Spatial Patterns of Grapevines with Eutypa Dieback in Vineyards with or without Perithecia

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ABSTRACT

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Eight vineyards in northern and central California were surveyed during three consecutive years, 1989–1991, and the presence or absence of Eutypa dieback symptoms was recorded for each vine in contiguous blocks of 1,250–3,150 vines. The vineyards were located in areas with different levels of mean annual rainfall; some vineyards contained inoculum sources (perithecia) of *Eutypa lata;* others did not. The spatial patterns of infected vines were examined by ordinary runs, two-dimensional distance class, spatial autocorrelation, and geostatistical analyses. Disease incidence ranged from 3.4% in 1989 to 81.5% in 1991. During the study, disease incidence more than doubled in five of the vineyards. Vineyards with perithecia had higher disease incidence. A disease gradient or edge effect was detected in two vineyards that did not contain inoculum sources; one of these was found to be adjacent to a vineyard with *E. lata* perithecia. The different analyses consistently described the relative randomness of the patterns of diseased vines among the vineyards. Those vineyards that contained perithecia had a higher proportion of vineyard rows with nonrandom disease patterns according to runs analysis. Two-dimensional distance class analysis showed that vineyards with perithecia contained clusters of diseased vines or other nonrandom patterns. Vineyards with perithecia also consistently had more significant spatial autocorrelation coefficients and semivariograms that indicated spatial dependence at distances up to 25 m. A nonrandom pattern was consistently found in one vineyard that was not near any known inoculum source. Three other vineyards with no known inoculum sources nearby were consistently considered to have random patterns, according to the spatial pattern analyses. In these vineyards, there was no evidence that would indicate disease spread by means other than airborne ascospores from distant sources.

Additional keywords: deadarm, epidemiology, *Eutypa armeniacae.*

Eutypa dieback is a perennial canker disease that affects a wide range of woody plants. Its primary economic hosts are grape (*Vitis* L. spp.) and apricot (*Prunus armeniaca* L.) (6). The disease is found on at least four continents (6,8) and in all seven major wine grape-growing areas in northern and central California (Napa, Sonoma, Mendocino-Lake, Foothills, Central Valley, Monterey, and Lodi-Delta) (25,26). Eutypa dieback is a serious constraint on vineyard longevity in all but the Foothill region. High levels of Eutypa dieback were previously restricted to coastal areas with high rainfall (>500 mm/yr), but over the last 20 yr, the disease has become very severe in many vineyards in the

Central Valley. Mean annual rainfall in the grape-growing areas of northern and central California ranges from 270 mm in the Central Valley to 970 mm in Mendocino County.

Eutypa lata (Pers.:Fr.) Tul. & C. Tul. (= *Eutypa armeniacae* Hansf. & M.V. Carter) is disseminated by ascospores produced in perithecia, which form in a black pseudostroma on the wood of infected trees and vines. The development of perithecia is favored by annual rainfall of 500 mm or more (5,24,33). Perithecia occur rarely in the Central Valley, usually in areas under sprinkler irrigation (13,29,30,33). Even in high-rainfall areas, perithecia are not common on grapevines. Perithecia develop very slowly, requiring at least 5 yr from infection to ascospore maturity. The stromata can then produce new perithecia annually for a number of years (6). The anamorphic state, *Libertella blepharis* A.L. Smith (syn. *Cytosporina* Sacc. sp.), produces large numbers of filiform spores from small pycnidia on the inner bark of infected vines or in the perithecial stromata. The spores are exuded in a gelatinous matrix typical of splash-dispersed conidia. The function of these spores is unknown; they may function as conidia or spermatia, but they do not germinate readily in culture (5,25). The distribution of the teleomorph in California is much more limited than that of the anamorph.

There is no evidence for host specificity within *E. lata,* although isolates display variation in pathogenicity (6,7,12,13). In California, apricots have been proposed as the most important source of inoculum for Eutypa dieback of grape (32,33). Cherries have also been implicated (29,30).

The disease spreads only during the dormant season (October to March in California) when vines are pruned. Ascospores of *E. lata* are discharged during rain events and are dispersed by wind to infect susceptible pruning wounds. However, some researchers have reported that Eutypa dieback of apricot can be spread by conidia and by pruning tools (1,2). Several researchers have reported that conidia of *E. lata* are not capable of germination (5,25), but conidial germination has been reported by others (19). Because of many failures to germinate conidia and failures to spread the disease with pruning tools, most researchers have assumed that ascospores are the only functional inoculum (6,19).

Symptoms of Eutypa dieback develop very slowly; the incubation period in grapevines is 3 yr or more (6,25). Symptoms are most evident during the spring, when healthy shoots are about 30 cm long. Shoots on infected vines are stunted, with shortened internodes and small, distorted, chlorotic leaves. These symptoms may persist for several years, but eventually the infected portion of the vine will die, resulting in "deadarm" (25,26).

Eutypa dieback can increase relatively rapidly in some vineyards so that over 90% of the vines are infected by the time the vineyard reaches 20 yr of age (11). However, the disease is not believed to spread from vine to vine within most vineyards, because of the absence of perithecia in these vineyards (33). The rate of increase of Eutypa dieback in vineyards in California's Central Valley is difficult to explain, since sources of ascospores are typically more than 50 km distant.

Spatial pattern analyses have been used to provide information regarding inoculum sources and spread of plant pathogens (4,9, 15,17,20–22,34). This strategy is based on the assumption that different mechanisms of disease spread will typically produce different spatial patterns of diseased plants. For example, spread of Eutypa dieback by ascospores from distant sources might produce a random or uniform pattern of diseased vines, while spread from internal sources (ascospores or conidia) would tend to produce a clustered pattern and spread by pruning tools might result in a striped pattern along the rows. Spread by ascospores from a nearby source might result in a disease gradient. Clustering can be detected by analysis for spatial autocorrelation (4,9,15,16, 20,22,34) or by nonparametric methods that test for independence in disease status among adjacent plants or quadrats (17,21,31). Gradients or linear spread patterns can also be detected by these methods or by regression analysis.

The objective of this research was to examine the spatial patterns of vines affected by Eutypa dieback in order to elucidate possible mechanisms for disease spread under a variety of environmental conditions and to compare spatial patterns among vineyards with or without known inoculum sources in the vineyard. A preliminary report has been published (28).

