Preliminary Spatial and Temporal Analysis of Citrus Variegated Chlorosis (CVC) in São Paulo, Brazil

T. R. Gottwald, F. B. Gidtti, J. M. Santos, and A. C. Carvalho

ABSTRACT. Disease progress and spatial spread of citrus variegated chlorosis (CVC) disease was monitored yearly for 6 yr in a 20-ha grove consisting of 4,309 Natal sweet orange/Cleopatra mandarin trees in the state of São Paulo, Brazil. Linear, exponential, monomolecular, logistic, and Gompertz temporal models were fitted to the data set by linear and nonlinear regression analysis. Overall, the nonlinear Gompertz model was the most appropriate, based on correlation of observed versus predicted values and examination of residuals of regression for patterns. The Gompertz rate parameter k for the grove was 0.49. Disease gradients were very steep but extended rapidly during the third year of the epidemic resulting in the flattening of gradient slopes over time. This flattening is consistent with spread from a point focus which would be expected for insect-vectored contagions but inconsistent with spread from seed-borne pathogens. Spatial analysis by the ordinary runs method indicated a high degree of within- and across-row association of CVC-diseased trees and demonstrated that disease in trees immediately adjacent to one another was highly associated. This association of adjacent CVC-diseased trees increased over time. Spatial proximity patterns resulting from spatial autocorrelation analysis indicated associations among CVC-diseased trees immediately adjacent as well as at some distance. The association among CVC-diseased trees located immediately adjacent to one another suggests a pattern of vector movement to immediately adjacent trees with only limited movement over longer distances.

Citrus variegated chlorosis disease (CVC) was first seen in the states of São Paulo and Minas Gerais, Brazil in 1987 (18). Samples were examined by transmission electron microscopy in 1990 and bacteria-like organisms were seen in the xylem which resembled *Xylella fastidiosa* (17). Isolation and culturing of the organisms from diseased xylem tissue of affected citrus from Brazil confirmed the presence of *X. fastidiosa* (3). Pathogenicity tests with cultured *X. fastidiosa* have recently demonstrated that bacteria from cultures induce typical CVC symptoms in greenhouse-inoculated citrus plants (3).

Symptoms associated with CVC are similar to symptoms induced by other xylem-limited diseases. The most notable symptoms include a mottled chlorosis and reduced size of new foliage, resembling a zinc deficiency pattern. Small brown necrotic, gummy lesions often develop on the lower leaf surface in conjunction with the chlorotic patterns on the upper surface. Fruit are small, early maturing, and hard, making them difficult to juice and often causing damage to juicing machines. The disease is more prevalent in younger trees, i.e. less than 6 to 8 yr old, in which the entire tree is often affected. In more vigorous trees or trees older than six years CVC is often associated with individual branches.

No vector has been associated with CVC transmission, but other diseases caused by *X. fastidiosa*, such as Pierce's disease of grape, are usually transmitted by leafhopper (Cicadellidae) vectors. Leafhoppers must therefore be considered potential vectors for transmission of CVC.

Little is known of the epidemiology of CVC and virtually no data have been collected. Some data have been collected concerning the number of groves infected over time, the age distribution of CVC-infected trees, and the geographic location of infected groves. The objectives of this study were *i*) to examine the data from one outbreak in

Mention of a trademark, warranty, proprietary product, or vendor does not constitute a guarantee by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

one grove to make a preliminary spatial and temporal assessment against which future outbreaks can be compared, *ii*) to determine if the spatial and temporal characteristics of CVC increase and spread are consistent with a particular type of transmission, and *iii*) to examine the findings in light of other citrus diseases to help put the threat of CVC to the citrus industry of Brazil into perspective.

MATERIALS AND METHODS

Incidence and spatial position of CVC-affected trees was recorded yearly for 6 yr from 1987 to 1992 in a 20-ha grove of Natal sweet orange on Cleopatra mandarin rootstock in the São Jorge farm near Bebedouro in São Paulo State. The plot consisted of 4309 trees in 82 north-south rows of uneven length planted on a 6 m × 8 m pattern. Incidence was recorded as positive or negative for CVC, based on visual symptoms.

