Ecology and Epidemiology

Effects of Tillage on the Spatial Pattern of Microsclerotia of *Macrophomina phaseolina*

O. M. Olanya and C. Lee Campbell

Graduate student and associate professor, Department of Plant Pathology, North Carolina State University, Raleigh 27695-7616.
Paper 10,989 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh 27695-7601.
We sincerely appreciate the excellent assistance of Charles R. Harper throughout the study and the helpful comments of H. D. Shew and A. G. Wollum. We also thank Jerry and Bryan Weaver and Joe Powell for their cooperation.
Accepted for publication 7 September 1987 (submitted for electronic processing).

ABSTRACT

Olanya, O. M., and Campbell, C. L. 1988. Effects of tillage on the spatial pattern of microsclerotia of *Macrophomina phaseolina*. Phytopathology 78:217-221.

The effects of soil tillage on the spatial pattern of sclerotia of *Macrophomina phaseolina* were investigated at eight sites in 8-× 8-m grids divided into 64 1-× 1-m contiguous quadrats. One soil core (2.5 × 10–14 cm) was removed from the center of each quadrat before tillage and after one or two diskings. Inoculum density (ID) was determined by assaying a 10-g subsample of air-dried soil using a selective assay procedure. Mean ID was similar among tillage treatments within a site and, based on values of Morisita's index, low to moderate aggregation of inoculum was detected consistently. Aggregation of propagules decreased after one tillage operation at most locations. Physical redistribution of inoculum by disking was most evident in plots with high initial inoculum density and a high degree of initial aggregation of inoculum.

Conventional tillage of soil with plows, discs, chisels, or other implements disturbs the physical, chemical, and biological properties of soil (6,19). Although reduced or no tillage agriculture has gained popularity (18), conventional tillage is still widely used. Research on tillage has been oriented primarily toward studying the physical properties of soil, and each tillage operation alters these properties in a characteristic manner (6). Although crop yield has been used as the primary indication of the impact of tillage on soil properties, recent efforts have sought to characterize and predict the effects of tillage on specific physical properties of soils (5,17).

With respect to biological and biochemical effects, tillage may reduce the stratification of organic residue on the soil surface, which in turn may influence temperature, soil moisture (19) and soil chemistry (2), populations of soil animals, and the structure of microbial communities (9). Some overall changes in the population dynamics of soil microorganisms caused by tillage have been identified, but few investigations have examined the effects of tillage on the spatial pattern or physical arrangement of soil microorganisms. Spatial pattern is a key factor in determining the reliability of inoculum density estimates, in designing field studies, and in assessing the impact of plant disease on crop yield (13,21). Cultivation equipment was identified as a major means of disseminating *Cylindrocladium crotalariae* (Loos) Bell in fields of peanuts rotated with soybeans (12). *Pratylenchus scribneri* Steiner was less dispersed in conventional tillage plots than in zero-tillage plots (untilled), whereas *Hoplolaimus galeatus* (Cobb) Filipjev was more dispersed (1).

Macrophomina phaseolina (Tassi) Goid. is a soilborne pathogen that causes root and stalk rots of over 250 crops throughout the world (22). Recent reports indicate the propagules of *M. phaseolina* are aggregated in field soils (3,13). The ubiquitous nature of the fungus, the fact that it survives in soil primarily as discrete propagules (microsclerotia) (8), and the availability of a selective assay (4) suggested that *M. phaseolina* would be a suitable pathogen for which to investigate the effects of soil tillage on aggregation of soilborne plant pathogens. The purpose of this study was, therefore, to determine the effects of disking of soil on the spatial pattern of *M. phaseolina*. Primary concerns were the effects of disking operations on degree of propagule aggregation and redistribution of propagules in soil. A preliminary report of this study has been published (16).

MATERIALS AND METHODS

Site description. Four field locations in North Carolina were selected: Coolmore Farm (Edgecombe County), two sites; Weaver Farm (Wayne County), two sites; Upper Coastal Plain Agricultural Research Station (Edgecombe County), three sites; and NCSU Research Unit 1 (Wake County), one site. Soil texture was silt loam at NCSU Research Unit 1 and Coolmore Farm 2. All other experimental plots had loamy sand and sandy soil texture (Table 1). Cropping history of the fields was as follows: NCSU Research Unit 1 was fallow in 1983 and 1984. Coolmore farms 1 and 2 were cropped to corn (1983), cotton (1984), and corn (1985). Weaver farms 1 and 2 had corn (1983), tobacco (1984), and corn (1985). Rocky Mount sample 0 was cropped to corn (1983), while samples 1 and 2 were fallow in 1984. Although the fields had been sampled and found to contain microsclerotia of *M. phaseolina*, the occurrence of disease in previous seasons had not been recorded.

