Techniques

# Analysis of the Spatial Pattern of Plant Pathogens and Diseased Plants Using Geostatistics

D. O. Chellemi, K. G. Rohrbach, R. S. Yost, and R. M. Sonoda

Former graduate research assistant, Department of Plant Pathology; assistant director, College of Tropical Agriculture and Human Resources; and associate professor, Department of Agronomy and Soil Science, University of Hawaii-CTAHR, Honolulu 96822; and professor, University of Florida, Institute of Food and Agricultural Sciences, Agricultural Research and Education Center, Ft. Pierce 33454. Present address of first author: Plant Pathology Department, University of California, Davis 95616.
Journal series paper 3190 of Hawaii Institute of Tropical Agriculture and Human Resources.
Accepted for publication 28 July 1987 (submitted for electronic processing).

## ABSTRACT

Chellemi, D. O., Rohrbach, K. G., Yost, R. S., and Sonoda, R. M. 1988. Analysis of the spatial pattern of plant pathogens and diseased plants using geostatistics. Phytopathology 78:221-226.

Geostatistical techniques were used to examine the spatial variability among members of a population of plant pathogens and diseased plants in simulated and actual field conditions. Variability was determined by measuring the average of the squared differences between samples over a series of distances and directions with semivariograms. A random pattern with a variance-to-mean (v/m) ratio of 1.01 and a Moran I statistic of 0.019 was used to simulate propagule counts from a hypothetical field and resulted in semivariograms with constant values, indicating propagule counts from each quadrat were independent of neighboring quadrats. An aggregated pattern with a v/m ratio of 2.37 and a Moran I statistic of 0.907 resulted in a linear semivariogram ($r^2 = 0.95$) with a slope of 1.22, indicating the variability between propagule counts increased linearly as the distance between quadrats increased. Application to disease incidence data was demonstrated by measuring the variability in patterns of dead pepper seedlings created by amending soil with toxic levels of copper. Semivariograms were constant for random patterns of dead seedlings but showed a nonlinear increase in variability for aggregated patterns of dead seedlings. Two field plots were monitored to determine the variability of initial inoculum of *Phytophthora nicotianae* var. *parasitica* and its relation to the incidence of pineapple heart rot. Inoculum had a v/m ratio of 5.22 and 27.26 and a Moran I statistic of 0.044 and 0.309 for fields 1 and 2, respectively. Semivariograms revealed that aggregation of inoculum was not homogeneous but varied over four directions analyzed. However, semivariograms for disease incidence showed that aggregation of diseased plants was homogeneous for the same four directions.

Characterization of the spatial position taken up by members of a population of organisms such as plant pathogens and diseased plants can facilitate the determination of relationships between inoculum density and disease incidence; optimum sampling parameters; the influence of cultural, biological, and environmental factors on population dynamics; and the risk assessment of genetically altered microorganisms. Various methods of spatial pattern analysis are used to characterize the spatial position of plant pathogens and diseased plants (2,8,10,13,17,18,20,21). A major limitation in the use of methods based on point patterns is the difficulty in relating patterns of initial inoculum to disease incidence, because of differences in the spatial scales and methods used to describe nonrandomness of inoculum and nonrandomness of disease (7,8,13,19). In addition, these methods fail to recognize the degree of dependency among neighboring observations (i.e., spatial dependence). Spatial autocorrelation functions use the linear correlation between a spatial series and the same series at a further distance interval to detect spatial dependence and have been applied to studies in plant pathology (5,6,17). Their use assumes that second-order stationarity is present (25). However, second-order stationarity does not apply if a finite variance and covariance cannot be defined, as in the case when samples do not come from the same probability distribution (5,11,25).

One method of spatial analysis that does account for the position of a sample but is not bound by the requirements of stationarity is geostatistics. Geostatistics is based on the theory of regionalized variables and differs from autocorrelation in that the only required assumption is that the variance of the difference between samples is a function of their distance of separation (5,11). It has been applied in studies in geology, geography, hydrology, forestry, and soil science (5,11,25,26).

Geostatistics detects spatial dependence by measuring the variation among samples separated by the same distance (5,11,14). Geologists use this variability among samples for the estimation of ore reserves within an unsampled area, based on a limited number of peripheral samples (5). Spatial variability is measured by determining the average of the squared difference in values between pairs of samples separated by a given distance. With $h$ representing a particular distance between samples and their

relative orientation, and on the assumption that the difference in value between two samples depends only upon $h$, the mean difference for all pairs separated by a specific distance is defined as

$$m(h) = (1/n) \sum_{i=1}^{n} [g(x) - g(x+h)] \quad (1)$$

where $x$ denotes the position of one sample in the pair, $x + h$ the position of another sample $h$ units away, $g(x)$ the measure of a value at location $x$, and $n$ the total number of pairs separated by this distance. The variance of these differences is defined as

$$\gamma(h) = (1/2n) \sum_{i=1}^{n} [g(x) - g(x+h)] \quad (2)$$

The term $\gamma(h)$, called the semivariance, is a measure of the expected difference between all values separated by the same distance in a selected direction. The semivariogram for a given direction is usually displayed as a plot of the semivariance versus distance and provides a picture of the spatial variation within a field (Fig. 1). In situations where no sampling error exists, the curve passes through the origin, because two samples taken at the exact same location ($h = 0$) have the same value, and the difference between these values is zero. As the distance between samples is increased slightly, some difference between the two values is expected, and the semivariogram has a small positive value. As the samples move farther apart, the differences usually increase. Ideally, when the distance becomes very large, the sample values become independent of one another, and the semivariogram approaches a constant value. Semivariograms can be calculated for all directions combined or for specific directions to test for anisotropy (nonhomogeneity in all directions).