Disease progression models were tested on data from the São Jorge plot over time. The appropriateness of the nonlinear forms of the exponential, monomolecular, logistic, and Gompertz models was examined for disease incidence by nonlinear regression analysis using the SAS NONLIN procedure (SAS Institute, Inc. Cary, North Carolina, USA: version 6.02) (12). The appropriateness of each model was assessed by examining standard residual plots and tested by correlation analysis of observed versus predicted values. The models with the highest coefficient of correlation were chosen as superior (13).

The centroid of disease occurrence (average location of disease incidence) was calculated for 2×2 quadratized data for each year of the epidemic (1). The calculation results in a mathematical determination of the relative center of disease in a field by row and column designators.

Analysis of CVC-disease gradients was accomplished by subjecting 2×2 quadratized data to the GRADCALC PC-based program which calculates the distance from a proposed focus to every other point in the spatial matrix (7). For the São Jorge data analysis the northernmost infected tree in the small cluster of trees in the northeast end of the plot was assumed as the focus. A subset of the data was determined by GRADCALC by striking a gradient from the proposed focus toward the southeast (225 degrees magnetic direction) based on centroid movement over time. This gradient line was the midline of a 90 degree angle whose apex was the focus tree. The weighted average disease incidence was then calculated for those trees within 20-m concentric annuli around the focus that fell between the two lines delimiting the 90 degree angle. Because the Gompertz model was considered the most appropriate temporal model tested, the disease gradient data from the GRADCALC output were transformed by a modified Gregory model, Gompit (y) vs. ln (x), where y = disease incidence and x = distance from the proposed focus (10). Individual gradients can at times be discontinuous, i.e. zero values can be found between positive values along a gradient. To avoid the problem of taking a gompit (–ln(–ln(x))), where x = 0, a small number (0.0004 to 0.04) equivalent to the lowest reading for disease incidence for each year was added to all incidence values (7). Gradients were calculated from transformed values for each year by the SAS REG procedure (SAS Institute, Inc. Cary, North Carolina, USA: version 6.02), and represented graphically (Freelance Graphics for Windows Release 1.0, Lotus Corp., Cambridge, Mass., USA).

Aggregation of diseased plants was determined by ordinary runs analysis for each year (12). Aggregation was assessed as the proportion of rows in the north-to-south and east-to-west orientations with significant ($P = 0.05$) clustering. To visualize disease severity, three-dimensional response surface maps were generated for each year to better understand the directionality of disease spread within and across rows and spatially within the matrix. The 3-dimensional plots were prepared

by using a data contouring software package (Surfer, version 4, Golden Software, Golden Colorado, USA). Prior to spatial analysis, the data for each year was divided into 2×2, 4×4, 8×8, and 16×16 tree quadrats starting at the southwest corner of the plot. The spatial distribution of CVC-diseased quadrats of each size was analyzed by Morisita's index of dispersion (15) and spatial lag autocorrelation analysis of 2×2 quadrats for each year was accomplished with the LCOR2 program (8,14).

RESULTS AND DISCUSSION

The present distribution of CVC in Brazil includes the newer citrus growing areas in São Paulo and Minas Gerais states that have been established in what was previously grassland-savanna (Fig. 1). At the time of writing, less than six percent of the groves in the area presently show symptoms (Fig. 2A) and these are predominately in the north (Table 1). Nearly half of the diseased blocks (48.5%) are 3-5 years of age (Fig. 2B) and of these, the majority of blocks have fewer than 1% diseased trees (Fig. 2C).

CVC progressed slowly for the first two years of the epidemic then entered a logarithmic phase in the third year (Fig. 3). Because the epidemic had not approached an asymptote, all of the nonlinear models fit well. The two nonlinear models which were the most appropriate for the CVC data were the logistic and Gompertz, based on residual plot analysis and correlation analysis of observed vs predicted values (Table 2). Both of these models are appropriate for disease progress curves that are asymmetrically sigmoid, which is the shape that a CVC progress curve is expected to take as more data are collected. The Gompertz model is known to be the most flexible of those tested and was chosen for this reason and for its presumptive flexibility with the addition of future data.