Plot establishment, tillage, and sampling. An 8- × 8-m^2 plot containing 64 (1- × 1-m) contiguous quadrats was established at each site. Grids were marked with flags so that the precise location of each plot could be identified after tillage operations. The tillage equipment used was: John Deere 3.66-m tandem disc for Rocky Mount sites 0, 1, and 2; John Deere 7.31-m tandem disc for Coolmore Farm sites 1 and 2; John Deere 7.31-m tandem disc for Weaver Farm sites 1 and 2; and a 3.66-m tandem disc for NCSU Unit 1.

Tillage treatments consisted of 0, 1, or 2 passes over the plots with the disc. The plot was sampled before tillage and after the first and second tillage. The samples were designated tillage 0, 1, and 2, respectively. The direction of implement movement was the same for both tillage operations at a site. The depth of tillage was approximately 15 cm.

For each tillage level (0, 1, or 2), one soil core (2.5 cm diameter × 10–14 cm deep) was removed from the center of each quadrat. Soil samples were placed in plastic bags and transported to the laboratory for analysis. For each experimental site, soil moisture was determined on a dry weight basis (10), and soil texture was determined using the hydrometer method (7). Percent humic material and soil pH were determined by the Agronomic Division of the North Carolina Department of Agriculture.

Assay procedure for identification of *M. phaseolina*. To assay for *M. phaseolina* (4), soil samples were air dried and one 10-g subsample was blended for 5 sec in a high-speed blender with 250 ml of tap water. The contents were dumped onto an 80 mesh sieve (177-μm opening) nested over a 325 mesh sieve (44-μm opening), and the soil was washed for 1 min with running tap water. Residue on the 80 mesh sieve was discarded while contents from the 325 mesh sieve were washed with 0.5% sodium hypochlorite into a plastic beaker. The mixture was stirred on a magnetic stir plate for 8 min. The stir bar was removed, and the contents were dumped onto a 325 mesh sieve and washed with running tap water for 1 min. The contents were emptied into a sterile 250-ml flask. Approximately 100 ml of molten potato-dextrose agar (PDA) was added to the contents. The PDA was cooled by placing bottles in a water bath maintained at 50 C. To inhibit bacterial and fungal contaminants, 3.8 ml of streptomycin sulfate solution (10 g/L of water) and 1.5 ml of chloroneb solution (15.4 g/L of water) were added to the flask. The flask was swirled gently to mix the contents, and the contents of the flask were poured into five sterile petri dishes. Plates were incubated in the dark for 5 or 6 days at 30–32 C. Colonies did not overlap on plates, and the number of colonies per sample was counted and recorded after incubation.

Data analysis. The variance to mean ratio and Morisita's index

TABLE 1. Sample date, soil moisture (%), soil bulk density, soil phase, taxonomic classification, and soil surface texture, percent humic matter (HM), and soil pH at various locations in North Carolina selected for studies on the effect of tillage on the spatial pattern of *Macrophomina phaseolina*

