Temporal and Spatial Dynamics of Microsclerotia of *Macrophomina phaseolina* in Three Fields in North Carolina Over Four to Five Years

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**ABSTRACT**


The spatial and temporal dynamics of microsclerotia of *Macrophomina phaseolina* were investigated in three fields planted to common agronomic crops in North Carolina over 4 to 5 yr. Two fields, C-1 and C-2, were divided into 180 contiguous quadrats (6.1 × 6.1 m) arranged in an 18 × 10 grid, and one field, W, was divided into 144 contiguous quadrats (4.6 × 4.6 m) arranged in a 12 × 12 grid. During the period of study, inoculum density ranged from 35.3 to 77.5, 20.4 to 59.7, and 6.4 to 18.0 microsclerotia per 10 g of air-dry soil in C-1, C-2, and W, respectively. In C-1 and C-2, no characteristic seasonal fluctuation or general trend in number of propagules was found. In W, an increase in inoculum density in the first half of each year was generally followed by a decrease in the second half of the year. A small but significant decrease in inoculum density was found over the 4 yr of sampling in W. In each field, the propagules remained aggregated over the years of sampling as indicated by values of Lloyd’s index of patchiness. The degree of aggregation fluctuated, which indicated the existence of factors with opposing effects on the level of aggregation. The level of aggregation usually decreased when the soil was tilled but was also probably affected by nonrandom deaths of propagules. Mean location (e.g., the centroid of occurrence) of the propagules remained nearly constant in each field over the period of study. Spatial correlation analysis and density maps demonstrated the development of new foci near old ones in C-1. In C-2, no new foci formed. In W, the spatial pattern of the microsclerotia was characterized by a relative high density on one side of the field and a relative low density on the other side during the years of sampling. A sensitivity of spatial correlation analysis to extreme values was observed. The observed spatial pattern of microsclerotia of *M. phaseolina* could not be explained by spatial variation in physical and chemical properties of soil.

*Macrophomina phaseolina* (Tassi) Goidanich is a soilborne fungus that causes root and stalk rots on more than 300 crops throughout the world (7). The fungus survives in soil primarily as microsclerotia. Microsclerotia are formed abundantly in the host plant (7), especially under conditions that accelerate maturity of the host plant (30), at high temperatures (8), and during dry periods (8). Mixing of the infected and colonized crop debris through the soil by tillage leads to an increase in inoculum density in the soil (9,22,31). The survival of microsclerotia in soil is variable, and many factors appear to influence the persistence of microsclerotia of *M. phaseolina* (1,5,6,10).

Temporal dynamics of field populations of microsclerotia of *M. phaseolina* have been examined by several researchers (9,21,22,29,31) over periods that varied from 1 to 5 yr. Over 2-, 2.5-, and 4-yr periods of study, an increase in propagule density was found by Mihail (22), Francel et al (9), and Meyer et al (21). No increase in inoculum density was found by Short et al (29) over 2.5 yr in a field in which soybeans were grown, but a characteristic seasonal fluctuation (i.e., an increase from January to April and a decrease from April to July) was found.

The spatial pattern of microsclerotia of *M. phaseolina* is generally aggregated (22,23,25), although at small scales (e.g., within the rhizosphere of maize plants), the pattern of propagules may be nearly random (26). Tillage decreases the level of aggregation of propagules of *M. phaseolina* (25). In a study of the spatial and temporal dynamics of *M. phaseolina* over a period of 2 yr in a field in which the highly susceptible crop *Euphorbia lathyris* was grown, Mihail (22) concluded that continual cultivation redistributes inoculum in such a way that quadrats in close proximity would be less likely to have similar densities of propagules. We are unaware, however, of a study that has examined the spatial and temporal dynamics of propagules of *M. phaseolina* in soil planted with common agronomic crops and over more than a 2-yr period. Thus, in this study the temporal and spatial dynamics of populations of microsclerotia of *M. phaseolina* were examined in naturally infested soils over 4 to 5 yr in three fields with common agronomic crops and standard cultural operations.