Fig. 1. Progressive map showing location of tne state of São Paulo, the relative citrus growing areas within São Paulo, and the areas within the citrus producing area where citrus variegated chlorosis has been seen.

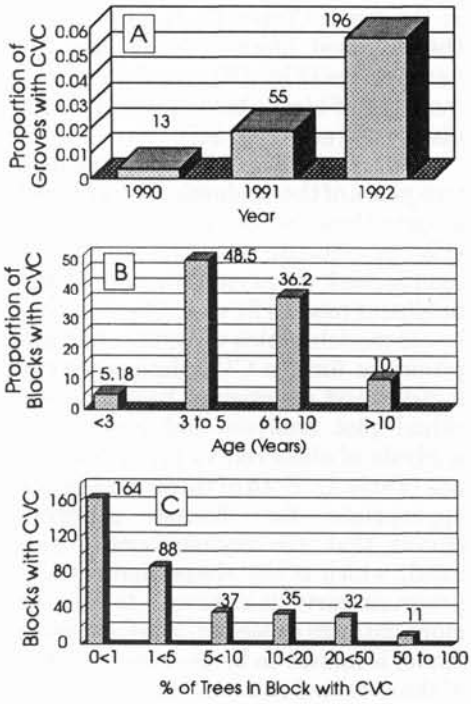

Fig. 2. A. The increase over time of the proportion and number of groves in São Paulo state expressing symptoms of citrus variegated chlorosis (CVC). B. The proportion of individual blocks of citrus affected by CVC and their relative distribution into age categories. C. The distribution of disease incidence rankings within blocks expressing CVC.

The Gompertz model has been used to describe the temporal progress of other insect-vectored citrus diseases as well (4, 5). The Gompertz rate of CVC increase ($k = 0.489$) falls near the same range as that calculated for citrus tristeza virus increase in Spain ($k = 0.07$ to 0.41) and the logistic rate of CVC increase ($r = 1.187$) falls within the range of citrus greening disease increase ($r = 1.035$ to 2.527) (4, 5). The rate of CVC disease increase is considerably less than that of foliar citrus bacterial diseases such as citrus canker and citrus bacterial spot which can approach an asymptote of 95 to 100% in one to two years (6, 9). Thus the rate of CVC increase is not unusual for vectored citrus diseases and considerably slower than rates of disease increase of foliar bacterial diseases of citrus. The Gompertz model was used to predict continued disease increase in the São Jorge plot based on data from the first 6 yr. In this plot CVC was predicted to reach an asymptotic level of disease (0.98 to 0.99) within 13 to 14 yr (Fig. 3). This is similar to the time required by greening (6 to 13 yr) to reach an asymptotic level (4).

Spatial spread of CVC in the São Jorge plot can best be seen in the 3-dimensional surface response plots of quadratized disease incidence (Fig. 4A). CVC started in the northeast corner of the plot and spread toward the southwest over time. The general trend of disease movement was substantiated by centroid analysis which demonstrated a southwestern move-

TABLE 1
DISTRIBUTION AND INCIDENCE OF CITRUS VARIEGATED CHLOROSIS BY REGION IN THE STATES OF SÃO PAULO AND MINAS GERAIS, BRAZIL

Region	Total Groves Examined	Groves Infected	Percent Infected
Fernandopolis	216	1	0.46
San Jose Rio Preto	330	42	12.73
Catanduva	333	39	11.71
Severinia	287	17	5.92
Bebeduro	295	54	18.30
Itapolis	421	1	0.24
Taquaritinga	430	29	6.74
Matão	104	13	12.50
Araraquara	114	0	0.00
Puerto Ferreira	233	0	0.00
Limeira	465	0	0.00
Mogi Mirim	234	0	0.00
Total	3462	196	5.66