Location	Sample date	Soil moisture at sampling (%)	Bulk density of soil gm/cm^3	Soil phase	Taxonomic classification	Soil surface texture	Sand (%)	Silt (%)	Clay (%)	HM (%)	pH
NCSU Research Unit 1 (Wake County)	9/20/84	1.93	···	Cecil clay loam, 2–6% slopes	Clayey, kaolinitic thermic, typic hapludults	Silt loam	35	51	14	0.4	6.3
Coolmore 1 (Edgecombe County)	3/28/85	14.00	1.08	Wickam sandy loam, 0–4% slope	Fine-loamy, mixed thermic, typic hapludults	Loamy sand	74	23	3	0.2	5.9
Coolmore 2 (Edgecombe County)	3/28/85	12.38	1.75	Wickam sandy loam, 0–4% slope	Fine-loamy, mixed thermic, typic hapludults	Silt loam	35	51	14	0.1	6.4
Weaver 1 (Wayne County)	3/4/85	12.52	1.68	Wagram loamy sand, 0–6% slope	Loamy, siliceous, thermic, arenic paleudults	Sand	89	8	3	0.8	5.6
Weaver 2 (Wayne County)	3/4/85	15.16	1.78	Norfolk loamy sand, 2–6% slope	Fine-loamy siliceous, thermic typic paleudults	Loamy sand	82	14	4	0.9	5.9
Rocky Mount 0 (Edgecombe County)	9/27/84	7.38	1.72	Norfolk sandy clay loam, 0–2% slope	Fine-loamy, siliceous, thermic aquic paleudults	Sandy clay loam	58	16	26	0.3	6.3
Rocky Mount 1 (Edgecombe County)	6/26/85	3.57	1.89	Rains fine sandy loam	Fine-loamy siliceous thermic typic paleudults	Sandy loam	63	30	7	0.5	6.2
Rocky Mount 2 (Edgecombe County)	6/26/85	4.38	···	Rains fine sandy loam	Fine-loamy siliceous thermic typic paleudults	Sandy loam	59	27	14	0.7	6.1

(15,21) were calculated for each tillage level at each location. Morisita's index, I_δ, was computed according to the formula

$$I_\delta = n[(\Sigma(x^2) - \Sigma x)/((\Sigma x)^2 - \Sigma x)]$$

where x is the number of colonies per 10 g of soil in each sampling unit and n is the number of sampling units. The variance and the mean were computed using the Univariate frequency procedure of the Statistical Analysis Systems (20).

Inoculum counts from contiguous grids at each level of tillage were arranged into a frequency table with eight class intervals of equal size between the highest and lowest propagule counts. Five frequency distributions (negative binomial, Poisson, Neyman type A, Thomas double poisson, and positive binomial) were used (11) to attempt fitting of the frequency distribution to the inoculum density counts for data at each level of tillage. Distributions were chosen to represent a range of possible spatial patterns including aggregated (negative binomial, Neyman type A, and Thomas double poisson), random (Poisson), and regular (positive binomial). A chi-square goodness-of-fit test was then applied to the observed and expected values from the frequency distributions to determine if the observed data differed significantly from the fitted probability distribution. A $p \geq 0.05$ indicated that the distribution of sample data did not differ significantly from the tested distribution.

Average mean inoculum densities calculated for each site and at each level of tillage were divided into high and low ID groups. Five locations were placed in the high ID group and three in the low ID group; 44 propagules per 10 g of soil was used as the arbitrary dividing line for the two groups. The correlation between mean inoculum density and Morisita's index in each ID group was calculated for each of the three levels of tillage.

RESULTS

The mean inoculum density was similar for the three treatment levels of tillage at each location; however, variations in mean ID were observed among locations (Table 2). NCSU Research Unit 1, Coolmore sample 2, and Rocky Mount sample 3 had the highest mean inoculum density with a range of 56.2–85.9 propagules per 10 g of air-dry soil. Weaver Farm sample 2 and Rocky Mount sample 2 had medium inoculum density, ranging from 44.3 to 53.8 sclerotia per 10 g of air-dry soil. Weaver Farm sample 1, Rocky Mount sample 1, and Coolmore sample 1 had the lowest mean inoculum density, ranging from 22.5 to 22.8 sclerotia per 10 g of air-dry soil.

The values of the variance to mean ratio and Morisita's index were used as indications of the spatial pattern of *M. phaseolina*. Both indices of dispersion had values greater than 1 (Table 2) indicating that at all locations the propagules of *M. phaseolina* had an aggregated spatial pattern. The degree of aggregation ranged from moderate to low. A decrease in the degree of aggregation, as indicated by decreasing values of the indices of dispersion, generally occurred with each successive level of tillage (Table 2). The variance to mean ratios ranged from 4.3 to 48.3, 3.3 to 36.1, and 2.5 to 31.6 at tillage 0, 1, and 2, respectively. Similar trends were observed for values of Morisita's index. At one location, Rocky Mount 2, values of the variance to mean ratio remained nearly constant, indicating no effect of tillage treatment on aggregation of propagules as measured by the indices of dispersion.