MATERIALS AND METHODS

Eight vineyards representing three different wine-growing areas in northern and central California were chosen (Table 1). The vineyards were selected on the basis of the levels of mean annual rainfall at their locations and the presence or absence of *E. lata* perithecia. The vineyards represented the following wine-growing regions: Napa (Yountville-CS, Oak Knoll-Sb, Suisun-PS, and Carneros-Ch), Lodi-Delta (Delta-Cb and Sacramento-CS), and Monterey (King City-PS and King City-Cb). During the spring of each year from 1989 to 1991, a block of vines was surveyed in each vineyard. The Oak Knoll-Sb and Suisun-PS vineyards were surveyed only in 1990 and 1991. The presence or absence of Eutypa dieback symptoms was recorded for each vine in the block. In the first year, missing vines were recorded as symptomless. In subsequent years, missing or replanted vines retained the rating they received the previous year. In general, the number of missing plants was very low in relation to the total number of plants in each vineyard block. For the purposes of the analyses, vines that showed symptoms in one year were considered diseased in subsequent years. Five to 10 wood specimens were collected from each vineyard block to confirm the association of *E. lata* with the symptoms. Disease incidence was calculated for each combination of vineyard and year. Two-dimensional maps of the spatial patterns of diseased vines were generated for each combination of vineyard and year. The spatial patterns were also characterized by the calculation of the total number of diseased plants along each row and across the rows in each vineyard.

Several statistical methods were used to interpret the spatial patterns of vines with Eutypa dieback: ordinary runs analysis, two-dimensional distance class analysis, spatial autocorrelation, and geostatistics.

Ordinary runs analysis was used to evaluate the aggregation of diseased vines along rows or transects across rows (14,21). The number of runs in each row or transect was determined on the basis of the sequence of diseased and healthy vines. If diseased vines were aggregated, there were few runs, compared to the number expected under the null hypothesis of randomness. A *Z* statistic

TABLE 1. Characteristics of eight vineyards surveyed for Eutypa dieback

Location	Cultivar	Year planted	Mean annual precipitation (cm)	Perithecia	Vine spacing (m)	Block size (no. of vines)
Carneros-Ch	Chardonnay	1974	61.0	No	1.83 × 3.05	3,150
Delta-Cb	Chenin blanc	1969	43.2	No	2.44 × 3.66	3,150
King City-Cb	Chenin blanc	1981	27.9	No	1.83 × 3.05	1,989
King City-PS	Petite Sirah	1973	27.9	No	1.83 × 3.05	2,350
Oak Knoll-Sb	Sauvignon blanc	1972	63.5	Yes	1.83 × 3.05	1,450
Sacramento-CS	Cavernet Sauvignon	1975	43.2	Yes	1.83 × 3.66	2,400
Suisun-PS	Petite Sirah	1972	61.0	Yes	2.44 × 3.66	1,250
Yountville-CS	Cabernet Sauvignon	1974	63.5	No	1.83 × 3.66	2,200

was used to compare the observed vs. the expected number of runs (14,21). The analysis was conducted separately for each row or transect. The proportions of vineyard rows or transects across rows with significantly fewer runs than expected were calculated for each vineyard in each year.

Two-dimensional distance class analysis is another method that employs binomial data (each plant is either diseased or healthy) for evaluation of the randomness of diseased plants (17). Distance classes are defined in terms of horizontal and vertical units between plants in their two-dimensional lattice (the vineyard). Pairs of diseased plants are assigned to distance classes on the basis of the number of lattice units separating them. The number of pairs in each distance class is divided by the total number of possible pairs in that distance class. This process is repeated with each infected plant in the lattice as the origin. Standardized count frequencies (SCF) are calculated by computer simulation for each distance class under the null hypothesis of randomness. The observed SCFs are then compared to the expected SCFs for each distance class, and the significance level for the difference between observed and expected SCFs is calculated (17). The proportion of distance classes with SCFs significantly different from the expected value is a relative measure of aggregation. The particular distance classes that have SCFs greater than expected can indicate the approximate cluster size, orientation of aggregation, and relative position of clusters of diseased plants (17). Software (2DCLASS) developed by Nelson et al (31) was used to perform this analysis on the Eutypa dieback data.

Spatial autocorrelation and geostatistical analyses are designed to interpret continuous quantitative data, so the binomial disease incidence data from the surveys were converted into counts of diseased vines within quadrats. A quadrat size containing nine vines was chosen for each vineyard. The quadrat size varied from 5.49 × 9.15 m to 7.32 × 10.98 m.

If the occurrence of a certain level of disease in a quadrat can be used to accurately predict the disease level in another quadrat, disease is said to be spatially autocorrelated (22). Spatial and spatiotemporal autocorrelation analyses have been developed

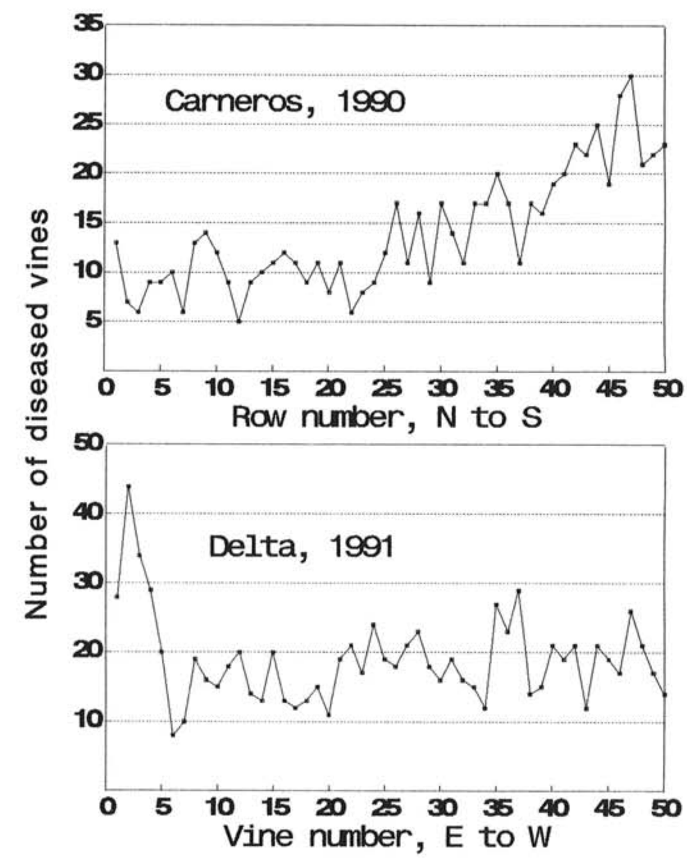


Fig. 1. Total diseased vines for rows (Carneros-Ch, 1990) or transects across rows (Delta-Cb, 1991) in two locations displaying a disease gradient or edge effect.