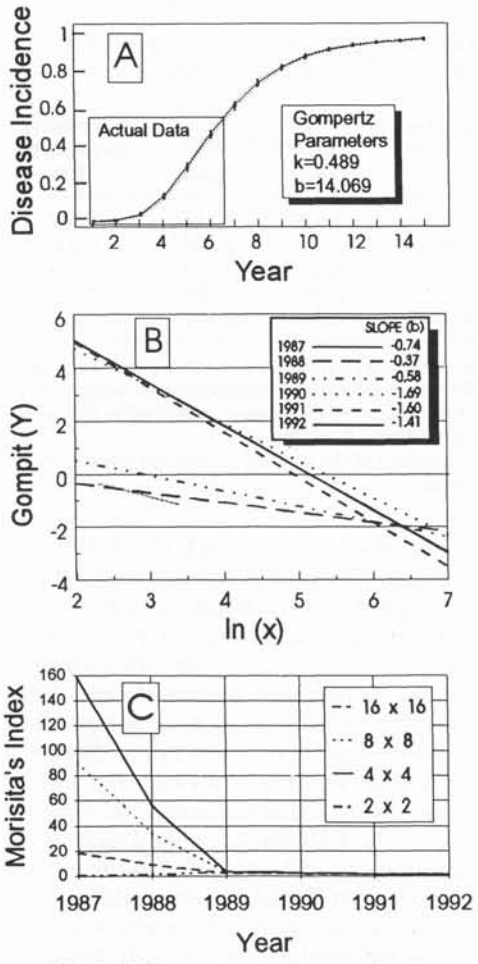

Fig. 3. A. Disease progress curve for citrus variegated chlorosis (CVC) in the São Jorge block. Solid line indicates the predicted points based on the data from the first six years (marked actual data in the boxed area). Dotted lines indicate 95% confidence limits to the prediction. B. Gompit-linearized disease gradients for CVC to the southeast from a proposed focus of infection in the northeast corner of the São Jorge plot. X is measured in meters from the focus. C. Relative aggregation based on Morisita's index of dispersion (MI) for different quadrat sizes over time. MI > 1 indicates aggregation among diseased quadrats, MI = 1 indicates a random distribution of diseased quadrats, and MI < 1 indicates regular arrangement of diseased quadrats.

ment of the mathematical center of disease over time (Fig. 5). CVC disease gradients were fit to the gradient model [gompit (y) vs. ln (x)] by linear regression (Fig. 3B). Gradients to the southeast were the most extensive for all years indicating a general spread of the disease from the focus to the southwest (data only shown for southwest [magnetic 225°] oriented gradients). A considerable amount of spread occurred during the third (1989) and fourth (1990) years of the epidemic which corresponds to the greatest movement of the centroid of disease during 1989 (Fig. 5). As a result the slopes of linearized gradients became steeper and the gradient lines became more extensive relative to the focus. The change in the disease gradients and the centroid movement over time demonstrate a highly directional movement to disease spread in the São Jorge plot. The diagonal (northeast to southwest) spread across rows is inconsistent with spread caused by mechanical or cultural practices. Such demonstrable spread from a focus of infection is also inconsistent with seed transmission other than possibly for the establishment of the focus itself. Such directional spread is consistent with aerial-windborne and insect-vectored pathogens.

Aggregation was examined by ordinary runs analysis. A slightly greater amount of aggregation occurred in rows running north-south compared to those running east-west (Table 3). This is interesting because trees are 8 m apart in the north-south direction and only 6 m apart in the east-west direction, suggesting a definite directionality to CVC spread in the plot regardless of planting distance. Morisita's index of dispersion (MI) is often utilized to examine the relative intensity of aggregation. MI values > 1 indicate aggregation of diseased trees, whereas an MI $= 1$ indicates a random distribution of disease. Cluster sizes of 4×4, 8×8, and 16×16 trees were prevalent for the first two years of the epidemic, with the smaller quadrat sizes of 4×4 and 8×8 resulting in the highest MI values and thus being the most significant indicators of approximate cluster size for those years (Fig. 3C). All indications of aggregation began to fall off as disease levels approached 50% which is normal for all plant diseases. The distribution of cluster sizes did not

TABLE 2
NONLINEAR REGRESSION ANALYSIS OF DISEASE INCIDENCE OF CITRUS VARIEGATED CHLOROSIS IN SÃO JORGE FARM, SÃO PAULO, BRAZIL