The objective of this study was to examine the potential for the use of geostatistics in evaluating spatial patterns of soilborne plant pathogens and diseased plants. Portions of this work were reported previously (3,4).

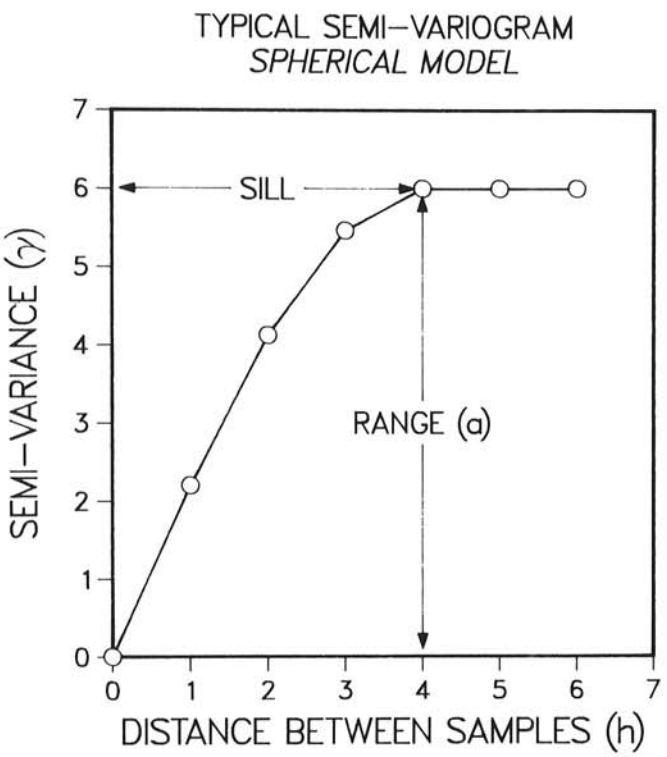


**Fig. 1.** A typical shape for a semivariogram. The distance at which samples become unrelated to each other is denoted by $a$ and is called the range of influence. The semivariogram value at which the curve levels off is called the sill.

## MATERIALS AND METHODS

**Evaluation of two simulated inoculum patterns.** The ability of semivariograms to measure spatial dependence when data are obtained as propagule counts was investigated with a random and an aggregated spatial pattern simulating arrangements of a soilborne plant pathogen in a hypothetical field (Fig. 2). Both patterns were composed of 144 samples arranged in a 12× 12 grid. The random pattern had a variance-to-mean (v/m) ratio of 1.01 and a Moran I statistic of 0.019; the aggregated pattern had a v/m ratio of 2.37 and a Moran I statistic of 0.907 (Table 1). The Moran I statistic was determined by comparing the inoculum level of each quadrat with that of its four immediate neighbors, using a computer program written by Nicot et al (17). Semivariograms were calculated for all directions combined, with a computer program written by R. S. Yost. An IBM PC-compatible version of this program is available upon request from the authors.

TABLE 1. Assessment of spatial patterns of propagule counts from a hypothetical field and of patterns of pepper seedlings with copper-induced toxicity[a]

| Variable | Pattern | v/m[b] | I[c] ± SE | *P* value [I = *E*(I)] |
|---|---|---|---|---|
| Propagule counts | Random | 1.01 | 0.019 ± 0.007 | 0.841 |
| | Aggregated | 2.37 | 0.907 ± 0.007 | 0.000 |
| Disease incidence | Random | ND[d] | 0.020 ± 0.028 | 0.700 |
| | | ND | −0.122 ± 0.028 | 0.447 |
| | Aggregated | ND | 0.580 ± 0.028 | 0.000 |
| | | ND | 0.572 ± 0.028 | 0.000 |

[a] Mortality recorded 8 days after transplanting, as 0 (healthy) and 1 (dead).
[b] Variance-to-mean ratio.
[c] Moran's I statistic.
[d] Not determined.