TABLE 2. Incidence of vines with Eutypa dieback and results of ordinary runs analysis for eight California vineyards

			Proportion of rows with significant Z[a]	
Location	Year	Incidence	Along rows	Across rows
Carneros-Ch	1989	14.9	0.32	0.32
	1990	27.5	0.20	0.14
	1991	39.5	0.24	0.18
Delta-Cb	1989	23.4	0.11	0.08
	1990	34.7	0.31	0.08
	1991	39.3	0.22	0.12
King City-Cb	1989	3.4	0.02	0.02
	1990	6.4	0.02	0.08
	1991	16.9	0.12	0.10
King City-PS	1989	22.5	0.11	0.10
	1990	37.1	0.11	0.14
	1991	59.7	0.22	0.18
Oak Knoll-Sb	1990	59.2	0.25	0.30
	1991	80.6	0.24	0.18
Sacramento-CS	1989	36.5	0.25	0.14
	1990	68.5	0.23	0.14
	1991	81.5	0.21	0.28
Suisun-PS	1990	54.8	0.32	0.36
	1991	73.0	0.23	0.24
Yountville-CS	1989	16.6	0.30	0.12
	1990	31.9	0.11	0.24
	1991	48.5	0.11	0.24

[a] Runs analysis was performed separately along each row or each transect across rows for each combination of vineyard and year. Values are the proportions of tests that indicated significant aggregation of diseased vines at $\alpha = 0.05$.

TABLE 3. Spatial aggregation of grapevines with Eutypa dieback in eight California vineyards as measured by two-dimensional distance class analysis

		Proportion of SCFs[a]		
Location	Year	Higher	Lower	Clustered at origin[b]
Carneros-Ch	1989	0.14	0.08	Yes
	1990	0.11	0.10	Yes
	1991	0.13	0.11	Yes
Delta-Cb	1989	0.18	0.07	No
	1990	0.32	0.18	Yes
	1991	0.33	0.19	Yes
King City-Cb	1989	0.11	0.00	No
	1990	0.09	0.01	No
	1991	0.05	0.01	No
King City-PS	1989	0.15	0.01	No
	1990	0.11	0.05	Yes
	1991	0.16	0.07	No
Oak Knoll-Sb	1990	0.12	0.12	Yes
	1991	0.12	0.13	Yes
Sacramento-CS	1989	0.17	0.04	Yes
	1990	0.18	0.09	No
	1991	0.12	0.07	No
Suisun-PS	1990	0.32	0.12	Yes
	1991	0.09	0.05	Yes
Yountville-CS	1989	0.06	0.02	No
	1990	0.13	0.18	No
	1991	0.06	0.12	No

[a] Tabular values indicate the proportions of distance classes with standardized count frequencies (SCFs) that were significantly ($\alpha = 0.05$) higher or lower than expected.

[b] Distance classes with significant SCFs clustered at the origin indicate aggregation of diseased vines.

to model the development of patterns of diseased plants through both space and time (15,16,22,34). Detailed descriptions of these methods are available (3,10,23,34). Spatial autocorrelation analysis was performed with STAUTO software developed by Reynolds and Madden (34). For this analysis, the binary distance weighting option was invoked. Proximity patterns examined were within-row, across-row, and square. These parameters are all options provided by the STAUTO program.

Geostatistical analysis was originally developed for use in geology but has proved useful in plant pathology (9,18,20). This method can compensate for the variable distances between plants that are encountered in the comparison of different vineyards. Spatial dependence is expressed as a function of distance in linear units, rather than the unitless spatial or temporal lags used in STAUTO. The basis of geostatistics is the development of the semivariogram. The semivariogram is a plot of semivariance, $\nu(h)$, versus distance, h. The semivariance is the variance about the mean difference in disease level between all sampling units separated by the distance h. If disease level is spatially dependent, $\nu(h)$ is positively correlated with h, with either a linear or curvilinear relationship (35). GEO-EAS (US EPA, Las Vegas, NV) was used to perform this analysis. Semivariograms were constructed on the basis of the untransformed data from the nine-vine quadrats. The presence or absence of anisotropic patterns was determined by examination of the semivariograms for zero, 45, 90, and 135° azimuth, where zero represents the direction in which the vineyard rows were oriented.

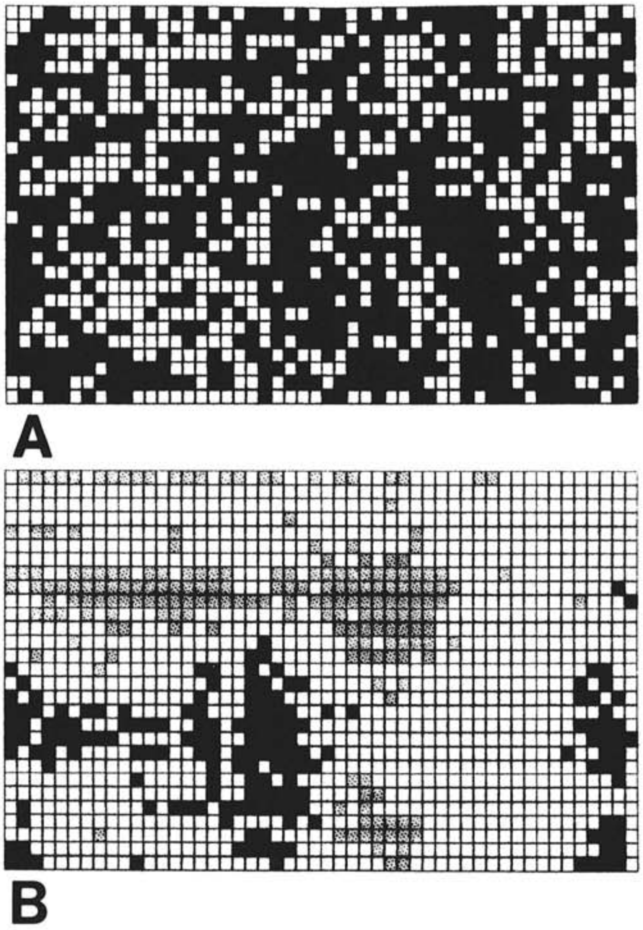


Fig. 2. Oak Knoll-Sb, 1990. **A,** Map of diseased vines. Black squares indicate vines with Eutypa dieback; white squares indicate symptomless vines. The horizontal axis corresponds to the orientation of the vineyard rows. **B,** Representation of the results of two-dimensional distance class analysis. Black squares indicate distance classes with a standardized frequency significantly greater ($\alpha = 0.05$) than expected. Shaded squares indicate distance classes with a standardized frequency significantly less ($\alpha = 0.05$) than expected. The origin is located at the bottom left.

