Subject Category: Epidemiology

Spatial patterns of soilborne inoculum of *Verticillium dahliae* in four commercial potato fields of Central Wisconsin

P. C. Nicot, D. I. Rouse, and B. S. Yandell

First and second authors, former research assistant and assistant professor, respectively, Department of Plant Pathology, 1630 Linden Drive, University of Wisconsin-Madison, Madison, WI 53706. Third author, assistant professor, Department of Horticulture and Department of Statistics, University of Wisconsin-Madison, Madison, WI 53706.

Supported by Hatch project 142-2500 and by funds from the Wisconsin Potato Board.

Accepted for publication

ABSTRACT

Nicot, P. C., Rouse, D. I., and Yandell, B. S. 1985. Spatial patterns of soilborne inoculum of *Verticillium dahliae* in four commercial potato fields of Central Wisconsin. Phytopathology 75:000-000.

Square "study areas" of three different sizes were established in each of four commercial potato fields of Central Wisconsin. Depending on its size, each study area was divided into 25, 49, 100, or 400 contiguous square quadrats. A sample of soil was taken from each quadrat, air dried, and assayed for *Verticillium dahliae* Kleb. by a soil dilution plating technique. The variability of inoculum levels across each study area was assessed by analysis of variance and by frequency distribution analysis. Within each large study area (540 m x 540 m divided into 400 quadrats in 1982 and 100 in

1983), large, significant differences in inoculum density were found among samples. Significant differences in inoculum density among samples within a study area were found in fewer small study areas (50 cm x 50 cm in 1982 and 12.5 cm x 12.5 cm in 1983, all divided into 25 quadrats) than medium-sized study areas (4.5 m x 4.5 m divided into 25 quadrats in 1982, and 6.3 m x 6.3 m divided into 49 quadrats in 1983). The observed level of variability of inoculum densities decreased as the size of the quadrats decreased. Each study area had a unique arrangement of quadrats with high and low inoculum densities of V. dahliae. The spatial pattern of inoculum in each study area was characterized as "aggregated", "random", or "regular", based on a visual assessment and analysis of spatial autocorrelation. A large scale aggregation was observed in the large study areas of three of the four fields examined. The pattern was random in most medium-sized and small study areas. The results of this study suggest that caution should be taken when collecting soil samples to assess the populations of V. dahliae in potato fields, as large zones with either high or low inoculum densities might be missed by particular sampling schemes.

Information on the location of individual propagules of plant pathogens in soil may be critical for studies of the epidemiology and ecology of soilborne pathogens. It has been postulated that clumping of inoculum in field soil may be associated with low correlations between inoculum density and disease incidence (21), or that it may affect the shape of the curves relating disease incidence to soil inoculum density (12, 25). The occurrence of inoculum in clumps has also been shown to increase the influence of the sampling

procedures on the accuracy of the estimates of average soil inoculum levels in field studies (7, 9, 13). Commonly used mathematical models of root infection by soilborne fungi, recently reviewed by Gilligan (8), assume that the propagules of the pathogen in the vicinity of the host roots occur uniformly in the soil at the apices of tetrahedra (3, 4, 15), or randomly (2, 11). To account for possible clumping of inoculum in naturally infested soils, Gilligan expanded an earlier model by approximating the distribution of inoculum with a negative binomial distribution (8). The results of Gilligan's study suggested that the selection of a particular model of host infection should be based on prior knowledge of the patterns of inoculum in the soil surrounding the host (8).

Frequency distribution analysis has often been used as a tool to test whether propagules of a pathogen occurred at random or in clumps in a volume of soil (19). As a consequence, the word "distribution" has often been used both to designate a probability distribution (frequency distribution), in a mathematical sense, and to designate the spatial arrangement of propagules of a pathogen in a volume of soil. To avoid confusion in this paper, the use of the term distribution will be restricted to its mathematical meaning and the spatial arrangement of propagules of a pathogen in soil will be referred to as spatial pattern.

Differences in inoculum levels of Verticillium dahliae at different depths in the soil have been reported by several researchers (14, 27). Based on a bioassay with tomato seedlings, Wilhelm (27) observed a degree of infectivity 3-4 times higher in samples taken from the 0-30cm layer of soil than below 30cm. The fungus could either be detected at very low levels, or not detected

at all, at depths greater than 30cm at 15 of the 20 sites sampled. In another study 98% of the population of V. dahliae, assessed with a dilution plating technique, were found in the top 30cm of soil (14). Differences in inoculum levels occuring across a field have also been studied. Evans and Gleeson (7) estimated soil populations of V. dahliae in an intensively sampled (12m x 12m) study area. Although spatial patterns of the pathogen were not mentioned, the authors tested a "row effect" and a "column effect" in an analysis of variance and reported a significant column effect. This is an indication that the pattern of the fungus was probably not random over the study area. The frequency distribution of soil inoculum levels of V. dahliae in an intensively sampled potato field in Ohio was shown to give a poor fit to the Poisson distribution (23). This suggests that the pattern of occurence of the fungus may be nonrandom at some scale smaller than the sampling unit, but says little about the large scale pattern in the field. Although the spatial arrangement of zones of high and of low inoculum density was not discussed in either of these studies, their results support the hypothesis that spatial patterns of V. dahliae were nonrandom.

The present study was initiated to examine the variability of soil inoculum levels of V. dahliae across several commercial potato fields of central Wisconsin and to characterize the patterns of inoculum in each field. As patterns may appear random or aggregated depending on the scale at which they are examined (20, 26), the study was done in each field at three scales of biological importance: the whole field ("large scale"), the volume of soil explored by the roots of an individual potato plant ("medium scale"), and a single soil core ("small scale").