Model	Parameter[a]	Estimate	Asymptotic standard error	Asymptotic 95% Confidence Limits		Observed vs. predicted[b] (R^2)
				Lower	Higher	
Exponential	r	1.091	0.018	1.045	1.136	0.908
Monomolecular	r	0.061	0.014	0.024	0.098	0.908
Logistic	r	1.187	0.168	1.143	1.230	0.989
Gompertz	k	0.489	0.061	0.321	0.658	0.991
	b	14.069	4.236	2.308	25.830	

[a]Models were estimated by nonlinear regression of the integrated equations $y = y_0e^{rt}$, $y = 1-(1-y_0)e^{rt}$, $y = 1/[1 + \exp{-(\ln)y_0/(1-y_0))^{+rt}}]$, and $y = \exp^{-Be-kt}$ for the exponential, monomolecular, logistic, and Gompertz models, respectively, where r and k are rate parameters, y is disease measured as incidence of diseased trees, t is time in years, and for the Gompertz model $B = -\ln(y_0)$.
[b]Coefficients of determination of correlation of predicted values of the various models against observed values used to examine the appropriateness of the models.

appear to change over time in relation to disease incidence. Thus diseased trees most often occurred in 16 to 64 tree groups.

Spatial proximity patterns resulting from spatial lag autocorrelation analysis of 2×2 quadrats show significant positive correlations of quadrats with each other and indicate a rapid increase in distances over which CVC-diseased trees were associated with one another (Fig. 4B). Proximity patterns for 1987 and 1988 are elongated in the north-south direction and confirm the results from ordinary runs analysis that CVC spread was preferential in the north to south direction. From 1989 through 1992 diseased trees were significantly positively correlated with all others up to 15 lags (30 trees) away with no breaks. The lack of discontinuous proximity patterns is inconsistent with other citrus diseases previously analyzed and may indicate that vector movement is more general from tree to adjacent tree without significant jumps of distance (5). This general tree-to-tree movement would explain the underlying spatial processes which lead to the continuous and unbroken gradients seen from the original focus of disease to the south or southeast.

This study is based on data collected from a single epidemic in a single large block of citrus trees and therefore should be considered preliminary. Unfortunately it represents an analysis of the only set of quantitative epidemiology data that exists for CVC. Although conclusions should not be drawn from a single data set, the analyses provided can be used as a basis for comparison of future data and insights into the range of future experiments and data needed to make more accurate predictions. Since this is a new citrus disease, few previous reports exist and those that do are mostly descriptive. Unfortunately, CVC has provoked alarm and predictions of devastation of the citrus industry of Brazil in some public presentations where reports were based on fears rather than data (11, 16). We caution that, although this disease is new, apparently spreading, and should be carefully monitored, *no* data presently exist to make any such predictions. Such unfounded predictions of doom only serve as sensationalism and adversely affect the economics of citriculture in Brazil and its markets, and the credibility of the research community.

ACKNOWLEDGMENTS

The authors wish to express their gratitude to the Cutrale Citrus Corporation for providing the data and Procitrus and especially M. Naday of Fazenda Sete Lagoas Agricola for providing the means to examine the disease in Brazil and efforts in organization of research visits. Thanks also to P. Bell, T. Riley, and D. Zies for help with the data analyses.