RESULTS

Disease incidence ranged from 3.4% in one King City vineyard in 1989 to 81.5% in the Sacramento-CS vineyard in 1991 (Table 2). Disease incidence increased in all eight vineyards during the

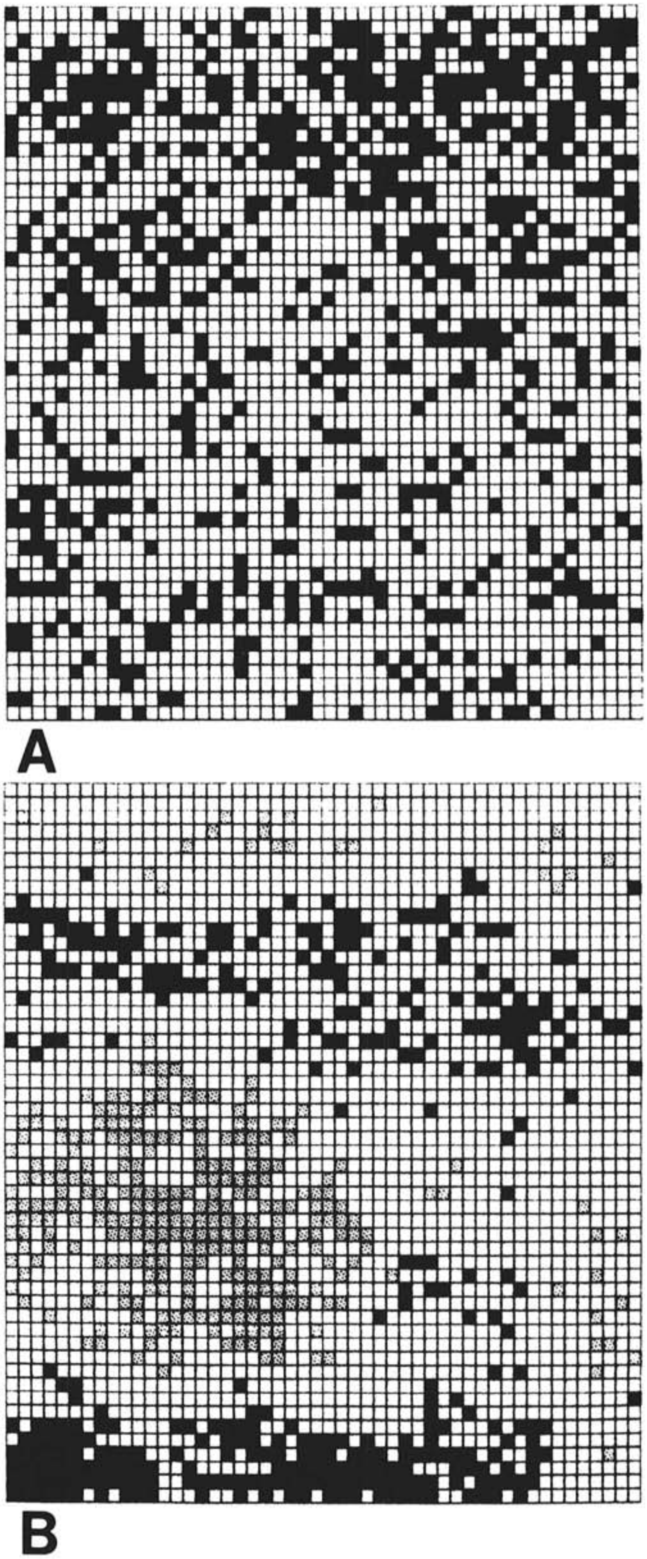


Fig. 3. Carneros-Ch, 1990. **A,** Map of diseased vines. Black squares indicate vines with Eutypa dieback; white squares indicate symptomless vines. The horizontal axis corresponds to the orientation of the vineyard rows. **B,** Representation of the results of two-dimensional distance class analysis. Black squares indicate distance classes with a standardized frequency significantly greater ($\alpha = 0.05$) than expected. Shaded squares indicate distance classes with a standardized frequency significantly less ($\alpha = 0.05$) than expected. The origin is located at the bottom left.

survey period and more than doubled in the Carneros-Ch, King City, Sacramento-CS, and Yountville-CS vineyards.

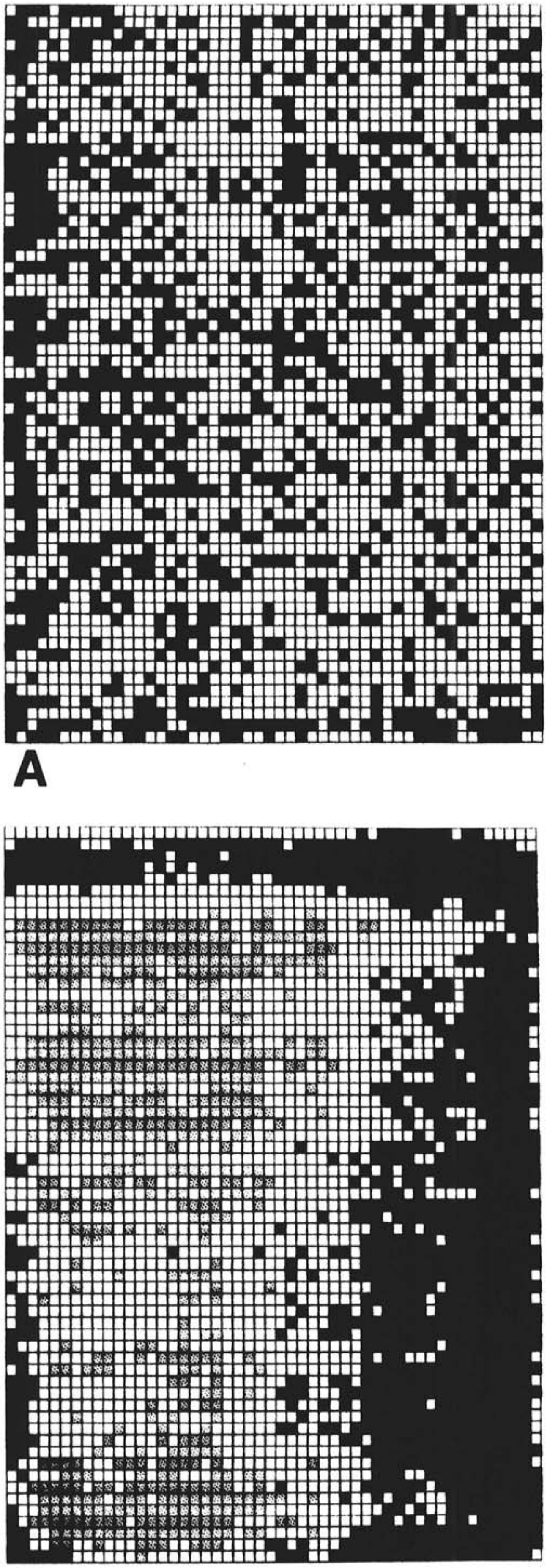
A disease gradient was present in one vineyard, and another vineyard contained a pronounced "edge effect," where diseased vines were concentrated at one side of the vineyard (Fig. 1). In the other six, no disease gradients were evident along or across the vineyard rows.