Assessment of the physical redistribution of the sclerotia was demonstrated by isodensity mapping. For example, at NCSU Research Unit 1, in plots with high inoculum density and a relatively high degree of propagule aggregation, redistribution of the sclerotia was particularly evident (Fig. 1), although mean ID did not change. In plots where the inoculum had a lower degree of aggregation, such as Coolmore 2, Weaver 2, Rocky Mount 2, and Rocky Mount 3, physical redistribution was not evident.

The Poisson distribution did not differ significantly from the frequency count data in seven of 24 instances (Table 2). Other data sets were not fitted by any of the tested probability distributions. This lack of fit by probability distributions, taken to indicate spatial aggregation or regularity, may have been due to the number

TABLE 2. Mean and variance, indices of dispersion values, and goodness-of-fit to the Poisson distribution for propagule densities of *Macrophomina phaseolina* with three levels of tillage at eight sites

Farm	Tillage level[w]	Mean[x]	Variance	Indices of dispersion: Variance mean	Indices of dispersion: Morisita's index[y]	Poisson distribution prob. > x[z]
NCSU Research Unit 1	0	81.44	3,928.95	48.25	1.57	0.0001
	1	76.56	2,762.88	36.09	1.45	0.0001
	2	85.91	2,715.48	31.61	1.35	0.0001
Coolmore 1	0	22.50	407.91	18.13	1.75	0.0001
	1	24.83	274.53	11.06	1.40	0.0775
	2	26.14	315.39	12.07	1.42	0.0013
Coolmore 2	0	71.42	468.03	6.55	1.08	0.0327
	1	67.97	476.54	7.01	1.09	0.1150 NS
	2	72.17	346.43	4.80	1.05	0.0002
Weaver 1	0	30.14	274.94	9.12	1.27	0.1046 NS
	1	23.38	128.21	5.48	1.19	0.1762 NS
	2	32.79	130.99	3.99	1.09	0.1642 NS
Weaver 2	0	49.16	291.47	5.93	1.10	0.0370
	1	51.61	294.27	5.70	1.09	0.0345
	2	50.34	124.32	2.47	1.03	0.0001
Rocky Mount 0	0	20.03	111.49	5.50	1.20	0.0001
	1	23.61	178.50	7.60	1.27	0.0891 NS
	2	23.04	82.49	3.50	1.11	0.5199 NS
Rocky Mount 1	0	53.78	231.95	4.31	1.06	0.0026
	1	44.27	191.75	4.33	1.07	0.0001
	2	51.48	221.08	4.29	1.06	0.0356
Rocky Mount 2	0	65.08	446.77	6.87	1.09	0.0006
	1	73.47	245.30	3.34	1.03	0.0008
	2	56.16	279.49	4.98	1.07	0.0031

[w] 0 = Plot not tilled (control); 1 = plot tilled once; 2 = plot tilled second time.
[x] Mean number of propagules of *M. phaseolina* in 10 g of air-dry soil.
[y] All values were greater than 1.00 ($P > 0.05$) according to a chi-square test.
[z] $P \geq 0.05$ indicates that the frequency count data were not significantly different from the Poisson. No frequency count data set was fitted significantly by the negative binomial, Neyman type A, Thomas double poisson, or positive binomial probability distributions.

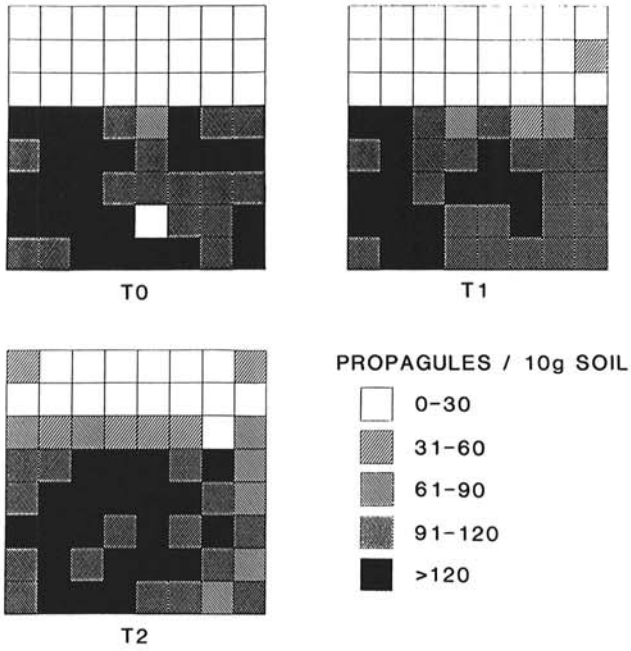

Fig. 1. Maps of propagule density of *Macrophomina phaseolina* in 64 (1-× 1-m) quadrats before (T0), after one (T1), and after two (T2) diskings of soil at NCSU Research Unit 1. Direction of each tillage was from bottom to top of each map.