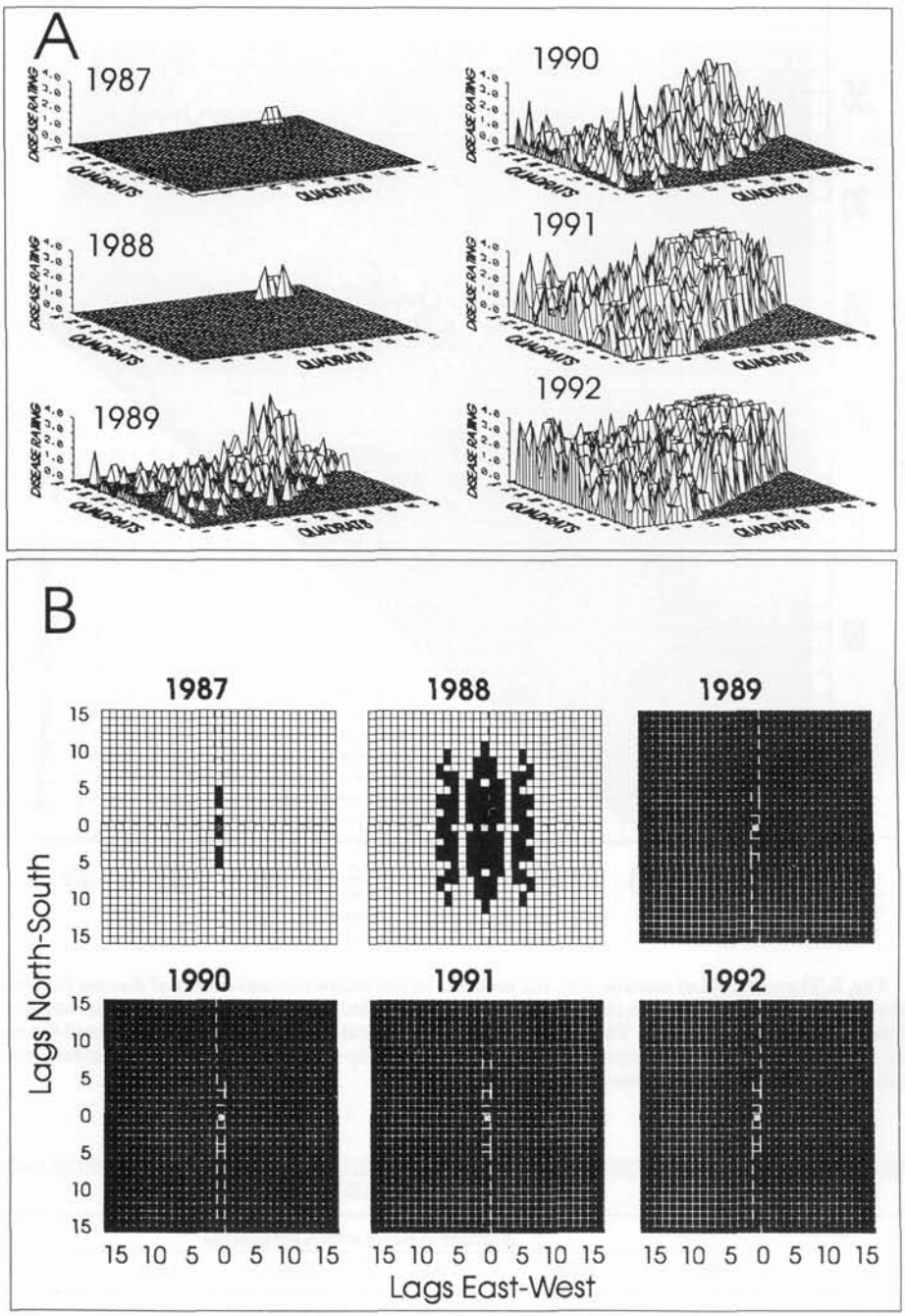

Fig. 4. A. Three-dimensional surface response models of disease incidence of citrus variegated chlorosis (CVC) based on 2 × 2 quadrats in the São Jorge plot. Notice the focus of infection in the northeastern corner of the plot in 1987 which increases in disease incidence and spreads across much of the plot in 1989. B. Disease proximity patterns prepared from results of correlograms from spatial lag autocorrelation analysis of disease incidence in 2 × 2 quadrats of the São Jorge plot. Hatched central square represents the 0,0 lag distance (origin). Black squares indicate significant positive autocorrelations ($P = 0.05$) to the origin. Note the north-south orientation of autocorrelated quadrats in 1987 which extends further in the north-south axis in 1988 and begins to extend obliquely as well. Proximity patterns for 1989-1992 show all quadrats are autocorrelated with the origin indicating extensive contiguous diseased quadrats.