On the basis of ordinary runs analysis, the Suisun-PS and Oak Knoll-Sb vineyards generally had the highest proportion of rows and transects containing clusters of diseased vines. The proportions of significant rows and transects ranged from 0.02 in the King City-Cb vineyard in 1989 to 0.36 in the Suisun vineyard in 1990 (Table 2). The proportions did not display a consistent trend over time.

Two-dimensional distance class analysis detected nonrandomness in several vineyards (Table 3, Figs. 2–4) and provided a measure of cluster size and other patterns. The degree of aggregation of diseased vines varied considerably among the vineyards. The 2DCLASS software outputs a matrix containing the observed and expected SCFs and the significance level for each distance class. Maps of diseased vines (Figs. 2A–5A) and significant distance classes (Figs. 2B–5B) are shown graphically for several vineyards. These significant distance classes should not be interpreted to correspond to the actual positions of infected vines. Rather, they represent only the distance classes with significantly higher or lower SCFs than expected. The proportion of distance classes with greater than expected SCF values varied from 0.05 in the King City-Cb vineyard in 1991 to 0.33 in the Delta-Cb vineyard in 1991 (Table 3). The Delta-Cb location had the highest proportion in 1989 and 1991, and the Suisun-PS and Oak Knoll-Sb locations had the highest proportions in 1990. Proportions of distance classes with SCFs less than expected were lower overall, ranging from less than 0.01 in the King City-Cb vineyard in 1989 to 0.19 in the Delta-Cb vineyard in 1991. Within vineyards, there was no consistent trend over time for the proportions of distance classes with SCFs either greater than or less than expected. The position of the significant distance classes in the two-dimensional lattice is also important in the interpretation of these results. In particular, significant distance classes clustered near the origin are an indication of the "core" cluster size (31). In the Carneros-Ch, Oak Knoll-Sb, Delta-Cb, and Suisun-PS locations, significant distance classes were clustered near the origin and sometimes at the higher distance classes (Figs. 2–4). In the King City-Cb and Yountville-CS locations, significant distance classes did not occur in clusters near the origin (Fig. 5). In the other locations, clustering of significant distance classes varied from year to year (Table 3).

Disease levels in adjacent nine-vine quadrats were consistently spatially autocorrelated in the Carneros-Ch, Delta-Cb, Sacramento-CS, Oak Knoll-Sb, and Suisun-PS vineyards. In the King City vineyards, disease levels were only occasionally autocorrelated (Fig. 6). Autocorrelation coefficients were similar for the different proximity patterns but were generally higher for the square pattern. Significant coefficients were associated with all the vineyards except Yountville-CS in at least 1 yr. Coefficients did not consistently increase or decrease in magnitude over time.

Nonoriented semivariograms indicated some degree of spatial dependence for the Carneros-Ch, Delta-Cb, Oak Knoll-Sb, Suisun-PS, and possibly Sacramento-CS vineyards (Fig. 7). The shape of semivariograms and the magnitude of the semivariance for a given vineyard changed significantly over time. The semivariance increased over time in five vineyards but decreased in



← **Fig. 4.** Delta-Cb, 1990. **A,** Map of diseased vines. Black squares indicate vines with Eutypa dieback; white squares indicate symptomless vines. The horizontal axis corresponds to the orientation of the vineyard rows. **B,** Representation of the results of two-dimensional distance class analysis. Black squares indicate distance classes with a standardized frequency significantly greater ($\alpha = 0.05$) than expected. Shaded squares indicate distance classes with a standardized frequency significantly less ($\alpha = 0.05$) than expected. The origin is located at the bottom left.

the three vineyards with the highest disease incidence (Oak Knoll-Sb, Sacramento-CS, and Suisun-PS). Oriented semivariograms (Fig. 8) in general did not illustrate strong anisotropic patterns. However, a greater degree of spatial dependence was evident in the 0 and 90° semivariograms than in the 45 and 135° semivariograms for the Oak Knoll-Sb and Suisun-PS vineyards (Fig. 8I, M, and N). In the Carneros-Ch and Delta-Cb vineyards, some anisotropy was detected (Fig. 8B and D), reflecting the presence of a gradient or edge effect.

DISCUSSION

The vineyards that contained perithecia of *E. lata* had consistently higher disease incidence than those that did not. This may reflect the contribution of these internal inoculum sources to disease incidence. However, other factors such as geographic location, cultivar, favorable environmental conditions, and vineyard age may have contributed to high disease levels in these vineyards (5,6,27,33). For example, the Suisun-PS vineyard was located in an area known to contain large amounts of perithecia on apricots (33). Also, perithecia develop slowly and generally do not appear until late in an epidemic. Because of these factors, high disease incidence can not be attributed directly to the presence of perithecia. However, the presence of perithecia was also associated with more clustered spatial patterns.

In general, the relative randomness of diseased vines among the vineyards was consistently described by the several analytical approaches used. Nonrandom patterns were detected more frequently in vineyards containing perithecia.

On the basis of the ordinary runs analysis, the Oak Knoll-Sb and Suisun-PS vineyards, both of which contained perithecia, were consistently among the vineyards with the highest proportions of nonrandom rows (Table 2).

In two-dimensional distance class analysis, nonrandomness is indicated by a high proportion of significant distance classes (Table 3). The proportion regarded as sufficient to conclude nonrandomness has been reported as 5–10% (31). This criterion is exceeded in nearly every vineyard in this study. However, the