of 0-class observations or to the particular selection of class intervals for this analysis.

When all eight locations were considered, correlations between mean ID and Morisita's index were negative at all three tillage levels but were not significant. Locations were divided into two groups based on low ($<$ 44 propagules per 10 g of soil) and high ($>$ 44 propagules per 10 g of soil) ID. At the low ID level ($n = 3$), correlations at tillage 0 and 2 were negative but nonsignificant, whereas at tillage 1 the correlation was 0.97 ($p = 0.15$). At the high ID level ($n = 5$), all correlations (r) were positive and were 0.73 ($p = 0.16$), 0.48 ($p = 0.41$), and 0.83 ($p = 0.04$) at tillage 0, 1, and 2, respectively.

DISCUSSION

Little quantitative information is available on the effects of tillage operations on inoculum density, spatial pattern, cluster size, and the magnitude and direction of sclerotial redistribution of soilborne plant pathogens. These effects have several important implications for the study of *M. phaseolina* and other soilborne organisms. Tillage is one of the most important physical phenomena that could alter the apparent ability of a soilborne pathogen to cause disease. If the epidemiological competence of the soilborne pathogen is low, then an increase in dispersal of the pathogen through multiple tillage operations could reduce the likelihood of disease. If, however, the epidemiological competence of the pathogen is high, then dispersal of the pathogen could increase the likelihood of disease by exposing greater numbers of host plants to inoculum.

In our study, a general decrease in the degree of aggregation of *M. phaseolina* was observed with tillage. At five of eight locations values for the variance to mean ratio and Morisita's index decreased between tillage level 0 and at least one other level of tillage. This decrease in aggregation was observed when initial values of Morisita's index were 1.10 or greater and probably resulted from the spread of microsclerotia through soil movement or expulsion of soil debris from the discs. Soil movement by tillage equipment was also observed to break the soil clods and may, thus, have dispersed aggregated propagules. However, if, as at three of eight locations, the propagules were nearly random ($I_\delta < 1.10$) at the outset, either a smaller change or no change occurred in degree of propagule aggregation.

When the direction of spread of inoculum was examined by comparing variances about the mean ID values for rows and columns ($n = 8$) at each site, no consistent trend was discerned for redistribution with versus across the direction of tillage. Lag correlation analysis (14) also did not give a consistent indication as to the extent and direction of redistribution with or across the direction of tillage. Perhaps the plot size was too large or too small in relation to the cluster size of *M. phaseolina* to detect significant lag correlations. No information is currently available on the cluster size for inoculum of this fungus in cultivated field soils.

The mean inoculum density of *M. phaseolina* was similar among the three levels of tillage within each location. Soil samples were taken within 30 min after each tillage operation and were taken to include most of the approximately 15 cm tillage depth. No significant changes would be expected to occur in soil biological properties or nutrient status of the disked layer within this period of time.

In general, more study is required on the effects of tillage on spatial relationships of soilborne pathogens. Our results indicate that the effects of tillage on spatial pattern of *M. phaseolina* are dependent on the initial degree of microsclerotial aggregation. These results are perhaps expected intuitively but have not been previously demonstrated empirically. Knowledge of the effects of multiple tillage operations over a longer period of time would enhance our understanding of how tillage operations affect inoculum density and spatial pattern of soilborne pathogens. The effects of tillage direction in relation to previous plant row orientation on spatial pattern of inoculum and the resulting disease in a row crop should be examined to provide suggestions for a comprehensive cultural management scheme for specific soilborne pathogens. An increased understanding of the effects of soil tillage on spatial pattern of soilborne pathogens could ultimately lead to better strategies for management of root diseases and possibly to a better understanding of the reasons for success or failure of current management strategies.