MATERIALS AND METHODS

Site selection and sampling. Four commercial potato fields with a history of Verticillium wilt were chosen for this study in the Central Sands area of Wisconsin. Research was conducted in field "A" during 1982, and in fields "B", "C", and "D" in 1983. These fields consisted of Plainfield loamy sand and were flat, circular (ca. 800m in diameter), and irrigated with center pivot systems. The eastern half of field C was fumigated with 50 gals/acre metham-sodium in October 1983.

In each field, square "study areas" of 3 different sizes (corresponding to the scales at which the pattern of inoculum was to be studied) were delimited, and each divided into contiguous squares referred to as "quadrats". One "large" study area, approximately centered on the irrigation pivot, was delimited in each field. The large study area was divided into 400 "large" (27m x 27m) quadrats on a 20x20 grid pattern in field A, and into 100 large (54m x 54m) quadrats on a 10x10 grid pattern in fields B, C, and D. Ten "medium-size" and ten "small" study areas were established in field A in 1982 and three medium-size and three small study areas were established in each of fields B, C, and D in 1983. The medium-size study areas were divided into 25 "medium-size" (90cm x 90cm) quadrats on a 5x5 grid in 1982 and into 49 (90cm x 90cm) quadrats on a 7x7 grid in 1983. The small study areas consisted of (50cm x 50cm) squares in 1982 and (12.5cm x 12.5cm) squares in 1983, each divided into 25 "small" quadrats on a 5x5 grid.

Soil samples were taken from field A in early June 1982 and from fields B, C, and D, in early June 1983. In 1982 each sample of soil consisted of a single 30cm-deep soil core, 2.5cm in diameter, which was removed from the

center of each quadrat in every study area. In 1983, each sample removed from a large or a medium-size quadrat was a composite of 9 soil cores taken as indicated in Fig. 1; a single soil core was taken from the center of each small quadrat. To evaluate the variability of inoculum levels across each quadrat of the large study areas, an additional soil sample was removed from each large quadrat in two fields in 1983. Each additional sample was a composite of 9 cores taken at random throughout the (54m x 54m) quadrat.

All the samples taken in 1982 and 1983 were placed in separate polyethylene bags labeled with a study area number and quadrat coordinates, and transported to the laboratory.

Soil assay for Verticillium dahliae. The samples were air dried for 4-5 weeks at 30-50% relative humidity and 20-24°C to eliminate drought-sensitive conidia and mycelial fragments (5, 22), and stored at room temperature until assayed. Just before each sample was assayed, the soil aggregates were gently broken by hand to a particle size smaller than 500µm and the soil was homogenized by hand shaking.

For each sample assayed in 1982, one 10g subsample was suspended in 100ml of water, from which five 1ml aliquots were taken with a pipet and plated separately onto an NPX-pectate medium selective for V.dahliae (5). In 1983, three 10g subsamples were taken from each sample and two 1ml aliquots were plated for each subsample. Therefore, the estimate of the soil inoculum of V. dahliae in a sample was obtained by averaging colony counts from 5 plates in 1982 and from 6 plates in 1983. The change in design between 1982 and 1983 was made to increase the precision of the estimate of inoculum density in each sample (18).

All the plates were incubated at 20-22°C for at least two weeks and microsclerotial colonies of V. dahliae were counted with the aid of a dissecting microscope (magnification 15X). The number of colony forming units (CFU) of the pathogen per plate was converted into a number of propagules per gram of dry soil (ppg) by multiplying the number of CFU per plate by 10.

As the assay of the 900 samples taken in 1982 and 1175 taken in 1983 lasted over a period of several months, the possibility of a loss of viability of the fungus in storage was considered: To avoid systematically underestimating the inoculum density of V. dahliae in all the samples of whole study areas, the samples to be assayed were drawn at random. The viability of the fungus was tested by re-assaying the first 25 samples at the end of the period needed to assay all the samples.

Statistical analysis of the spatial patterns. An analysis of spatial autocorrelation (19) was performed for each study area, on the square-root transformed estimates of soil inoculum density of V. dahliae in the soil samples from each quadrat. The data were square-root transformed to stabilize the variance (18).

We tested for spatial autocorrelation in each study area, using Moran's I statistic (6, 16, 24) where X_i was the square root of the total number of

$$I = \frac{n}{S_o} \frac{\sum_{i=1}^{n} \sum_{j=1}^{n} w_{ij} (X_i - \bar{X})(X_j - \bar{X})}{\sum_{i=1}^{n} (X_i - \bar{X})^2},$$

CFU counted from the assay of the soil sample taken from quadrat i; X was the average of all X_i's for the study area; n was the total number of quadrats

in the study area; w_{ij} was a weight coefficient between quadrat i and quadrat j; and S_o was the sum of all w_{ij}'s for i=1 to n and for j=1 to n. The weight coefficients were determined prior to the computation of the statistic, based on which quadrats were to be compared. In this study, the inoculum level of V. dahliae in every quadrat i was correlated to those in its four immediate neighbors, that is, those quadrats having an edge in common with i. The coefficient w_{ij} was therefore given a value of one if i and j were immediate neighbors, and zero otherwise. The probability distribution of the I statistic can be approximated by a normal distribution, under certain conditions (6). This property was used to test for the occurence of spatial autocorrelation in each study area, as described by Cliff and Ord (6). Based on the weight coefficients used in this study, a significantly positive I implied that either high or low inoculum levels of V. dahliae tended to occur in groups of neighboring quadrats indicating an aggregated pattern. A significantly negative I implied that inoculum levels in neighboring quadrats tended to be alternatively high and low in an arrangement similar to that of black and white squares on a chessboard ("regular pattern").