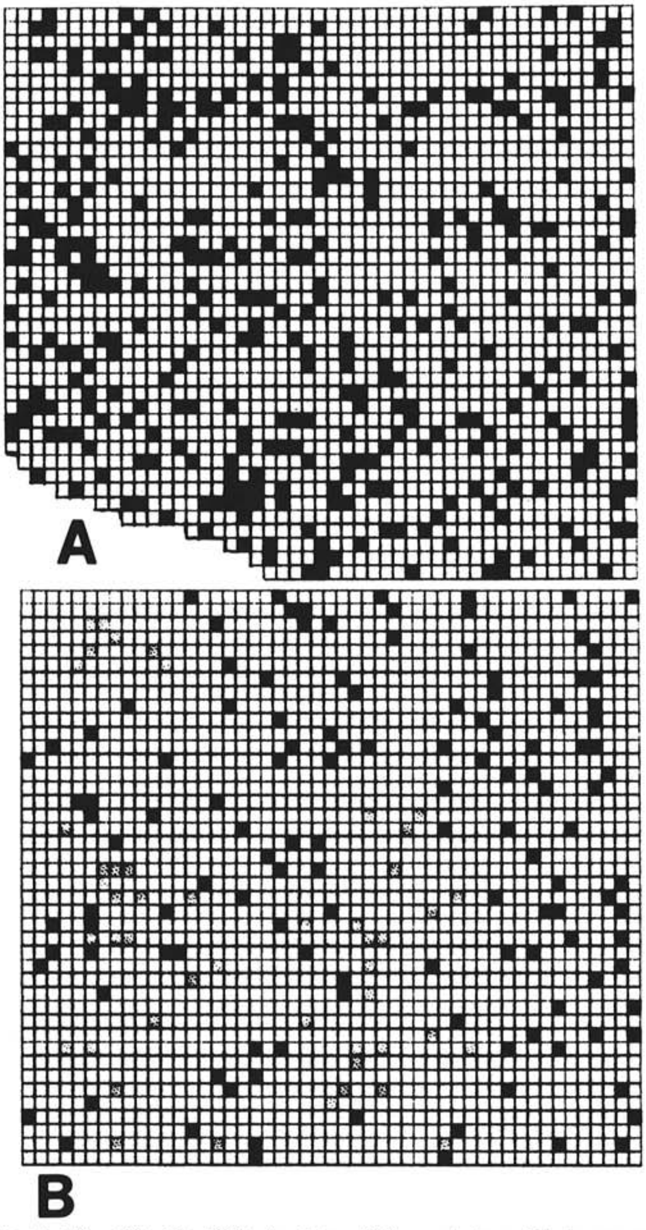


Fig. 5. King City-Cb, 1991. **A,** Map of diseased vines. Black squares indicate vines with Eutypa dieback; white squares indicate symptomless vines. The horizontal axis corresponds to the orientation of the vineyard rows. **B,** Representation of the results of two-dimensional distance class analysis. Black squares indicate distance classes with a standardized frequency significantly greater ($\alpha = 0.05$) than expected. Shaded squares indicate distance classes with a standardized frequency significantly less ($\alpha = 0.05$) than expected. The origin is located at the bottom left.

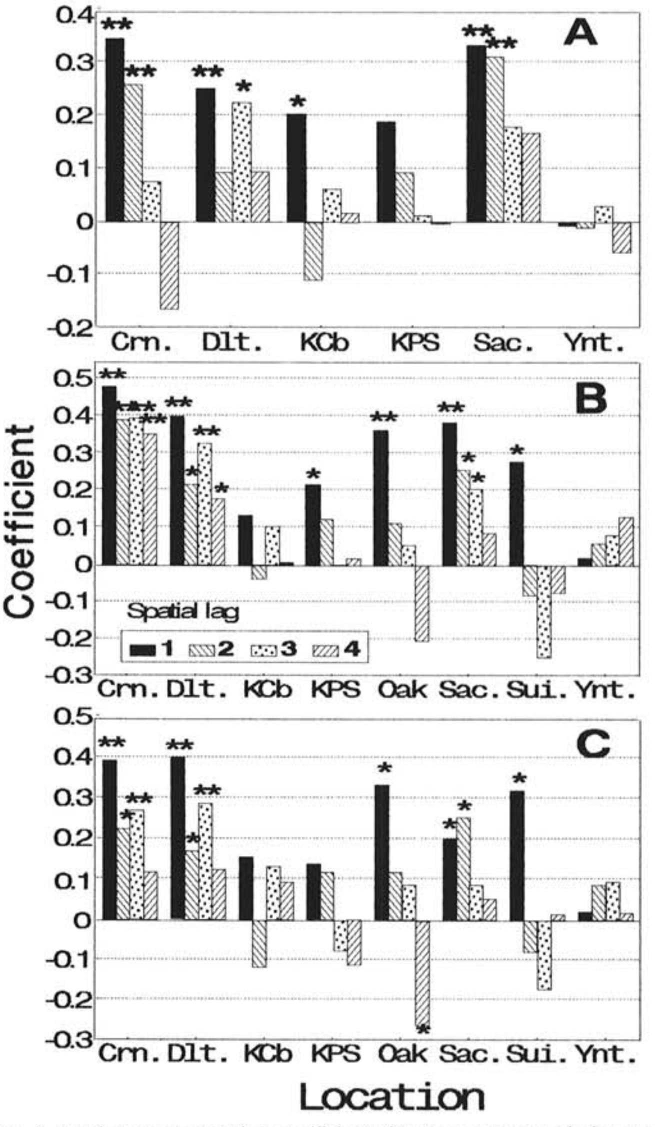


Fig. 6. Spatial autocorrelation coefficients for the square proximity pattern for all locations in **A,** 1989, **B,** 1990, and **C,** 1991. Crn. = Carneros-Ch; Dlt. = Delta-Cb; KCb = King City-Cb; KPS = King City-PS; Oak = Oak Knoll-Sb; Sac. = Sacramento-CS; Sui. = Suisun-PS; and Ynt. = Yountville-CS. * = Coefficients significant at $\alpha = 0.05$; ** = coefficients significant at $\alpha = 0.01$.

proportion varied considerably among the vineyards, and it was higher in vineyards with perithecia. The proportion did not consistently increase or decrease over time. In the Suisun-PS vineyard, for example, the total proportion of significant distance classes dropped from 0.44 in 1990 to 0.14 in 1991. This indicates that the spatial pattern was less clustered in 1991. This effect can be attributed to the very high levels of disease in this vineyard in 1991 (31).

Possibly more important than the proportion of significant distance classes is their position in the lattice (17). If the distance classes around the origin have SCFs significantly higher than expected, it indicates the "core" or average size of clusters of diseased plants. This clustering around the origin occurred consistently in the Carneros-Ch, Oak Knoll-Sb, and Suisun-PS vineyards (Table 3, Figs. 2B and 3B). The average cluster size was five to 11 vines for the Oak Knoll-Sb vineyard in 1990 (Fig.