**MATERIALS AND METHODS**

Plots, cultivation, and sampling. Three field locations in North Carolina were selected: two fields at the Coolmore farm (Edgecombe County), designated C-1 and C-2; and one field at the Weaver farm (Wayne County), designated W. Crops and cultivation operations are shown in Figure 1. All the crops that were planted were susceptible to infection by *M. phaseolina*. At the Weaver farm, rye was used as a cover crop each winter. At the Coolmore farm, no cover crop was grown between field seasons.

Grids of 180 (18 × 10) contiguous quadrats (6.1 × 6.1 m) were established in fields C-1 and C-2 (crop row spacing, 100 cm), and a grid of 144 (12 × 12) contiguous quadrats (4.6 × 4.6 m) was established in field W (crop row spacing, 90 cm). Grid corners were identified and mapped in relation to permanent landmarks (e.g., irrigation well head and fence posts) to facilitate precise reestablishment of the grid on each sample date. Soil samples were taken 18 times in C-1 from 20 March 1983 to 5 April 1988, 12 times in C-2 from 24 July 1984 to 5 April 1988, and 13 times in W from 12 September 1983 to 16 December 1987. On each sample date, one soil core (2.5 cm in diameter × 10–14 cm deep) was removed from the center of each quadrant. Soil samples were placed in plastic bags, transported to the laboratory, and assayed for *M. phaseolina* (3). On one date in fields C-1 (22 February 1985) and W (4 March 1985), soil moisture (%) was determined gravimetrically for each sample (11). On one sampling date (2 October 1985 for C-1, 30 October 1985 for C-2, and 3 October 1985 for W), a soil sample from each quadrant was submitted to the agronomic division of the North Carolina Department of Agriculture for standard soil analysis; analyses...
were performed for humic matter (%), weight/volume, cation exchange capacity (CEC), base saturation (%), exchangeable acidity (meq/100 cm³), pH, Ca and Mg as percentage of CEC, and indices for relative concentrations of P, K, Mn, Zn, and Cu. At the same time soil samples were submitted to the North Carolina Department of Agriculture for analysis, portions of the soil samples for 12 groups of 15 quadrats each in fields C-1 and C-2 and of 12 quadrats in field W were combined, mixed well, ground in a hammer mill, and oven-dried. Combined samples were used for determination of soil particle-size composition by the hydrometer method (4).

Data analysis. For each sampling date, the mean number of microsclerotia and variance among quadrats were computed with the Univariate procedure of the Statistical Analytical System (28). Lloyd’s index of patchiness (LIP) was calculated for each sampling (15).

The average location of microsclerotia (i.e. the centroid of microsclerotial occurrence) is proposed as a new measure for the examination of the spatial pattern of soilborne pathogens and was determined for each grid of quadrats and sampling time. For this calculation, each row and column was represented by its ordered position from one corner in the field. The row and column numbers in C-1 and C-2 ranged from 1 to 18 and 1 to 16, respectively; and in W, both ranged from 1 to 12. To compute the centroid location, the total number of propagules per row and columns was multiplied by row and column numbers, respectively; then the obtained values were summed over all rows and columns, respectively, and both summations were divided by the total number of propagules.

\[ R = \frac{\Sigma (x_i \times i)}{\Sigma x_{ij}} \]

and

\[ C = \frac{\Sigma (x_j \times j)}{\Sigma x_{ij}}, \]

in which \( R \) is the mean row number of propagule occurrence, \( C \) is the mean column number of propagule occurrence, \( i \) is the row number, \( j \) is the column number, and \( x_{ij} \) is the number of propagules in quadrat \((i,j)\).