The underlying model used for this analysis was the spatial autoregressive model:

$$(X_i - \mu) = \sum_{i=1}^{n} \sum_{j=1}^{n} w_{ij} (X - \mu)_j + e_i$$

where μ was the expected value of X_i, and e_i is unexplained error. Because the eastern half of field C had been fumigated, V. dahliae was not detected in the eastern half of the field except at a low inoculum density in a few quadrats. High inoculum densities were observed in the western half of

the field. Due to this systematic difference between the two halves of field C, the model was not appropiate to use for the whole field. Thus, an analysis of spatial autocorrelation was performed separately for the non-fumigated (western) part of field C.

RESULTS

Survival of V. dahliae in stored dry soil. The inoculum level of V. dahliae declined by an average of 10% in the 25 soil samples assayed at the begining and at the end of the 6-month period needed to assay all the samples taken in 1983. This decline, evaluated by a paired t-test on the estimates of soil inoculum level (in ppg) in each sample and on their square root transform (effected to stabilize the variance [28]), was not statistically significant ($p>0.10$).

Differences in soil inoculum levels of V. dahliae across four commercial potato fields. The range of inoculum levels and the average for each study area are shown in Tables 1-3. The widest ranges for each field were observed in the large study areas. The highest inoculum level found in this study was 80ppg, in field C.

For most techniques used to quantitate V. dahliae in field soil, repeated assays of a sample of soil yield individual estimates of inoculum density of the fungus that are not identical (18). Therefore, differences between estimates of levels obtained from the assay of different samples could reflect actual differences in the samples, but they could also be an artifact resulting from the variability of the soil assay. Two methods were used to test, for each study area, the hypothesis that the inoculum levels in the soil samples from every quadrat were identical, against the alternative hypothesis

that soil inoculum levels differed at the various sampling locations in the study area.

The first method, a frequency distribution analysis, relied on the assumption that counts per plate of colony forming units of V. dahliae for repeated assays of a given sample of soil, with the dilution plating technique, were approximately Poisson distributed (18). If the inoculum density of the fungus was identical in all the samples taken in an area then the frequency distribution of Verticillium counts per plate from the assay of these samples should be identical. The hypothesis was tested for each study area by fitting a Poisson model to the observed frequency distribution of Verticillium counts in each quadrat, based on the total number of V. dahliae CFU counted in the 5 or 6 plates examined for each sample. The observed frequencies were compared to their expected values, (calculated from a Poisson distribution with mean estimated as the average CFU count for the whole study area) and a chi-square goodness-of-fit test was performed.

The second method used to test the hypothesis that the inoculum levels of V. dahliae were identical in all the soil samples taken from a study area, was an analysis of variance based on the CFU count from the plates examined for each sample. The CFU counts per plate were square-root transformed prior to the analysis of variance, to stabilize the variance (28). A one-way analysis of variance was performed on the 1982 data. The 1983 data was analyzed by nested analysis of variance because of the nested design in the assay of the sample of soil taken in each quadrat of the study areas (three "subsamples" were taken from each sample, and each "subsample" was in turn subsampled when two 1ml aliquots were taken from the suspension).

The results of the frequency distribution analysis and the analysis of variance for each of the large, medium, and small study areas are shown in Tables 1, 2, and 3, respectively. The hypothesis was rejected with both types of statistical analysis in all large study areas (Table 1), and by analysis of variance in most medium size study areas, indicating the presence of significantly different inoculum levels across the study areas. In contrast, the hypothesis failed to be rejected in 10-13 (depending on which test was used) of the 19 small study areas examined, indicating that the soil inoculum levels in those study areas were not significantly different, given the level of precision associated with the soil assay used in this study.

The frequency of rejection, with either of the two statistical methods, of the hypothesis of apparent uniformity of inoculum levels in a study area, was compared for the three sizes of study areas (Table 4). The proportion of study areas in which the hypothesis was rejected by both methods decreased sharply as the size of the study area decreased.

To measure the variability of soil inoculum levels across a study area, a coefficient of variability (ratio of the standard deviation to the mean [27]) was computed for each study area, based on the total CFU counts for each sample (Tables 1-3). The average coefficients of variability were 1.48, 1.46, and 1.12 for the large, medium and small study areas, respectively,indicating a decrease in the level of variability of inoculum density as the distance between samples decreased.

Comparison of two sampling schemes in the large scale study areas. Two estimates of soil inoculum density of V. dahliae were compared for each quadrat of the large scale study area of fields B and C: that from the assay

of the sample taken near the center of the quadrat (9 bulked soil cores), and that from the assay of the additional sample (9 soil cores taken at random throughout the quadrat and bulked). The two types of estimates were compared separately for each individual quadrat, by nested analysis of variance (same nested design as described earlier). The hypothesis of equality of the two estimates of inoculum density provided for each quadrat by both types of sample, was rejected for 6 and 11 of the 100 large quadrats of fields B and C, respectively.

For each quadrat, the estimate of inoculum density obtained with one type of sample was plotted against the estimate obtained with the other type of sample (Fig. 2) The data points were widely scattered for both fields, suggesting little similarity between estimates. This coincided with significant ($P<0.01$; square-root transformed data) but low coefficients of correlation between sample types: $r_B = 0.532$ and $r_C = 0.698$ for fields B and C respectively. The regression lines (Fig. 2) were compared to the line $Y = X$. For field B the slope was significantly smaller than 1.00 ($P<0.01$) and the intercept was significantly greater than zero ($P<0.01$), indicating that on the average, each type of sampling resulted in distinct estimates of soil inoculum level in a large quadrat. The line $Y = X$ and the regression line for field C were not significantly different ($P>0.05$), indicating that on the average, both types of samples tended to give similar estimates of soil inoculum density in the large quadrats of that field.