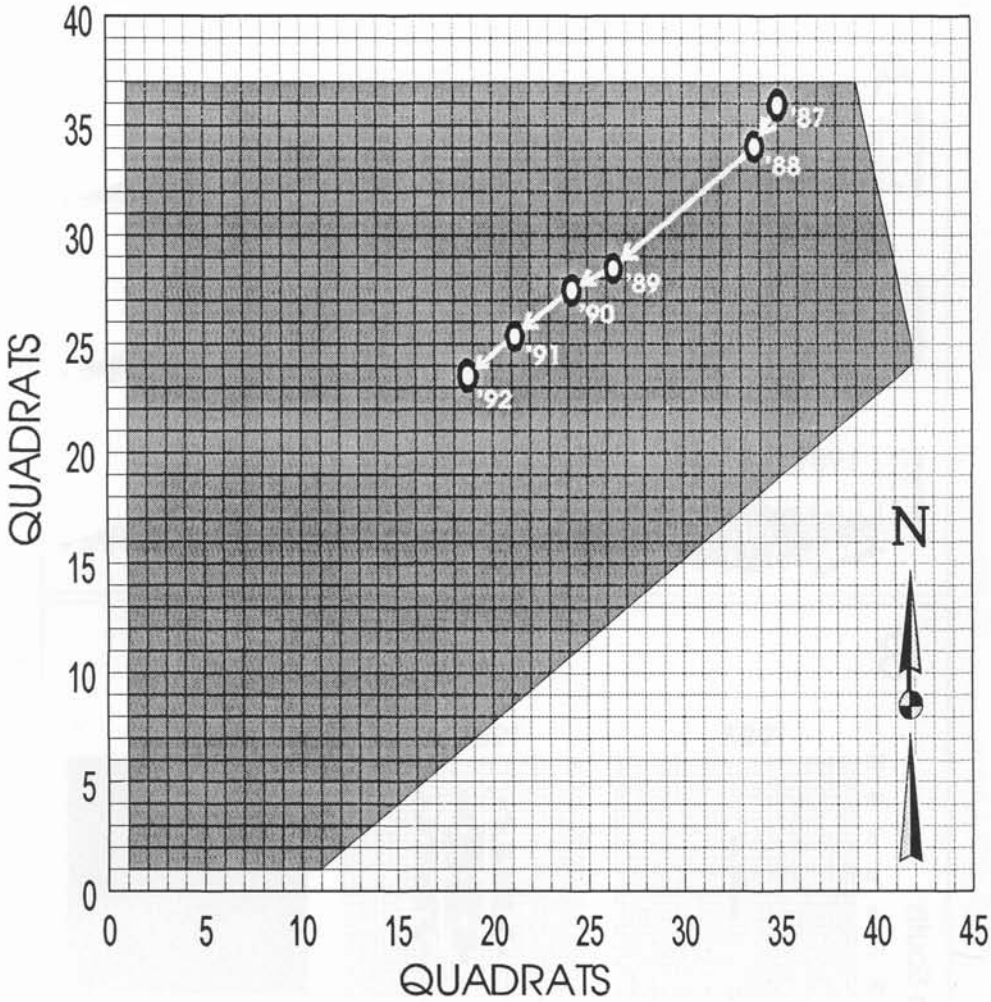

Fig. 5. The centroid of disease, i.e., the location of the mathematical center of disease incidence for each year of the epidemic in the São Jorge plot. The shaded area delimits the spatial arrangement of the 2 × 2 quadratized data. The circles indicate the calculated location of the centroid for each year. Note the general southwestern movement of the center of disease with the greatest movement occurring during the 1989 season.

TABLE 3
ORDINARY RUNS ANALYSIS OF CITRUS VARIEGATED CHLOROSIS IN SÃO JORGE FARM IN SÃO PAULO, BRAZIL

	Number of Rows with Aggregation[z]					
Year	1987	1988	1989	1990	1991	1992
N-S Rows	1/76	1/76	16/76	21/76	21/76	13/76
E-W Rows	0/82	0/82	9/82	17/82	17/82	11/82

[z]Diseased trees were considered to be aggregated within rows if the observed number of runs was significantly different from the expected at $P = 0.05$ on a one-sided test for $Z < -1.64$.

