0.558	−1.549	0.886
N-NE	337.5	45	−1.978	0.365	−0.041	0.700	−1.27	0.923
NE	315	45	−2.799	0.829	−0.091	0.915	−1.254	0.931
E-NE	292.5	45	−3.275	0.981	−0.124	0.864	−1.920	0.969
E	270	45	−4.9699	0.570	−0.123	0.890	−1.871	0.976
E-SE	249.5	45	−2.661	0.836	−0.084	0.923	−1.21	0.909
SE	225	45	−1.663	0.664	−0.093	0.768	−1.641	0.901
S-SE	202.5	45	−1.105	0.119	−0.055	0.560	−1.2	0.888

[a] Proposed focus on the western edge of the nursery bed 112.5 M from the northern end of the nursery corresponding to the area of highest disease severity. Magnetic direction of spread tested emanating from proposed focus.

[b] The arc tested consists of two lines radiating at a given angle from a proposed focal point (i.e. if the proposed direction of spread is 315° from magnetic north = (NW), and the arc tested is 45°: all plants between lines radiating at 292.5° and 337.5° from the proposed focal plant are included in the analysis including those points which fall on the lines.

[c] X = distance from the proposed focus, Y = disease severity, measured as the number of leaves infected/total number of leaves per plant, b = slope of the transformed gradient, and r^2 = the coefficient of regression.

Disease on scions was primarily restricted to the northwest corner of the plot, corresponding to the location with high disease levels on the rootstock seedlings. The intense handling of individual plants during budding, wrapping, unwrapping, and staking of new scion shoots likely contaminated the new scions in that area. The predominant within-row aggregation of scion disease may have occurred due to spread down the row during handling.

The more scattered aggregations of rootstock disease likely resulted from the random planting of a few contaminated rootstock seedlings. No evidence of natural spread was seen, likely because of the routine sprays of copper bactericides. The result was a passive dispersal of the pathogen by handling with subsequent spread inhibited by application of copper sprays.

The recovery of diseased plants to an apparent nondiseased or healthy state when transplanted from the Ocoee nursery to a simulated grove situation suggests that the disease may not be able to subsist in drier, less humid grove conditions. Thus, at least in the case of less aggressive isolates of *X. c. citrumelo* (12), infected nursery plants may pose no threat when outplanted in a grove situation, an observation that bears further study.

In the Lake Wales nursery, all diseased nursery beds were established from Swingle rootstock seedlings that originated in the same seed bed and all from a single seed lot. Disease was highly aggregated within rows (Fig. 5). A few diseased plants were probably established within a few nursery rows in each of the infected beds, and then the pathogen spread down the row by subsequent plant handling and horticultural practices. Although across-row aggregation was indicated, this is likely the result of dissemination of bacteria down adjacent rows rather than across-row spread. Often a severely diseased row would be adjacent to another severely diseased row on one side and a virtually nondiseased row would be present on the other side. Nursery managers confirmed that workers routinely stayed within rows during normal nursery operations. Thus, a passive distribution of contaminated seedlings, followed by further mechanical spread by workers down nursery rows, seems the most likely method of dissemination to explain the disease distribution in this nursery. There is no strong evidence in this case for any natural spread of CBS in this nursery from diseased plants across rows.

The Venice nursery demonstrates a different dissemination pattern than that seen in the other three nurseries. In this case, both ordinary runs analysis and spatial lag autocorrelation analysis indicate considerable across-row aggregation of disease. The nursery was not well maintained and no copper bactericides were applied. The highest disease levels were recorded on the edge of the western nursery bed (Fig. 3). If this area is considered a probable focus of disease, the flattest disease gradients were both across and down the rows to the southeast and northeast. Isopath maps showed that a major focus of infection on the western edge of the nursery with decreasing disease severity and less severe secondary foci to the southeast. Analysis of disease gradients further indicated spread more often to the southeastern directions toward the secondary foci. In this case, natural rather than mechanical spread of the disease from a discrete perimeter focus seems a more plausible explanation for the distribution of disease encountered.

Rather than complete destruction of the nursery, an alternative method of disease eradication was used. Rootstock seedlings were sprayed with copper bactericide and stripped of their foliage. Plants were then bud grafted and highly susceptible rootstock shoots above the budunion removed. The use of copper sprays was continued at regular intervals. At the time of this writing, the nursery had been inspected monthly for 18 mo; no subsequent disease had been detected on either the remaining portion of the rootstock or on the new scion shoots, and plants were transplanted to grove situations. Such practices appear to have great potential as alternative measures to plant destruction to reduce spatial spread of the pathogen or eliminate citrus bacterial spot from a nursery, even when the disease is well advanced.