Spatial patterns of soil inoculum levels. Each study area had a unique arrangement of quadrats with high and low inoculum densities. The pattern in each study area was examined and rated visually. The pattern was

characterized as "aggregated" if either high or low values of inoculum level tended to be found near each other. The pattern was characterized as "regular" if high inoculum levels tended to be regularly interspersed with low inoculum levels. The pattern was characterized as "random" if no particular trend could be detected.

The visual rating and the characterization of pattern based on the I statistic gave similar results in most study areas (Tables 5-7). The pattern of inoculum was aggregated in the large scale study areas of three of the four fields studied (Table 5). The pattern in field C appeared aggregated when the whole field was examined, because the western half of the field had high inoculum levels and the eastern half, which had been fumigated, had very low levels of V. dahliae. However, when only the western half of the field was examined (Cw and Co,w in Table 5), the pattern of inoculum appeared random. The pattern was random in most medium and small study areas (Tables 6 and 7), and the small study areas in which the pattern of inoculum appeared aggregated or regular (Table 7) were study areas for which inoculum levels among quadrats were not significantly distinct from each other (Table 3). A comparison of the frequency of each type of pattern in the large, medium, and small study areas (Tables 5-7) shows less aggregation and more regularity of the pattern as the quadrat size decreased.

DISCUSSION

The inoculum levels of V. dahliae in 25 air-dried soil samples declined on the average by 10% during a 6-month storage period at 20-22°C. However the differences between soil inoculum level before and after the storage period were not statistically significant. These results are in agreement with the

observations of others (1, 7, 10), who reported that populations of V. dahliae were stable in dry soil over periods of several months.

Two statistical methods, analysis of variance and frequency distribution analysis, were used to test whether differences in inoculum levels observed in the assay of soil samples taken from a study area could be attributed to actual differences in the field or if they were likely to have resulted from the variability of the soil assay. Both methods led to similar conclusions for each of the large study areas. However, the hypothesis was rejected nearly twice as often with the analysis of variance as with the frequency distribution analysis for the small and medium study areas (Tables 2, 3). A possible reason for these differences might be a difference in the number of quadrats in each type of study area. The medium and small scale study areas had only 49 or 25 quadrats, allowing for few classes of soil inoculum levels of the fungus in the frequency tables. As a consequense, the chi-square goodness of fit test in each of these study areas may have had less power (probability of rejecting the hypothesis when it is false [27]) than the analysis of variance, because of the small number of degrees of freedom (between 1 and 3) associated with the frequency tables.

A wide range and a high variability of inoculum levels were found in the soil samples taken 27m or 54m apart in the large study areas of four commercial potato fields. The discrepancy between inoculum levels in the samples taken near the center and those taken at random throughout the large quadrats of field B (and to a lesser extent in those of field C) suggests that significant spatial variations of inoculum levels may also have occurred within some of these quadrats. It also suggests that a soil sample taken from

the center of a 54m x 54m area in these fields may provide a poor estimate of the average inoculum level over the area.

The observed variability of inoculum levels in a study area decreased with the distance between sampling locations, determined by the sizes of quadrats and study areas. Soil inoculum levels in samples taken from (50cm x 50cm) or (12.5cm x 12.5cm) study areas differed significantly within only half of the areas examined in this study. These results suggest that there was little short-distance variability of inoculum levels across the fields studied.

The pattern of inoculum of V. dahliae appeared aggregated in the large study areas of three of the four fields examined, with distinct zones of high inoculum density and zones of low inoculum density covering large areas in the fields. The pattern appeared random in most medium and small study areas. In one of the fields studied the eastern half had been fumigated and had non-detectable levels of V. dahliae. The population size of the pathogen in the non-fumigated half of the field was high and the pattern of inoculum in the large quadrats appeared random. Such a pattern might be the result of the planting of Verticillium infested seed pieces (17) some year in the past, with a random dispersion of infested seed pieces. One could also speculate that the pattern in that field has evolved gradually over the years from an aggregated state with large zones of high inoculum levels as were observed in the three other fields studied. As potato crops were continuously planted in that field over many years, the soil populations of V. dahliae may have increased year after year and the inoculum may have been spread by implements throughout the field.

The combined information on variability of soil inoculum levels and

spatial patterns in study areas of different sizes indicates that there were large differences in inoculum levels over large distances across the four fields studied, but that inoculum levels tended to be more similar as the distance between samples decreased. These observations suggest that caution should be taken when collecting soil samples to assess the populations of *V. dahliae* in potato fields, as large zones with either high or low inoculum levels might be "missed" by particular sampling schemes.

LITERATURE CITED

1. Ashworth, L. J. Jr., Huisman, O. C., Grogan, R. G., and Harper, D. M. 1976. Copper-induced fungistasis of microsclerotia of Verticillium albo-atrum and its influence on infection of cotton in the field. Phytopathology 66:970-977.

2. Baker, R. 1978. Inoculum potential. Pages 137-157 in: Plant Disease, An Advanced Treatise. Vol. II. J. G. Horsfall and E. B. eds. Academic Press, New York. 436 pp.

3. Baker, R., and Drury, R. 1981. Inoculum potential and soilborne pathogens: The essence of every model is within the frame. Phytopathology 71:363-372.

4. Baker, R, Maurer, C. L., and Maurer, R. A. 1967. Ecology of plant pathogens in soil. VII. Mathematical models and inoculum density. Phytopathology 57:662-666.

5. Butterfield, E. J., and DeVay, J. E. 1977. Reassessment of soil assays for Verticillium dahliae. Phytopathology 67:1073-1078.

6. Cliff, A. D., and Ord, J. K. 1981. Spatial processes. Models and applications. Pion Ltd., London. 266 p.

7. Evans, G., and Gleeson, A. C. 1980. An evaluation of the sampling variation when estimating the population of Verticillium dahliae in field soil. Ann. Appl. Biol. 95:177-184.