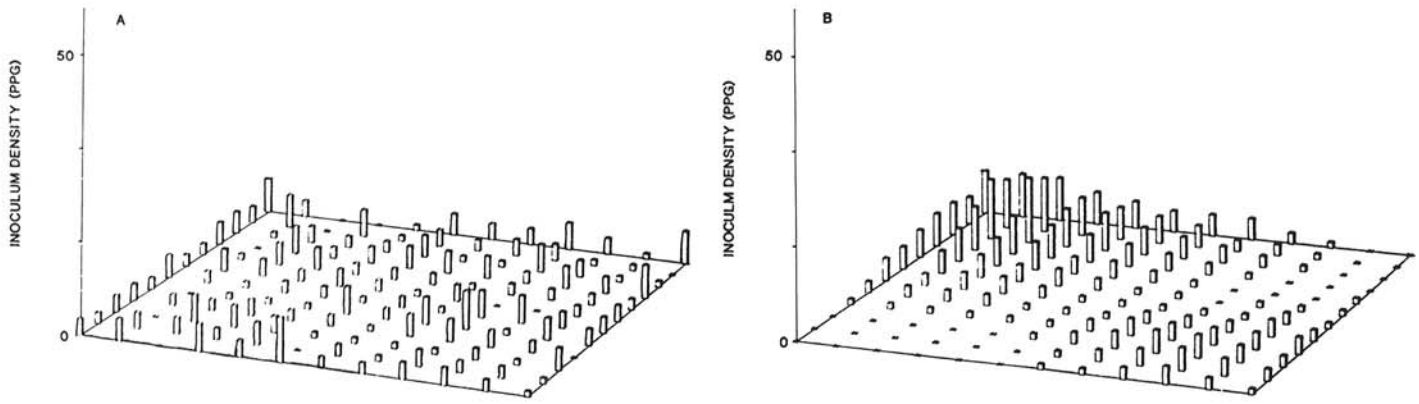


**Fig. 2.** Plots of inoculum densities from two hypothetical fields corresponding to random (**A**) and aggregated (**B**) spatial arrangements of a soilborne plant pathogen. PPG = propagules per gram.

**Evaluation of patterns of diseased plants.** To determine if geostatistics could measure spatial variability in patterns of diseased plants, random and aggregated patterns of dead seedlings were created in the greenhouse. A seedbed (61 × 61 cm) was divided into 144 quadrats of equal size. Various levels of copper, ranging from 0 to 120 ppm, were mixed with 200 g of silica sand and placed into each quadrat in the same random or aggregated pattern described previously. Then 4-wk-old pepper seedlings (*Capsicum annuum* L. 'Early Calwonder') were transplanted into the seedbed at 10-cm intervals. They were evaluated for copper toxicity symptoms after 8 days, on a scale of 0 (healthy) to 1 (seedling death). Semivariograms of seedling condition were generated for all directions combined. The test was repeated twice for each pattern.

**Application to a field situation.** Geostatistics was used to determine the spatial variability of initial inoculum density and its relation to disease incidence in the field. A 3.6-$m^2$ plot was selected in a field of pineapple (*Ananas comosus* (L.) Merr.) having a history of heart rot caused by *Phytophthora nicotianae* B. de Haan var. *parasitica* (Dast.) Waterh. Two identical experiments, separated by a 6-mo fallow period, were conducted in the plot, with fields 1 and 2 referring to the first and second experiments, respectively. The soil was a silty clay loam of volcanic origin (kolekole series) with a pH of 6.3 (1). The plot was sampled 24 hr prior to planting to determine initial density and dispersion of inoculum. A total of 144 samples were collected at points on a 12 × 12 grid. The sampling points were equidistant from each other, at 30-cm intervals. Each sample consisted of two adjacent 7-cm cores, 15 cm deep; these were placed in plastic bags, packed in coolers, and transported to the laboratory.

A leaf bait assay was used to facilitate the screening of large numbers of samples for the presence of *P. n.* var. *parasitica* (24). From each sample, 100 g of the sample was mixed with 30 ml of distilled water in a 0.48-L plastic bowl and allowed to stand overnight before flooding with 370 ml of distilled water. Six 2-cm pineapple leaf disks were floated on the surface. After 72 hr at room temperature three of the disks were removed, blotted dry, and placed on the surface of a petri plate containing the selective medium of Mitchell et al (16). After 48 hr of incubation at 25 C in the dark, the plates were examined for colonies of *P. n.* var. *parasitica*. In samples where *P. n.* var. *parasitica* was detected, a soil-dilution plating technique utilizing the selective medium was used to quantify inoculum propagules: 20 g of soil was mixed with 180 ml of distilled water, using a magnetic stir plate, and 1-ml aliquots of the resultant suspension were transferred to each of 10 petri plates, containing 15 ml of selective medium. The plates were incubated in the dark at 25 C for 72 hr, rinsed under a stream of tap water, and examined for colonies of *P. n.* var. *parasitica*. The total number of colonies obtained on 10 plates per sample was recorded as propagules per gram (ppg) of soil. The v/m ratio was obtained and Moran's I statistic was determined for a sample and its immediate four neighbors. Semivariograms were generated for the data in four directions (0, 45, 90, and 135°).

Cured crowns of pineapple were used as seed material and were planted in the test plot 24 hr after sampling. One crown was inserted 4–7 cm below the soil surface at each of the 144 sample points. The soil was saturated for 48–72 hr immediately following planting. The location of diseased crowns was recorded 11 days after planting. A value of 0 was assigned to healthy crowns and 1 to crowns with heart rot. The spatial relation between a crown and its four immediate neighbors was determined using Moran's I statistic. Semivariograms were generated for four directions (0, 45, 90, and 135°).