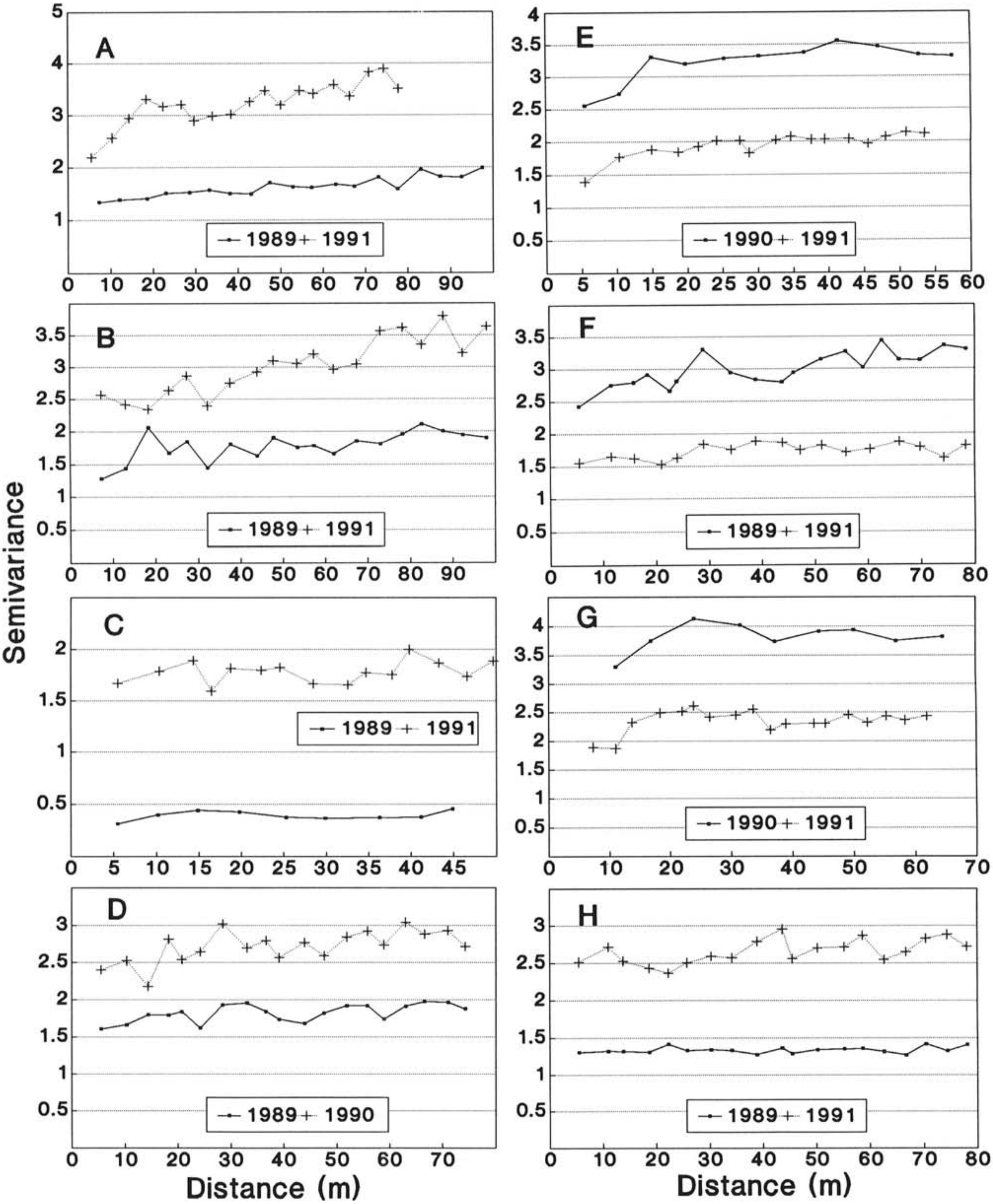


Fig. 7. Nonoriented semivariograms for all locations in selected years. **A,** Carneros-Ch; **B,** Delta-Cb; **C,** King City-Cb; **D,** King City-PS; **E,** Oak Knoll-Sb; **F,** Sacramento-CS; **G,** Suisun-PS; **H,** Yountville-CS.

2B), four to nine vines for the Suisun-PS vineyard in 1990, and 12–27 vines for the Delta-Cb vineyard in 1990 (Fig. 4B). Cluster size estimates are expressed as a range because the output indicates significant distance classes only to one side of the origin.

The size of the "core" clusters did not increase with time, as one might expect with a growing disease focus. In fact, it is unlikely that focus expansion could be detected during such a relatively short period of time. The incubation period for Eutypa dieback is 3 yr or more (25), so the "new" infections appearing in 1991 actually occurred prior to 1989.

Grouping of significant distance classes at the edges of the matrix indicates an edge effect, where disease levels are higher than expected at one or more edges of the vineyard block. This occurred with the Delta-Cb (Fig. 4B) and Suisun-PS vineyards. In the case of the Delta-Cb vineyard, this edge effect is evident from the map of diseased vines (Fig. 4A) and the diseased vine totals across the rows (Fig. 1). The probable source of this edge effect was an older vineyard that contained perithecia of *E. lata* located adjacent to the survey block on the east side. Perithecia were not detected in this adjacent vineyard until 1991. In the Carneros-Ch vineyard, a horizontal strip of significant distance classes occurred near the origin (Fig. 3B). This indicates within-row aggregation, which also is evident from the map of diseased vines (Fig. 3A) and the diseased-vine totals for the rows (Fig. 1). The source of this pattern was unknown. In contrast, the positions of the significant distance classes for the King City and Yountville-CS vineyards were not clustered in any evident manner (Fig. 5B). This indicates a random pattern (17,31). Clustering was not detected in these vineyards even when disease incidence reached 59%.

Two-dimensional distance class analysis offered several advantages over runs analysis. In addition to a statistical test for nonrandomness, it provided information on sizes of clusters of diseased plants, edge effects, and within-row aggregation. Some of these patterns or cluster sizes were not evident from examination of the maps of diseased vines.

The proportion of significant distance classes required to conclude nonrandomness is not clearly defined. Possibly, this will become more clear as this method gains wider use or this criterion can be clarified by analysis of simulated data sets of various sizes and disease levels. The 2DCLASS software can process very large data sets (31), but computer time may become excessive. For some of the data sets in this study (up to 3,150 data points), the analysis took more than 2 h on a microcomputer with an 80486 microprocessor. Data sets with a higher incidence of diseased vines took substantially longer to analyze.

Spatial autocorrelation analysis differs fundamentally from distance class analysis because it is not a test for randomness but a measure of the correlation between quadrats. Spatial autocorrelation can exist with a clustered pattern but also with a uniform pattern, especially at very low or high levels of the variable (10). Nevertheless, the spatial autocorrelation coefficients generally agreed with the results of the two-dimensional distance class analysis. First-order autocorrelation coefficients and partial autocorrelation coefficients were generally significant for Carneros-Ch, Delta-Cb, Oak Knoll-Sb, Sacramento-CS, and Suisun-PS. This indicated that clusters of diseased vines were small. In many cases, the second-order coefficients were significant as well, particularly for the Carneros-Ch vineyard. This was probably the result of the presence of a disease gradient.