8. Gilligan, C. A. 1985. Probability models for host infection by soilborne fungi. Phytopathology 75:61-67.

9. Goodell, P. B., and Ferris, H. 1981. Sample optimization for five plant parasitic nematodes in an alfalfa field. J. Nematol. 13:304-313.

10. Green, R. J. Jr., 1980. Soil factors affecting survival of microsclerotia of Verticillium dahliae. Phytopathology 70:353-355.

11. Gregory, P. H. 1948. The multiple-infection transformation. Ann. Appl. Biol. 35:412-417.

12. Grogan, R. G., Sall, M. A., and Punja, Z. K. 1980. Concepts for modeling root infection by soilborne fungi. Phytopathology 70:361-363.

13. Hau, F. C., Campbell, C. L., and Beute, M. K. 1982. Inoculum distribution and sampling methods for Cylindrocladium crotalariae in a peanut field. Plant Disease 66:568-571.

14. Jordan, V. W. 1971. Estimation of the distribution of Verticillium populations in infected strawberry plants and soil. Pl. Path. 20:21-24.

15. McCoy, M. L., and Powelson, R. L. 1974. A model for determining spatial distribution of soilborne propagules. Phytopathology 64:145-147.

16. Moran, P.A.P. 1950. Notes on continuous stochastic phenomena. Biometrika 37:17-23.

17. Nachmias, A, and Krikun, J. 1984. Transmission of Verticillium dahliae in potato tubers. Phytopathology 74:535-537.

18. Nicot, P. C., and Rouse, D. I. 1986. Precision and bias of three quantitative soil assays for Verticillium dahliae. Phytopathology 76:(accepted).

19. Nicot, P. C., Rouse, D. I., and Yandell, B. S. 1984. Comparison of statistical methods for the study of spatial patterns of soilborne plant pathogens in the field. Phytopathology 74:1399-1402.

20. Pielou, E. C. 1977. Pages 113-165 in Mathematical Ecology, J. Wiley and Sons, New York. 385 pp.

21. Roth, D. A., and Griffin, G. J. 1981. Cylindrocladium root rot of black walnut seedlings and inoculum pattern in nursery soil. Can. J. Plant Pathology 3:1-5.

22. Schnathorst, W. C. 1981. Life cycle and epidemiology of Verticillium. Pages 81-111 in M. E. Mace, A. A. Bell, and C. H. Beckman eds. Fungal wilt diseases of plants. Academic Press, New York. 640 pp.

23. Smith, V.L., and Rowe, R.C. 1984. Characteristics and distribution of propagules of Verticillium dahliae in Ohio potato field soils and assessment of two assay methods. Phytopathology 74:553-556.

24. Sokal, R. R., and Oden, L. 1978. Spatial autocorrelation in biology. 1. Methodology. 2. Some biological implications and four applications of evolutionary and ecological interest. Biol. J. Linn. Soc. 10:199-249.

25. Taylor, J. D., Griffin, G. J., and Garren, K. H. 1981. Inoculum pattern, inoculum density-disease incidence relationships, and population fluctuations of Cylindrocladium crotalariae microsclerotia in peanut field soil. Phytopathology 71:1297-1302.

26. Vandermeer, J. 1981. Elementary Mathematical Ecology. J. Wiley and Sons, New York. 294 pp.

27. Wilhelm, S. 1950. Vertical distribution of Verticillium albo-atrum in soils. Phytopathology 40:368-376.

28. Zar, J.H. 1984. Biostatistical analysis. Prentice-Hall, Inc. Englewood Cliffs, NJ. 718 pp.

TABLE 1. Variability of soil inoculum levels of Verticillium dahliae across the large study areas.

study area[1]	range of inoculum levels[2]	average inoculum level[2]	coefficient of variability[3]	Test of Hypothesis[4]: frequency distribution analysis[5] χ^2	$p(\chi^2 > \chi^2)$		analysis of variance[6] f	$p(F > f)$	
A	0-40	5.73	1.08	275.75	<.001	*	9.43	<.001	*
B	0-25	4.40	1.30	114.78	<.001	*	3.93	<.001	*
Br	0-18	2.97		70.02	<.001	*	4.44	<.001	*
C	0-80	10.47	1.35	436.17	<.001	*	11.53	<.001	*
Cw	0-80	19.93		22.42	<.001	*	4.67	<.001	*
Cr	0-33	8.18		169.98	<.001	*	5.98	<.001	*
Cr,w	0-33	13.63		12.25	.02	*	2.73	<.001	*
D	0-58	3.72	2.18	292.49	<.001	*	9.69	<.001	*

[1]Study areas from different fields are indicated by different letters. The soil samples were taken from the center of each quadrat, except for the study areas marked "r" for which each sample consisted of 9 cores taken at random throughout the quadrat and bulked. The eastern half of field C had been fumigated and had low populations of V. dahliae. The subscript "w" indicates that the data are shown separately for the western part of the field.

TABLE 1. (continued)

[2]Expressed as number of propagules per gram of dry soil.

[3]Ratio of the standard deviation to the mean, calculated on the basis of the total CFU counts for each sample.

[4]Hypothesis of identical soil inoculum level in all the samples taken from a study area. A star (*) indicates rejection of the hypothesis at 5% significance level.

[5]Chi square test for the fit of a Poisson model (see text for details).

[6]Based on the CFU counts from the plates examined for each sample. The counts were square-root transformed prior to the analysis.