The sensitivities of the assays used for the recovery of fungal propagules were compared. Chlamydospores of *P. n.* var. *parasitica* from an isolate previously collected from the plot were produced by the methods of Tsao (23) and purified by procedures recommended by Ramirez and Mitchell (21). The concentration of chlamydospores was determined by counting the number in 10 0.01-ml aliquots under 10× magnification. Chlamydospores were added to 500 g of air-dried soil obtained from a fallow pineapple field of similar soil type but having no history of heart rot, to produce samples with chlamydospore densities of 0, 0.01, 0.1, and 1 per gram of soil. Chlamydospores were then isolated from the samples with the leaf bait assay and selective medium as described previously. There were four replications for each chlamydospore density.

## RESULTS

The semivariogram from the simulated random pattern of pathogen propagules was constant, regardless of the distance separating samples (Fig. 3). Thus, variation between quadrats was random and not influenced by spatial dependence among neighboring samples. The semivariogram from the aggregated hypothetical arrangement was linear, with a coefficient of determination of $r^2 = 0.951$ and a slope of 1.223 (Fig. 3). This indicated that spatial dependence decreased linearly as the distance between samples increased.

The mean mortalities of pepper seedlings grown in a medium amended with copper in a random fashion and in an aggregated fashion were 40.2 and 57%, respectively. Semivariograms for seedling mortality in the random amendment of copper were constant, indicating the condition of a seedling was independent of that of its neighbors (Fig. 4A). Semivariograms for the mortality of seedlings transplanted to the aggregated arrangement of copper revealed spatial dependence up to a distance of three seedlings (Fig. 4B). The conditions of seedlings separated by a distance of three or more seedlings were unrelated.

The initial inoculum of *P. n.* var. *parasitica* in field 1 had a mean density of 0.96 ppg, a v/m ratio of 5.22, and a Moran I statistic of 0.044 (Fig. 5A and Table 2). The inoculum in field 2 had a mean density of 13.96 ppg, a ratio of 27.26, and a Moran I statistic of 0.309 (Fig. 5B and Table 2).

Semivariograms revealed that residual populations of *P. n.* var. *parasitica* were anisotropic (Fig. 6). In field 1, the semivariance for inoculum density for 0° increased slightly as the distance between samples increased, indicating a weak association between sample values (Fig. 6A). For 45° the semivariance values were more or less constant, suggesting that the sample values were independent of each other. Variability between samples in the 90° direction increased up to a sample distance of 1.2 m; at greater distances between samples, the semivariance declined, indicating the presence of another clump of inoculum (Fig. 6B). The semivariograms for 135° did not approach a constant value but continued to increase in a linear fashion, indicating that the greater

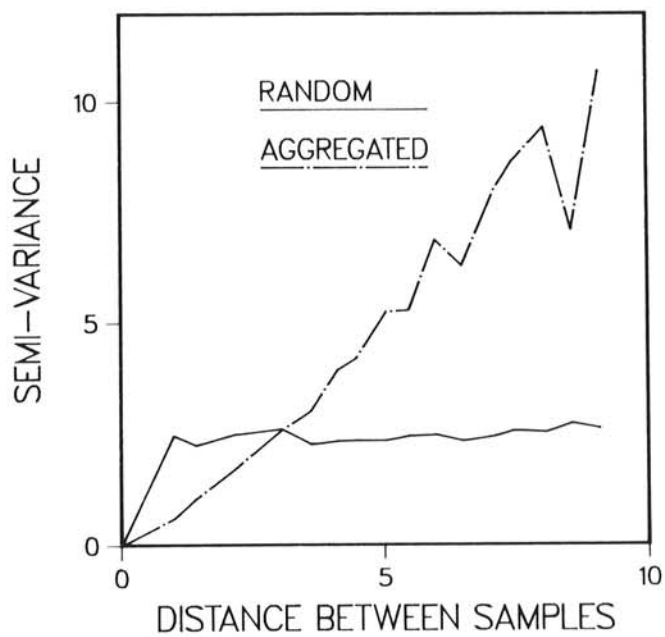


**Fig. 3.** Semivariograms from a hypothetical field corresponding to a random and an aggregated arrangement of a soilborne organism.

the separation of two samples, the greater the difference in inoculum density of the two samples, on the average.

In field 2, the semivariogram for 0° was linear; for 45°, it decreased up to a distance of 1.2 m and then increased; for 90°, it was constant up to a distance of 1.5 m and then increased; and for 135°, it remained constant, regardless of the distance between samples (Fig. 6C and D). Spatial dependence appeared to be greatest in the 0° direction. The $y$ intercept in all variograms from both fields did not equal 0 but instead had some positive value. This value is called a nugget variance and is due to measurement error, autocorrelation that occurs at intervals less than the smallest interval sampled (0.3 m), or combinations of both.