LITERATURE CITED

1. Campbell, C. L., and D. J. van der Gaag
1993. Temporal and spatial dynamics of microsclerotia of *Macrophomina phaseolina* in three fields in North Carolina over four to five years. Phytopathology (in press).

2. Chagas, C. M., V. Rossetti, and J. G. Beretta
1992. Electron microscopy studies of a xylem-limited bacterium in sweet orange affected with citrus variegated chlorosis disease in Brazil. J. Phytopathol. 134: 306-312.
3. Chang, C. J., M. Garnier, L. Zreik, V. Rossetti, and J. M. Bové
1993. Citrus variegated chlorosis (CVC): Cultivation of the CVC bacterium and attempts to experimentally reproduce the disease, p. 294-300. *In*: Proc. 12th Conf. IOCV. IOCV, Riverside.
4. Gottwald, T. R., B. Aubert, and Zhao Xue-Yuan
1989. Preliminary analysis of citrus greening (Huanglungbin) epidemics in the People's Republic of China and French Reunion Island. Phytopathology 79: 687-693.
5. Gottwald, T. R., M. Cambra, and P. Moreno
1993. The use of monoclonal antibodies to monitor spatial and temporal spread of citrus tristeza virus in symptomless trees in eastern Spain. p. 51-61. *In*: Proc. 12th Conf. IOCV. IOCV, Riverside.
6. Gottwald, T. R., R. G. McGuire, and S. Garran
1988. Asiatic citrus canker: Spatial and temporal spread in simulated new planting situations in Argentina. Phytopathology 78: 739-745.
7. Gottwald, T. R., C. Miller, R. H. Brlansky, D. W. Gabriel, and E. L. Civerolo
1989. Analysis of the spatial distribution of citrus bacterial spot in a Florida citrus nursery. Plant Dis. 73: 297-303.
8. 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.
9. Gottwald, T. R., L. W. Timmer, and R. G. McGuire
1989. Analysis of disease progress of citrus canker in nurseries in Argentina. Phytopathology 79: 1276-1283.
10. Gregory, P. H.
1968. Interpreting plant disease dispersal gradients. Annu. Rev. Phytopathol. 6: 189-212.
11. Lee, R. F., K. S. Derrick, M. J. G. Beretta, C. M. Chagas, and V. Rossetti
1991. Citrus variegated chlorosis: a new destructive disease of citrus in Brazil. Citrus Ind. 72(10): 12-13, 15.
12. Madden, L. V., R. Louie, J. J. Abt, and J. K. Knoke
1982. Evaluation of tests for randomness of infected plants. Phytopathology 72: 195-198.
13. Madden, L. V.
1986. Statistical analysis and comparison of disease progress curves. p. 55-84 *In:* K. Leonard and W. E. Fry (eds.) Plant Disease Epidemiology: Population dynamics and management. Macmillan, New York. 372 p.
14. Modjeska, J. S., and J. O. Rawlings
1983. Spatial correlation analysis of uniformity data. Biometrics 39: 373-384.
15. Morisita, M.
1959. Measuring of the dispersion of individuals and analysis of the distributional patterns. Mem. Fac. Sci. Kyushu Univ., Ser. E. Biol. 2: 215-235.
16. Roistacher, C. N.
1991. CVC: A warning and an appeal. Citrograph 77(4): 17-18.
17. Rossetti, V., M. Garnier, J. M. Bové, M. J. G. Beretta, A. R. Rocha Teixeira, J. A. Quaggio, and J. D. De Negri
1990. Présence de bactéries dans le xyleme d'orangers atteints de chlorose variégeé, une nouvelle maladie des agrumes au Brésil. C. R. Acad. Sci. Paris 310 Series III: 345-349.
18. Rossetti, V.
1993. Citrus variegated chlorosis - a new severe disease in Brazil. p. 449-452. *In*: Proc. 12th Conf. IOCV. IOCV, Riverside.
19. Upton, G. and B. Fingleton
1984. Spatial data analysis by example. John Wiley and Sons, Chichester, England. 400 p.