TABLE 2. Variability of soil inoculum levels of Verticillium dahliae across the medium-size study areas.

study area[1]	range of inoculum levels[2]	average inoculum level[2]	coefficient of variability[3]	Test of Hypothesis[4]: frequency distribution analysis[5] X^2	$p(\chi^2 > X^2)$	analysis of variance[6] f	$p(F > f)$
A1	0–10	1.92	1.33	1.15	.30	1.65	.05>p>.01 *
A2	0–14	2.24	1.32	3.42	.07	1.58	.10>p>.05
A3	0–14	2.72	1.31	2.49	.12	1.96	.05>p>.01 *
A4	0–12	2.72	1.08	0.53	.48	1.39	.25>p>.10
A5	0–10	2.72	1.06	4.69	.03 *	1.84	.05>p>.01 *
A6	0–10	2.72	1.24	4.94	.03 *	2.99	<.01 *
A7	0–14	3.28	1.04	0.81	.40	1.48	.10>p>.05
A8	0–12	3.44	1.07	2.30	.15	2.02	<0.01 *
A9	0–16	4.08	1.04	1.63	.22	2.63	<0.01 *
A10	0–16	4.88	0.90	3.66	.06	2.38	<0.01 *
B1	0–3	0.34	2.23	0.10	.96	0.85	>0.50
B2	0–7	1.02	1.73	8.13	.004 *	1.68	.05>p>.01 *
B3	0–45	17.65	0.49	25.74	<.001 *	2.57	<0.01 *
C1	0–3	0.17	3.60	3.10	.22	1.66	.05>p>.01 *
C2	0–45	10.85	0.87	51.17	<.001 *	4.68	<0.01 *
C3	0–64	17.31	0.84	42.36	<.001 *	6.53	<0.01 *

TABLE 2. (continued)

study area[1]	range of inoculum levels[2]	average inoculum level[2]	coefficient of variability[3]	Test of Hypothesis[4]			
				frequency distribution analysis[5]		analysis of variance[6]	
				χ^2	$p(\chi^2 > \chi^2)$	f	p(F > f)
D1	0-7	0.51	2.42	2.39	.13	1.70	.05>p>.01 *
D2	0-5	0.58	2.26	2.40	.13	2.14	<0.01 *
D3	0-14	1.46	1.84	11.05	<.001 *	3.09	<0.01 *

[1] Study areas from different fields are indicated by different letters.

[2] Expressed as number of propagules per gram of dry soil.

[3] Ratio of the standard deviation to the mean, calculated on the basis of the total CFU counts for each sample.

[4] Hypothesis of identical soil inoculum level in all the samples taken from a study area. A star (*) indicates rejection of the hypothesis at 5% significance level.

[5] Chi square test for the fit of a Poisson model (see text for details).

[6] Based on the CFU counts from the plates examined for each sample. The counts were square-root transformed prior to the analysis (see text for details).

TABLE 3. Variability of soil inoculum levels of Verticillium dahliae in the small study areas.

study area[1]	range of inoculum levels[2]	average inoculum level[2]	coefficient of variability[3]	Test of Hypothesis[4]				
				frequency distribution analysis[5]			analysis of variance[6]	
				χ^2	$p(\chi^2 > \chi^2)$		f	p(F > f)
AA1	0-6	0.72	2.10	1.37	.25		1.49	.10>p>.05
AA2	0-4	0.96	1.36	0.06	.83		0.97	>.25
AA3	0-10	1.84	1.37	0.69	.43		1.50	.10>p>.05
AA4	0-8	1.92	1.14	0.34	.58		1.12	>.25
AA5	0-6	2.00	1.12	0.90	.37		1.29	.25>p>.10
AA6	0-8	2.08	0.98	0.57	.47		1.14	>.25
AA7	0-12	2.64	1.43	8.30	<.01	*	2.82	<.01 *
AA8	0-14	4.96	0.96	8.68	.02	*	2.17	<.01 *
AA9	0-82	6.32	2.53	9.87	<.01	*	13.65	<.01 *
AA10	0-50	9.76	1.11	8.89	<.01	*	4.73	<.01 *
BB1	0-12	3.40	1.09	3.83	.05		1.42	.25>p>.10
BB2	0-28	6.13	1.31	7.71	<.01	*	7.41	<.01 *
BB3	12-33	20.27	0.26	1.02	.61		0.84	>.25
CC1	0-15	2.80	1.34	2.84	.09		4.60	<.01 *
CC2	0-17	5.00	0.89	1.56	.22		1.92	.05>p>.01 *
CC3	2-25	15.33	0.37	1.21	.55		1.30	.25>p>.10

TABLE 3. (continued)

study area[1]	range of inoculum levels[2]	average inoculum level[2]	coefficient of variability[3]	Test of Hypothesis[4]: frequency distribution analysis[5] χ^2	$p(\chi^2 > \chi^2)$		Test of Hypothesis[4]: analysis of variance[6] f	$p(F > f)$	
DD1	0-17	6.93	0.58	0.43	.52		1.11	>.25	
DD2	0-43	12.47	0.82	10.61	<.01	*	2.07	.05>p>.01	*
DD3	8-48	26.87	0.42	3.23	.08		2.35	<.01	*

[1] Study areas from different fields are indicated by different letters.

[2] Expressed as number of propagules per gram of dry soil.

[3] Ratio of the standard deviation to the mean, calculated on the basis of the total CFU counts for each sample.

[4] Hypothesis of identical soil inoculum level in all the samples taken from a study area. A star (*) indicates rejection of the hypothesis at 5% significance level.

[5] Chi square test for the fit of a Poisson model (see text for details).

[6] Based on the CFU counts from the plates examined for each sample. The counts were square-root transformed prior to the analysis (see text for details).

TABLE 4. Frequency of rejection of the hypothesis of a uniform inoculum density of Verticillium dahliae in four commercial potato fields.