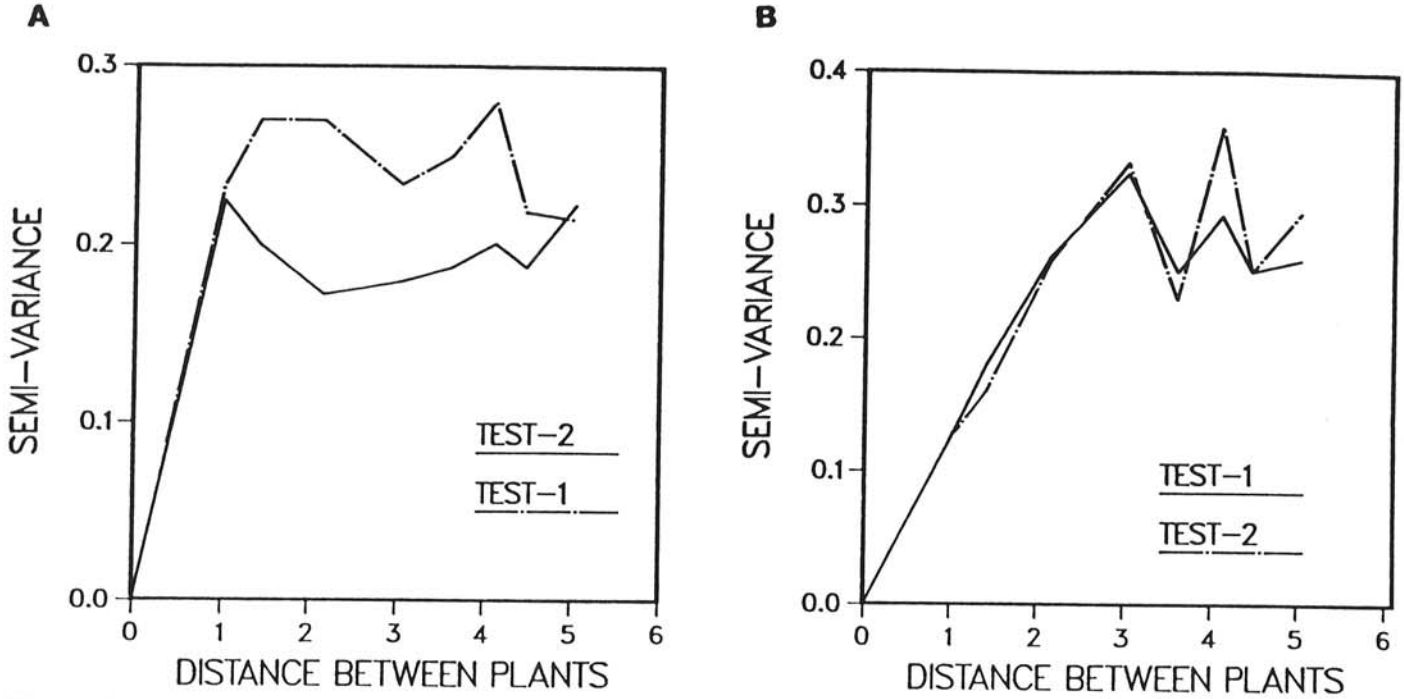


**Fig. 4.** Semivariograms of patterns of dead seedlings caused by a random (**A**) and an aggregated (**B**) pattern of toxic levels of copper incorporated into sand, on a rating scale of 0 (healthy) and 1 (dead).

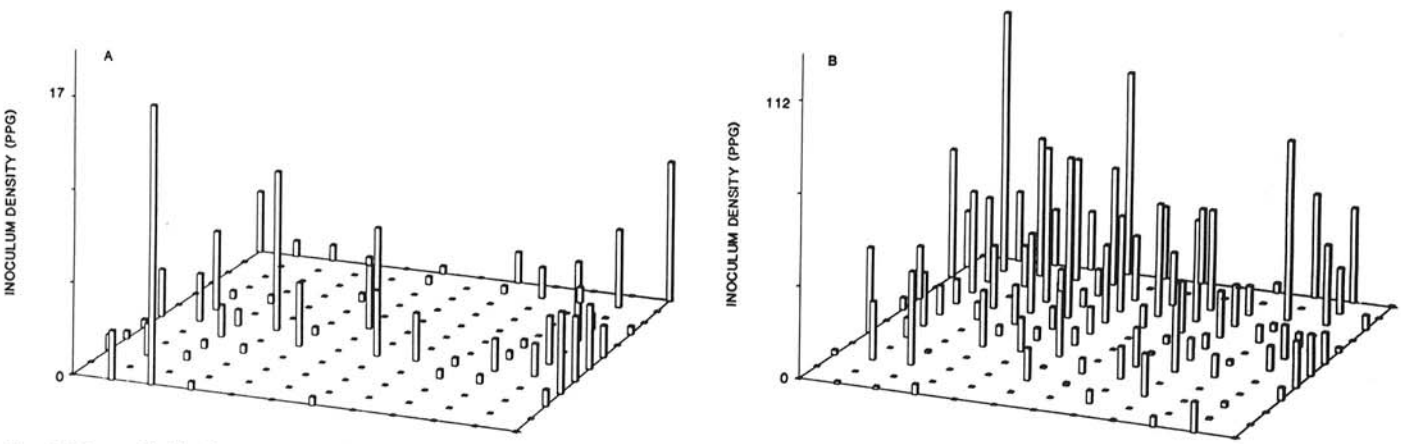


**Fig. 5.** Plots of initial inoculum density of *Phytophthora nicotianae* var. *parasitica* in a 3.6-$m^2$ section of pineapple field 1 (**A**) and field 2 (**B**). PPG = propagules per gram.