LITERATURE CITED

1. Alby, T., Ferris, J. M., and Ferris, V. R. 1982. Dispersion and distribution of *Pratylenchus scribneri* and *Hoplolaimus galeatus* in soybean fields. J. Nematol. 15:418-426.
2. Blevins, R. L., Frye, W. W., and Blitzer, M. J. 1980. Conservation of energy in no-tillage systems by management of nitrogen. Pages 14-20 in: Proc. 3rd Annu. No-Tillage Conf. R. N. Gallagher, ed. University of Florida, Gainesville.
3. Campbell, C. L. 1986. Spatial pattern dynamics of propagules of *Macrophomina phaseolina*. (Abstr.) Phytopathology 76:1129.
4. Campbell, C. L., and Nelson, L. A. 1986. Evaluation of an assay for quantifying populations of *Macrophomina phaseolina* from soil. Plant Dis. 70:645-647.
5. Cassel, D. K. 1983. Spatial and temporary variability of soil physical properties following tillage of Norfolk loamy sand. Soil Sci. Soc. Am. J. 47:196-201.
6. Cassel, D. K., and Nelson, L. A. 1985. Spatial and temporal variability of soil physical properties of Norfolk loamy sand as affected by tillage. Soil Tillage Res. 5:5-17.
7. Day, P. R. 1955. Report of the Committee on Physical Analyses (1954–1955). Soil Sci. Soc. Am. Proc. 20:167-169.
8. Dhingra, O. D. 1974. Saprophytic survival of *Macrophomina phaseolina* in soil. Ph.D. thesis. University of Illinois, Urbana-Champaign. 63 pp.
9. Edwards, C. A. 1980. Interactions between agricultural practice and earthworms. Pages 3-12 in: Soil Biology as Related to Land Use Practices. EPA 560/13/80-038. Daniel L. Didal, ed. U.S. Environmental Protection Agency, Washington, DC.
10. Gardner, W. H. 1965. Water content. Pages 82-127 in: Methods of Soil Analysis. C. A. Black, ed. Monograph 9, ASA, Madison, WI. 770 pp.
11. Gates, C. E., and Etheridge, F. G. 1970. A generalized set of discrete frequency distributions with FORTRAN program. Math. Geol. 4:1-24.
12. Krigsvold, D. T., Kenneth, H. G., and Griffin, G. J. 1977. Importance of peanut field cultivation and soybean cropping in the spread of *Cylindrocladium crotalariae* within and among peanut fields. Plant Dis. Rep. 61:495-499.
13. Mihail, J. D., and Alcorn, S. M. 1987. *Macrophomina phaseolina*:

Spatial pattern in a cultivated soil and sampling strategies. Phytopathology 77:1126-1131.
14. Modjeska, J. S., and Rawlings, J. O. 1983. Spatial correlation analysis of uniformity of data. Biometrics 39:373-384.
15. Morisita, M. 1959. Measuring the dispersion of individuals and analysis of distributional patterns. Mem. Fac. Sci. Kyushu Univ. Ser. E. 2:215-234.
16. Olanya, O. M., and Campbell, C. L. 1986. Effects of tillage on spatial pattern of *Macrophomina phaseolina*. (Abstr.) Phytopathology 76:1064.
17. Ojeniyi, S. O., and Dexter, A. R. 1979. Soil structural changes during multiple pass tillage. Trans. Am. Soc. Agric. Eng. 22:168-172.
18. Phillips, R. E., Blevins, R. L., Thomas, G. W., Frye, W. W., and Phillips, S. H. 1980. No tillage agriculture. Science 208:1108-1113.
19. Phillips, R. E., and Young, H. M., Jr. 1973. No-tillage farming. Reiman Assoc., Inc., Milwaukee, WI.
20. SAS Institute, Inc. 1985. SAS User's Guide: SAS GRAPH, G Contour. 1985 Versions, Statistical Analysis Systems Institute, Inc., Cary, NC.
21. Schuh, W., Frederiksen, R. A., and Jeger, M. J. 1986. Analysis of spatial patterns in sorghum downy mildew with Morisita's index of dispersion. Phytopathology 76:446-450.
22. Young, P. A. 1949. Charcoal rot of plants in east Texas. Tex. Agric. Exp. Stn. Bull. 712. 33 pp.