The aggressiveness of strains of *X. c. citrumelo* was examined for these four nurseries and other outbreaks of CBS in a concurrent study (12). Strains were characterized as aggressive for the Ocoee and Venice nurseries and moderately and weakly aggressive for the Frostproof and Lake Wales nurseries, respectively, based on attached leaf greenhouse assay and detached leaf inoculations (12). As previously mentioned, significant natural spread of CBS was considered to have been observed only in the Venice nursery associated with an aggressive strain of *X. c. citrumelo*. In the Venice nursery, no copper sprays were applied before disease detection, whereas copper bactericide was applied regularly in the Ocoee nursery where aggressive strains were also detected. Thus, the lack of natural spread in the Ocoee nursery and the natural spread observed only in the Venice nursery may have been related to the presence or absence of copper bactericides, respectively. The spatial patterns of disease in the Frostproof and Lake Wales nurseries were also consistent with the less aggressive bacterial strains prevalent in these nurseries. Disease spread in both cases was most likely attributable to mechanical means via machinery and handling. Thus, cultural practices used in individual nurseries can contribute to the different spatial patterns of CBS observed in different nursery situations. Evidence of natural spread of CBS has been recorded on only one previous occasion of the 23 recorded outbreaks (9,12). In this case, the bacterial isolates first taken from this outbreak were considered aggressive, although they have subsequently lost aggressiveness in culture (Civerolo and Gottwald, *unpublished*). Thus, there appears to be a diversity in strain aggressiveness.

LITERATURE CITED

1. Brlansky, R. H., Lee, R. F., and Civerolo, E. L. 1986. Detection of *Xanthomonas campestris* from citrus by membrane entrapment and immunofluorescence. (Abstr.) Phytopathology 76:1101.
2. Civerolo, E. L. 1984. Bacterial canker disease of citrus. J. Rio Grande Valley Hortic. Soc. 37:127-146.
3. Campbell, C. L., and Noe, J. P. 1985. Spatial pattern analysis of soilborne pathogens and root diseases. Annu. Rev. Plant Pathol. 23:128-148.
4. Gabriel, D. W., Hunter, J. E., Kingsley, M. T., Miller, J. W., and Lazo, G. L. 1988. Clonal population structure of *Xanthomonas campestris* and genetic diversity among citrus canker strains. Molec. Plant-Microbe Interac. 1:59-65.
5. Gabriel, D. W., Kingsley, M. T., Hunter, J. E., and Gottwald, T. R. 1989. Reinstatement of *Xanthomonas citri* (ex. Hasse) and *X. phaseoli* (ex. Smith) to species and reclassification of all *X. campestris* pv. *citri* strains. Int. J. Syst. Bacteriol. 39:14-22.
6. Gates, C. E., and Etheridge. F. G. 1970. A generalized set of discrete frequency distributions with FORTRAN program. Math. Geol. 4:1-24.
7. Gottwald, T. R., Civerolo, E. L., Garnsey, S. M., Brlansky, R. H., Graham, J. H., and Gabriel, D. W. 1988. Dynamics and spatial distribution of *Xanthomonas campestris* pv. *citri* group E strains in simulation nursery and new grove situations. Plant Dis. 72:781-787.
8. Gottwald, T. R., McGuire, R. G., and Garran, S. 1988. Asiatic citrus canker spatial and temporal spread in simulated new grove situations in Argentina. Phytopathology 78:739-745.
9. Gottwald, T. R., Miller, C., Brlansky, R. H., Gabriel, D. W., and Civerolo, E. L. 1989. Analysis of the spatial distribution of citrus bacterial spot in a Florida citrus nursery. Plant Dis. 73:297-303.
10. Gottwald, T. R., Timmer, L. W., and McGuire, R. G. 1989. Analysis of disease progress of citrus canker in nurseries in Argentina. Phytopathology 79:1276-1283.
11. Graham, J. H., and Gottwald, T. R. 1988. Citrus canker and citrus bacterial spot in Florida: Research findings—future considerations. Citrus Ind. 69(3):42–45, 48–51.
12. Graham, J. H., and Gottwald, T. R. 1990. Variation in aggressiveness of *Xanthomonas campestris* pv. *citrumelo* associated with citrus bacterial spot in Florida citrus nurseries. Phytopathology 80:190-196.
13. Gregory, P. H. 1968. Interpreting plant disease dispersal gradients. Annu. Rev. Phytopathol. 6:189-212.
14. Hartung, J. S., and Civerolo, E. L. 1987. Genomic fingerprints of *Xanthomonas campestris* pv. *citri* strains from Asia, South America, and Florida. Phytopathology 77:282-285.
15. Koizumi, M. 1981. Citrus canker. Pages 8-12 in: Citrus Diseases in Japan. T. Miyakawa and A. Yamaguchi, eds. Japan Plant Protection Assoc.
16. Madden, L. V., Louie, R., Abt, J. J., and Knoke, J. K. 1982. Evaluation of tests for randomness of infected plants. Phytopathology

72:195-198.
17. Modjeska, J. S., and Rawlings, J. O. 1983. Spatial correlation analysis of uniformity data. Biometrics 39:373-384.
18. Schoulties, C. L., Miller, J. W., Stall, R. E., Civerolo, E. L., and Sasser, M. 1985. A new outbreak of citrus canker in Florida. Plant Dis. 69:361.
19. Schoulties, C. L., Civerolo, E. L., Miller, J. W., Stall, R. E., Krass, C. J., Poe, S. R., and DuCharme, E. P. 1987. Citrus canker in Florida. Plant Dis. 71:388-395.
20. Taylor, L. R., Woiwod, I. P., and Perry, J. N. 1979. The negative binomial as a dynamic ecological model for aggregation, and the density dependence of k. J. Anim. Ecol. 48:289-304.
21. Upton, G., and Fingleton, B. 1985. Spatial Data Analysis by Example. John Wiley & Sons, Chichester, England. 410 pp.