TABLE 2. Sample statistics for inoculum density of *Phytophthora nicotianae* var. *parasitica* and disease incidence in field plots of pineapple

| | Inoculum density | | | | Disease incidence | | |
|---|---|---|---|---|---|---|---|
| Field | Mean (ppg)[a] | v/m[b] | I[c] ± SE | *P* value [I = *E*(I)] | Mean[d] (%) | I ± SE | *P* value [I = *E*(I)] |
| 1 | 0.96 | 5.22 | 0.044 ± 0.007 | 0.179 | 60.4 | 0.330 ± 0.007 | 0.000 |
| 2 | 13.96 | 27.26 | 0.309 ± 0.007 | 0.000 | 81.9 | 0.148 ± 0.007 | 0.010 |

[a] Propagules per gram.
[b] Variance-to-mean ratio.
[c] Moran's I statistic.
[d] Recorded 11 days after planting.

Disease incidence appeared to be aggregated, with a Moran I statistic of 0.33 and 0.148 in fields 1 and 2, respectively (Table 2, Fig. 7). Semivariograms showed that the incidence of heart rot was spatially related and appeared similar in all directions analyzed in field 1 (Fig. 8A and B). In field 2, spatial dependence was present at 0 and 45° but was not apparent at 90 and 135° (Fig. 8C and D). This might be due to the high incidence of disease reported in field 2.

Inoculum densities of 0.96 and 13.96 ppg resulted in 60.4 and 81.9% incidence of disease 11 days after planting.

Neither assay could recover chlamydospores at a density of 0.01 ppg of soil. The leaf bait assay had a recovery ratio of 50 and 100% at densities of 0.1 and 1 ppg of soil. The selective media failed to recover chlamydospores at a density of 0.1 ppg of soil and had a recovery ratio of 75% at a density of 1 ppg of soil.

## DISCUSSION

The simulated patterns provided an opportunity for the comparison of geostatistics to other test statistics used to assess spatial patterns in a situation where nugget variance could be controlled. The v/m ratio provides an indication of the departure from randomness but does not account for the geographic location of a sample. In addition, it may fail to detect randomness at a scale larger than the quadrat (17). Moran's I statistic indicates whether spatial dependence exists among neighboring samples and can be expanded to produce correlograms that detect the degree of spatial dependence between samples separated over various distances. In the random pattern, both indices as well as geostatistics indicated randomness. In the aggregated pattern, the v/m ratio detected a departure from randomness, Moran's I detected spatial dependence among neighboring samples, and geostatistics defined the degree of dependence by giving it quantifiable parameters (i.e., linear with a slope of 1.22). Defining the degree of spatial dependence provides opportunities for comparative studies of spatial variability between pathogens or fields as well as a base for predictions of inoculum levels in unsampled areas through kriging (25). The versatility of geostatistics was demonstrated by its ability to measure spatial variability and detect sample independence for both inoculum density and disease incidence data.

The benefits of the ability to detect and measure anisotropy was demonstrated in the actual field situation, where the relationship between the pattern of initial inoculum of *P. n.* var. *parasitica* and the incidence of pineapple heart rot was being studied. In field 1, the v/m ratio of 5.22 indicated a nonrandom dispersion of inoculum, but the Moran I statistic of 0.044 suggested a lack of spatial dependence. Inspection with semivariograms showed that inoculum was random in the 45° direction and displayed various levels of aggregation in other directions, ranging from slight (0°) to very noticeable (135°). The failure of inoculum to be aggregated in several directions explains the discrepancies between the v/m ratio and Moran's I.

Geostatistics also showed that while aggregation of diseased pineapple crowns was homogeneous in the four directions analyzed, aggregation of inoculum was not. Differences between the spatial pattern of inoculum and disease incidence were observed in other studies on the relations of soilborne plant pathogens to disease incidence (18,22). The differences observed in this study can be attributed to the rapid increase in inoculum potential associated with *Phytophthora* spp.; conditions conducive to the spread of inoculum in all directions, created when the plots were initially flooded for 72 hr immediately following planting; and differences between soil conditions within the plot.

The presence of a nugget variance in semivariograms for inoculum as well as disease incidence indicates that sampling errors existed. Additional steps to reduce errors, such as better techniques for detection of inoculum and disease incidence, as well as the use of a smaller sampling interval, should be incorporated into future studies of inoculum density and disease incidence with *P. n.* var. *parasitica* on pineapple.

The relation between mean inoculum density and disease incidence in this study correlates with examples in the literature where low initial levels of *P. nicotianae* in the field are capable of inciting large amounts of disease in tobacco fields (9,12) and with greenhouse studies of other *Phytophthora* spp. on various hosts, where inoculum densities resulting in 50% infection ranged from 0.1 to 0.9 chlamydospores per gram of soil, under optimum conditions (15,21). A 13-fold increase in inoculum density in test 2 resulted in only a 20% increase in disease incidence. The limited number of available host plants might explain the disproportionate increase in inoculum versus disease. However, the incidence of diseased plants would still be expected to be closer to 100%. Another explanation for the disproportionate increase is that the level of aggregation of inoculum imposes an upper limit on the overall disease development.