Hypothesis rejected with[1]	size of study areas		
	large[3]	medium	small
neither AV nor FDA	0	4	10
only AV[2]	0	8	3
both AV and FDA	4	7	6
Total	4	19	19

[1]The hypothesis was tested with analysis of variance (AV) and frequency distribution analysis (FDA). In both analyses the hypothesis was rejected for $P < 0.05$. (See Tables 1-3 and text for details).

[2]Rejection of the hypothesis with FDA and not with AV, did not occur.

[3]Results from study areas A, B, C, and D. (See Table 1).

TABLE 5. Patterns of soil inoculum of Verticillium dahliae in the large study areas

study area[1]	number of soil samples	visual rating[2]	spatial autocorrelation analysis[3] I ± SE	P value	rating[2]
A	400	A	.18 .03	<0.001	A
B	100	A	.37 .07	<.001	A
Br	100	A	.23 .07	<.001	A
C	100	A	.65 .07	<.001	A
Cw	50	r	.12 .10	0.18	r
Cr	100	A	.66 .07	<.001	A
Cr,w	50	r	-.09 .10	0.47	r
D	100	A	.45 .07	<.001	A

[1] Study areas from different fields are indicated by different letters. The soil samples were taken from the center of each quadrat, except for the study areas marked "r" for which each sample consisted of 9 cores taken at random throughout the quadrat and bulked. The eastern half of field C had been fumigated and had low populations of V. dahliae. The subscript "w" indicates that the data are shown separately for the western part of the field.

TABLE 5. (continued)

[2]The ratings are A= aggregated; r= random; and R= regular.

[3]I= Moran's coefficient of spatial autocorrelation; SE= standard error of I; P value is for test of hypothesis of no spatial autocorrelation. (See text for details).

TABLE 6. Patterns of soil inoculum of Verticillium dahliae in the medium-size study areas

study area[1]	number of soil samples	visual rating[2]	spatial autocorrelation analysis[3]			
			I	± SE	P value	rating[2]
A1	25	R	-.27	.15	0.12	r
A2	25	A	.32	.15	0.01	A
A3	25	r	-.19	.15	0.32	r
A4	25	r	-.02	.15	0.86	r
A5	25	r	.06	.15	0.52	r
A6	25	R	-.34	.15	0.05	R
A7	25	r	.11	.15	0.31	r
A8	25	A	.28	.15	0.03	A
A9	25	r	-.02	.15	0.90	r
A10	25	A	.04	.15	0.53	r
B1	49	A	.17	.10	0.07	r
B2	49	A	.23	.11	0.02	A
B3	49	r	.13	.11	0.16	r
C1	49	r	-.10	.09	0.40	r
C2	49	r	-.11	.10	0.40	r
C3	49	r	.07	.11	0.40	r

TABLE 6. (continued)

study area[1]	number of soil samples	visual rating[2]	spatial autocorrelation analysis[3] I ± SE	P value	rating[2]
D1	49	A	.13 .10	0.14	r
D2	49	r	.00 .11	0.83	r
D3	49	r	.05 .11	0.48	r

[1] Study areas from different fields are indicated by different letters.

[2] The ratings are A= aggregated; r= random; and R= regular.

[3] I= Moran's coefficient of spatial autocorrelation; SE= standard error of I; P value is for test of hypothesis of no spatial autocorrelation. (See text for details).

TABLE 7. Patterns of inoculum of Verticillium dahliae in the small scale study areas

study area[1]	number of soil samples	visual rating[2]	spatial autocorrelation analysis[3] I ± SE	P value	rating[2]
AA1	25	A	.26 .14	0.04	A
AA2	25	r	-.00 .15	0.81	r
AA3	25	R	-.43 .15	0.01	R
AA4	25	r	-.10 .15	0.70	r
AA5	25	r	-.32 .15	0.07	r
AA6	25	r	-.14 .15	0.50	r
AA7	25	A	.16 .15	0.17	r
AA8	25	r	-.18 .15	0.36	r
AA9	25	r	-.25 .11	0.06	r
AA10	25	r	.09 .14	0.34	r
BB1	25	r	.09 .15	0.38	r
BB2	25	A	.22 .15	0.07	r
BB3	25	r	-.27 .15	0.12	r
CC1	25	r	.19 .15	0.12	r
CC2	25	r	.15 .15	0.19	r
CC3	25	r	-.13 .14	0.52	r

TABLE 7. (continued)

study area[1]	number of soil samples	visual rating[2]	spatial autocorrelation analysis[3] I ± SE	P value	rating[2]
DD1	25	R	-.34 .14	0.03	R
DD2	25	r	-.01 .15	0.83	r
DD3	25	r	.10 .15	0.33	r

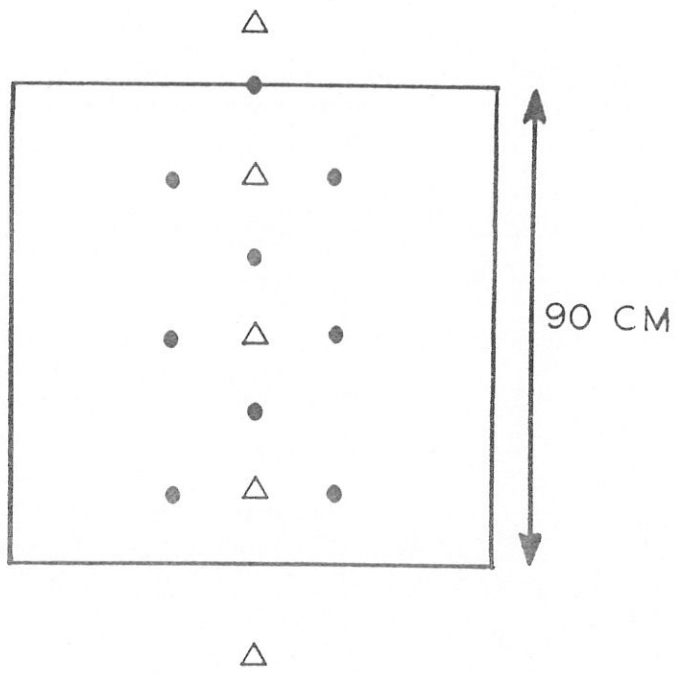
[1]Study areas from different fields are indicated by different letters.