Spatial correlation analysis (SCA) (24) was used to analyze pattern density of microsclerotia on each sampling date. The SCA was performed with the software program LCOR2 (12). This program calculates three spatial correlation matrices: \( \rho^l = [\rho(l,k)] \), \( \rho^c = [\rho(l,-k)] \), and \( \rho = \sqrt{\rho^l + \rho^c} \), in which \( l \) and \( k \) indicate the two-dimensional lags in number of rows (across rows) and in number of columns (within rows), respectively, in the rectangular grid of quadrats (Fig. 2). A difference in \( \rho^l \) and \( \rho^c \) indicates a skewness of the spatial pattern in relation to the edges of the field (12, 24).

For each field, Pearson product-moment correlation coefficients were computed between mean number of microsclerotia

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**Fig. 1. Temporal dynamics of Macrophomina phaseolina microsclerotia and cultivated crops over 4 to 5 yr in three fields: A, Coolmore 1, C, Coolmore 2, and E, Weaver. Cultivation operations between sampling dates are indicated by different letters: dl = disk leveling, d = disk, c = chisel plowing, p = planting of crop, and pl = bottom plowing. The direction of soil tillage is given by + (along plant rows) and - (perpendicular to plant rows). The number of times of disk per tillage operation is given in parentheses. Temporal change in the degree of aggregation of microsclerotia of Macrophomina phaseolina as measured by Lloyd’s index of patchiness (LIP) in three fields: B, Coolmore 1, D, Coolmore 2, and F, Weaver. To use a consistent scale, one value (7.24) is off the graph in F.**
Fig. 2. Patterns of spatial lags with significant correlations (P = 0.05) (black squares represent significant positive correlations and lined squares represent significant negative correlations) in the number of microclerotids of *Macrophomina phaseolina* in soil in two fields: A, Coolmore 1 (for six sampling dates) and B, Coolmore 2 (for eight sampling dates). Two-dimensional lags range from (0,−7) in the left upper corner through (0,0) to (0,7) in the right upper corner and from (15,−7) in the left lower corner to (15,7) in the right lower corner. The location (0,0) (empty box in center of first row) represents the quadrat of origin (which is each quadrat in succession during the analysis), and thus, the correlation of values for propagule density for any quadrat and itself is 1.0 by definition. Correlograms are presented for dimension (l,−k) to (l,k) only because of the symmetry of correlations such that $\rho = [\rho(l,k) = \rho(-l,-k)]$ and $\rho = [\rho(l,-k) = \rho(-l,k)]$. 
per quadrat over all sampling dates and the determined physical and chemical properties of soil in each quadrat using the correlation procedure of the Statistical Analytical System (28). The same calculations were made between mean number of propagules per group of 12 or 15 quadrats (described above) and percentage of clay, sand, and silt, as determined in the soil particle-size analysis.

RESULTS

Temporal dynamics of propagule density. During the period of the study, the average number of microsclerotia of *M. phaseolina* per 10 g of air-dry soil varied from 35.3 to 77.5 in C-1, from 20.4 to 59.7 in C-2, and from 6.4 to 18.0 in W. No characteristic seasonal fluctuations or general trends in mean number of microsclerotia was found in C-1 or C-2 over the period of the study (Fig. 1A and C). In W, an increase in the first half of each year was generally followed by a decrease in the second half of the year. A small but significant decrease ($R^2 = 0.40$, $P = 0.05$) in mean number of microsclerotia was found over the 4 yr of sampling in W (Fig. 1E).

Spatial dynamics of propagule density. LIP was greater than one for each sampling date in all three fields, which indicated spatial aggregation of microsclerotia (Fig. 1B, D, and F). In C-1, LIP decreased with spring tillage during the second and third years (1984 and 1985) of sampling and remained relatively stable after that (Fig. 1B). In C-2, LIP was nearly constant until 1987, when it increased and then decreased in 1988 (Fig. 1D). LIP fluctuated greatly in W (Fig. 1F); a clear decrease in the degree of aggregation was observed in 1983 and 1985 after the field had been disked (Fig. 1F). In October 1985, LIP decreased from 7.24 to 1.91 within 1 day after disked.