In this study, the utility of geostatistics as a method to measure

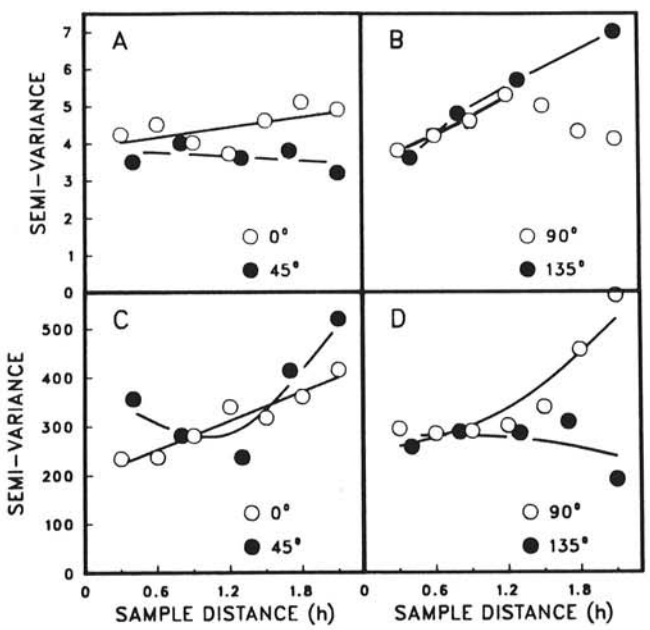


**Fig. 6.** Directional semivariograms for residual populations of *Phytophthora nicotianae* var. *parasitica* measured at 30-cm intervals in field 1 (**A** and **B**) and field 2 (**C** and **D**).

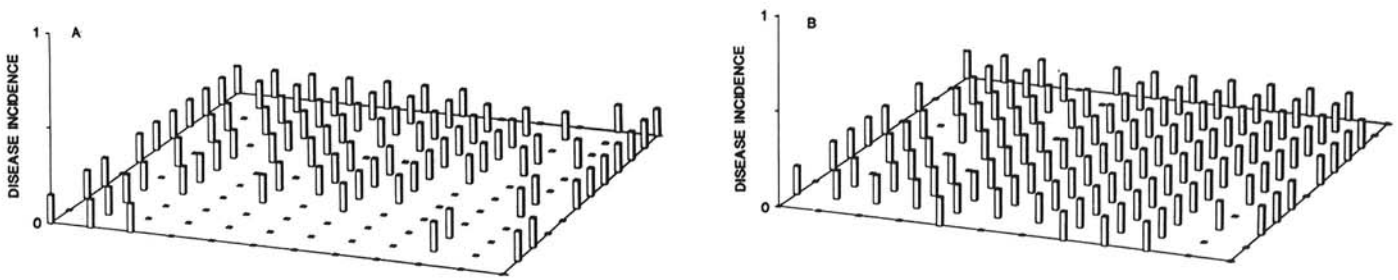


**Fig. 7.** Plot of incidence of pineapple heart rot in pineapple field 1 (**A**) and field 2 (**B**). A value of 1 indicates disease, and 0 indicates healthy crowns.

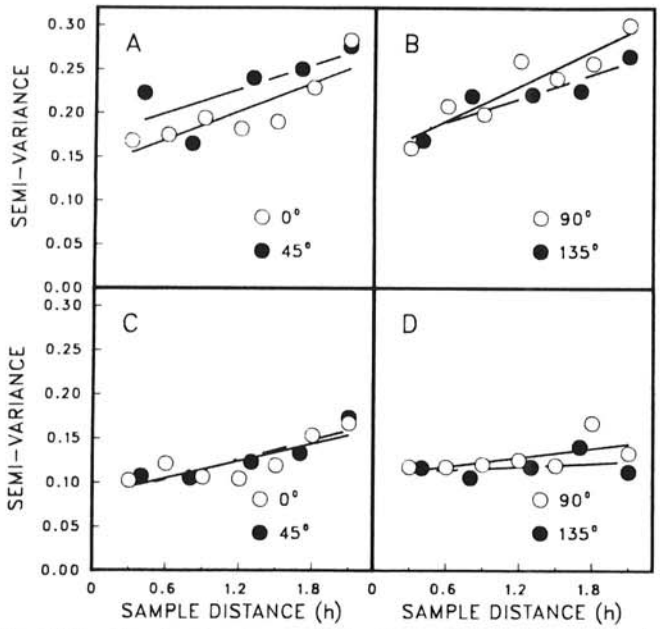


**Fig. 8.** Directional semivariograms for incidence of pineapple heart rot in field 1 (**A** and **B**) and field 2 (**C** and **D**).

differences between patterns of initial inoculum and patterns of diseased plants was demonstrated. It was also shown that although primary inoculum and diseased plants were aggregated in the field, the type of aggregation differed, and that inoculum randomly dispersed in a single direction can still result in a clustering of diseased plants. Finally, an initial density of *P. n.* var. *parasitica* of less than 1 ppg of soil was capable of inciting a significant level of disease in a pineapple field 11 days after planting.