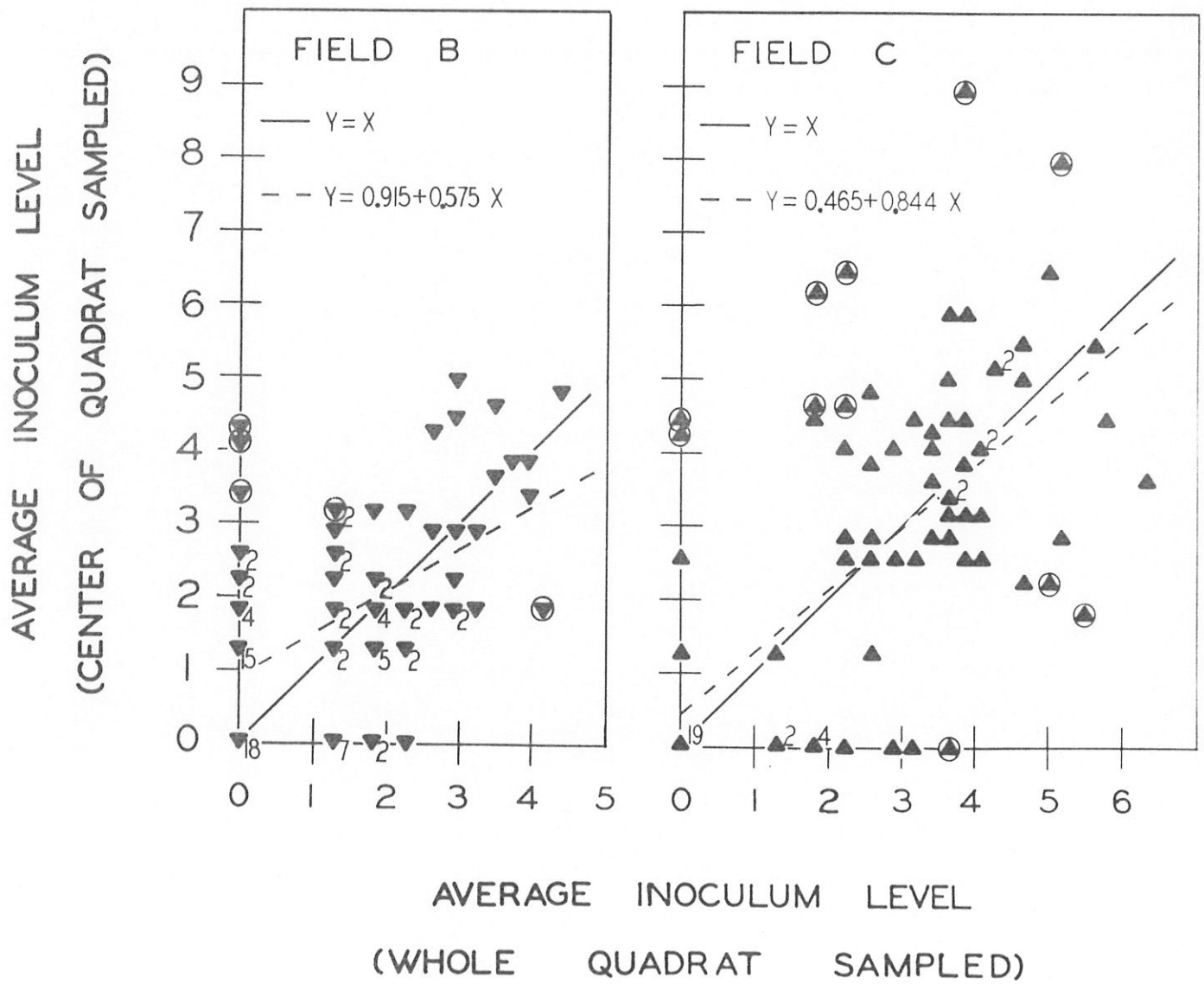
[2]The ratings are A= aggregated; r= random; and R= regular.

[3]I= Moran's coefficient of spatial autocorrelation; SE= standard error of I; P value is for test of hypothesis of no spatial autocorrelation. (See text for details).

Figure 1. Sampling scheme in the large and medium quadrats in 1983. Potato plants are represented by triangles (Δ) and locations of soil cores by circles (o). The nine soil cores taken from each quadrat were bulked into one single sample. A 90cm x 90cm sample area was located at the center of each large quadrat.

Figure 2. Effect of sampling location within quadrat on the estimate of soil inoculum density of *Verticillium dahliae* in the large quadrats of fields B and C. In both graphs, each point represents one of the 100 quadrats of the study area. (If more than one point falls on the same plotting position, the number of points at that position is indicated). The coordinates of each point are the square-root transform of inoculum densities of the fungus, expressed as propagules per gram of soil, in the two soil samples taken from that quadrat. The two soil samples consisted of 9 cores taken at random throughout the quadrat (abscissa) or near the center of the quadrat (ordinate). The circled points indicate quadrats for which the estimates of inoculum density in the two types of samples were significantly different (P=0.05).


90 CM


FIELD B
Y = X
Y = 0.915+0.575 X
FIELD C
Y = X
Y = 0.465+0.844 X
AVERAGE INOCULUM LEVEL
(CENTER OF QUADRAT SAMPLED)
AVERAGE INOCULUM LEVEL
(WHOLE QUADRAT SAMPLED)