Nonoriented semivariograms (Fig. 7) produced in geostatistical analysis correspond to the square proximity pattern for spatial autocorrelation analysis. Interpretation of semivariograms is less objective, since no test for significance is applied. When a variable is spatially dependent, the semivariogram is linear with a positive slope, or the semivariance increases rapidly when the value of h is low and then levels off near a constant value or sill (9,35). Several types of models can be fit to semivariograms (35), but no model-fitting was performed on the semivariograms in this study. The biological significance of fitting specific model types to semivariograms is unclear. On the basis of the semivariograms, the strongest spatial dependence occurred in the Carneros-Ch,

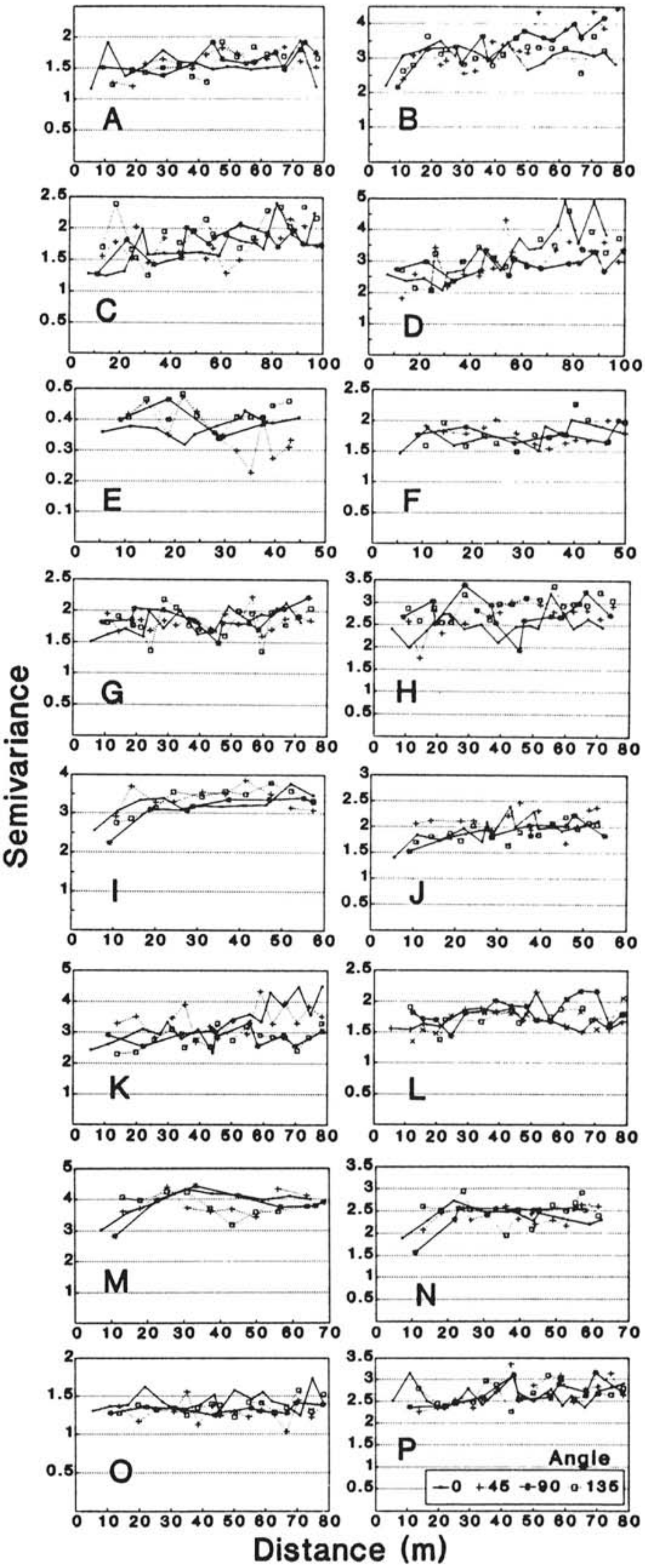


Fig. 8. Oriented semivariograms for all locations in selected years. **A and B,** Carneros-Ch, 1989 and 1991; **C and D,** Delta-Cb, 1989 and 1991; **E and F,** King City-Cb, 1989 and 1991; **G and H,** King City-PS, 1989 and 1990; **I and J,** Oak Knoll-Sb, 1990 and 1991; **K and L,** Sacramento-CS, 1989 and 1991; **M and N,** Suisun-PS, 1990 and 1991; **O and P,** Yountville-CS, 1989 and 1991. The orientation of the vineyard rows corresponds to an angle of zero.

Oak Knoll-Sb, and Suisun-PS vineyards (Fig. 7). The range of spatial dependence (35) was approximately 20 m for Carneros-Ch, 15 m for Oak Knoll-Sb, and 25 m for Suisun-PS. These distances agreed with the size estimates for clusters of diseased plants obtained by two-dimensional distance class analysis and with the spatial autocorrelation coefficients. For the Delta-Cb vineyard in 1990 and 1991 (Fig. 7B), the semivariance did not reach a sill but increased linearly, typical of a disease gradient or edge effect (20,35). This effect was not as evident in 1989 (Fig. 7B). For all vineyards, the form of the semivariograms changed substantially from year to year. Often, spatial dependence was indicated by the semivariograms in only one of the 3 yr.

The within-row aggregation demonstrated by two-dimensional distance class analysis for Carneros-Ch and Delta-Cb appeared as a slightly steeper slope in the oriented semivariograms for these vineyards (Fig. 8B and D).

The presence of perithecia in vineyards was associated with detectable nonrandom spatial patterns of diseased vines, determined by a number of analytical methods. These results support the conclusion that these internal inoculum sources contribute to epidemics of Eutypa dieback in these vineyards, and measures should be taken to prevent development of perithecia. Where they are already present, perithecia should be surgically removed from vineyards. Their development can be prevented by the avoidance of sprinkler irrigation and by the removal of infected wood from the vineyard as soon as dieback symptoms appear (6). Nonrandom patterns appeared to break down in some of these vineyards when disease incidence reached about 80%. Although the internal perithecia contributed to disease increase, it is likely that a substantial proportion of new infections were caused by external inoculum. This is a result of the long incubation (3 yr or more [25]) and latent periods (5 yr or more [6]) for Eutypa dieback. There will be a delay of 8 yr or more between the first infection in a vineyard and the appearance of a cluster of diseased vines caused by a spread of inoculum from perithecia. During the interim, a potentially high number of randomly distributed infections caused by external inoculum can occur. This might account for the low proportion of nonrandom rows indicated by runs analysis.

The Delta-Cb vineyard did not contain perithecia, but its nonrandom pattern could be attributed to the perithecia that were found in the adjacent vineyard. This provides further evidence that perithecia in vineyards are important in the increase of disease incidence.

On the basis of the spatial patterns, there was no evidence of vine-to-vine spread in vineyards without perithecia, except for the Carneros-Ch vineyard. If Eutypa dieback were being spread by conidia, one would expect clusters of diseased plants to develop more rapidly than if the disease were being spread by ascospores, since conidia are produced more quickly after infection and are splash-dispersed (19). There was no evidence for such spread, nor was there evidence for spread along the rows that might result from pruning tools. However, the results for the Carneros-Ch vineyard generally were typical of a nonrandom pattern characterized by a gradient across the vineyard. Diseased vines were aggregated in rows 25–50. The cause of this pattern is unknown. Perithecia of *E. lata* have not been detected in the immediate area, although conditions are believed to be conducive to their development. Spread by means other than ascospores remains a possibility in this case.

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