## LITERATURE CITED


1. Anonymous. 1972. U.S. Soil Conserv. Serv., Soil Surv., Hawaii.
2. Campbell, C. L., and Noe, J. P. 1985. The spatial analysis of soilborne pathogens and root diseases. Annu. Rev. Phytopathol. 23:129-148.
3. Chellemi, D. O. 1986. Spatial variability of *Phytophthora nicotianae* var. *parasitica* in the field and its relation to pineapple heart rot. M.S. thesis, University of Hawaii, Manoa. 93 pp.
4. Chellemi, D. O., Rohrbach, K. G., Yost, R. S., and Sonoda, R. M. 1986. Application of geostatistics to spatial studies in plant pathology. (Abstr.) Phytopathology 76:1097.
5. Clark, I. 1979. Practical Geostatistics. Elsevier Applied Science Publishers, Essex, England. 129 pp.
6. Cliff, A. D., and Ord, J. K. 1977. Spatial Autocorrelation. Pion, London. 178 pp.
7. Elliott, J. M. 1977. Some methods for the statistical analysis of benthic invertebrates, 2nd ed. Freshwater Biol. Assoc. Sci. Publ. No. 25. Ambleside, Cumbria, U.K. 160 pp.
8. Ferrin, D. M., and Mitchell, D. J. 1986. Influence of initial density and distribution of inoculum on the epidemiology of tobacco black shank. Phytopathology 76:1153-1158.
9. Flowers, R. A., and Hendrix, J. W. 1972. Population density of *Phytophthora parasitica* var. *nicotianae* in relation to pathogenesis and season. Phytopathology 62:474-477.
10. Gray, S. M., Moyer, J. W., and Bloomfield, P. 1986. Two-dimensional distance class model for quantitative description of virus-infected plant distribution lattices. Phytopathology 76:243-248.
11. Journel, A. G., and Huijbregts, C. H. 1978. Mining Geostatistics. Academic Press, New York.
12. Kannwischer, M. E., and Mitchell, D. J. 1981. Relationships of numbers of spores of *Phytophthora parasitica* var. *nicotianae* to infection and mortality of tobacco. Phytopathology 71:69-73.
13. 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.
14. Matheron, G. 1971. The theory of regionalised variables and its applications. Cahier No. 5. Cent. Morphol. Math. Fontainbleau. 211 pp.
15. Mitchell, D. J. 1978. Relationships of inoculum levels of several soilborne species of *Phytophthora* and *Pythium* to infection of several hosts. Phytopathology 68:1754-1759.
16. Mitchell, D. J., Kannwischer-Mitchell, M. E., and Zentmyer, G. A. 1986. Isolating, identifying, and producing inoculum of *Phytophthora* spp. Pages 63-66 in: Methods for Evaluating Pesticides for Control of Plant Pathogens. K. D. Hickey, ed. American Phytopathological Society, St. Paul, MN.
17. Nicot, P. C., Rouse, D. I., and Yandell, B. S. 1984. Comparison of statistical methods for studying spatial patterns of soilborne plant pathogens in the field. Phytopathology 74:1399-1402.
18. Noe, J. P., and Campbell, C. L. 1985. Spatial pattern analysis of plant parasitic nematodes. J. Nematol. 17:86-93.
19. Pielou, E. C. 1977. Mathematical Ecology. Wiley, New York. 358 pp.
20. Proctor, C. H. 1984. On the detection of clustering and anisotropy using binary data from a lattice patch. Commun. Stat.-Theor. Meth. 13:617-638.
21. Ramirez, B. N., and Mitchell, D. J. 1975. Relationship of density of chlamydospores and zoospores of *Phytophthora palmivora* in soil to infection of papaya. Phytopathology 65:780-785.
22. Shew, B. B., Beute, M. K., and Campbell, C. L. 1984. Spatial pattern of southern stem rot caused by *Sclerotium rolfsii* in six North Carolina peanut fields. Phytopathology 74:730-735.
23. Tsao, P. H. 1971. Chlamydospore formation in sporangium-free liquid cultures of *Phytophthora parasitica*. Phytopathology 61:1412-1413.
24. Tsao, P. H. 1983. Factors affecting isolation and quantitation of *Phytophthora* from soil. Pages 219-236 in: *Phytophthora:* Its Biology, Taxonomy, Ecology, and Pathology. D. C. Erwin, S. Bartnicki-Garcia, and P. H. Tsao, eds. American Phytopathological Society, St. Paul, MN.
25. Trangmar, B. B., Yost, R. S., and Uhara, G. 1975. Application of geostatistics to spatial studies of soil properties. Adv. Agron. 38:45-94.
26. Woolum, A. G., and Cassel, D. K. 1984. Spatial variability of *Rhizobium japonicum* in two North Carolina soils. Soil Sci. Soc. Am. J. 48:1082-1086.