In all three fields, there was little variation in the mean location or centroid of occurrence of microsclerotia among sampling dates. In C-1 and C-2, the mean location or centroid of occurrence ($R$, $C$) was near the field center (i.e., at 9.5, 5.5). The average location over all sampling dates in C-1 was $R = 9.90 \pm 0.41$ and $C = 5.45 \pm 0.18$. In C-2, the average location over all sampling

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**Fig. 3.** Maps of the density of microsclerotia of *Macrophomina phaseolina* in A, Coolmore 1 and B, Coolmore 2 on three sampling dates.
dates was $R = 9.77 \pm 0.59$ and $C = 5.24 \pm 0.25$. In W, the field center occurred at (6.5, 6.5), but mean location of microsclerotia was $(8.74 \pm 0.42, 6.22 \pm 0.32)$.

Spatial correlograms were examined for all three fields on each sampling date. Skewness of the spatial pattern of microsclerotia in relation to the field edges was shown by differences in $\rho$ and $\rho'$ matrices (Fig. 2). The presence of positively correlated, adjacent quadrats indicated that the size of clusters usually exceeded the quadrant size. The correlation matrices of C-1 were characterized by alternating positive and negative correlation coefficients in diagonal directions (i.e., as correlations are examined beginning at [0,0] and proceeding in a generally diagonal direction to [15,-7] or [15,7]) (Fig. 2A). The average size and shape of the clusters in C-1 varied between sampling dates, as indicated by differences in number and relative location of significantly correlated contiguous quadrats, but no general change in cluster size and shape was observed. Over time, new noncontiguous, significantly correlated elements that represented new inoculum foci appeared in C-1 (for example, between March 1986 and March 1987 and between March 1987 and April 1988 [Fig. 2A]). This is illustrated in Figure 3A as a general trend for the microsclerotial density maps. Correlograms computed for C-2 (Fig. 2B) showed a higher number of positively correlated lags in the length (across rows) than in the width (within rows). The correlogram lag in the length direction of the field were usually interrupted one or two times by one or more noncorrelated lags or on one sampling date (October 1985) by significant negatively correlated spatial lags (Fig. 2B). Thus, there existed one long patch or two or three smaller patches with relatively high propagule density within the field. Exceptions were the first sampling date (July 1984), when the pattern of spatial lag correlations showed a significantly correlated element near the lower left corner (Fig. 2B), and the last sampling date (April 1988), when spatial lag correlations indicated the occurrence of one, more or less circular, cluster (Figs. 2B and 3B). Spatial correlation matrices of W were not consistent. The spatial pattern of microsclerotia showed approximately one-half of the field with relative high densities and the other half with low densities for all sampling dates (Fig. 4).

The soil in the three fields varied in physical and chemical properties. For example, clay ranged from 5.5 to 11.1% in C-1, 3.5 to 11.1% in C-2, and 0.8 to 4.6% in W. Correlation coefficients between number of microsclerotia and physical and chemical properties of soil were generally low. Significant ($P = 0.05$) correlations were found, but correlation coefficients were not consistently positive or negative in all three fields, except for the index for Mn. Correlation coefficients between number of microsclerotia and index for Mn were negative and low, i.e., $-0.38, -0.15$, and $-0.20$ in C-1, C-2, and W, respectively.

**DISCUSSION**

Temporal dynamics of propagule density. Mean density in the three fields studied in North Carolina did not exceed 80 microsclerotia per 10 g of soil (Fig. 1). Frankel et al (9) found similar densities, but in other field studies with *M. phaseolina*, inoculum densities were about one order of magnitude higher (21,22,29,31).

Young and Alcorn (31) and Mihail (22) studied the dynamics of microsclerotia of *M. phaseolina* in fields planted with the highly susceptible crop *E. lactuca* and observed an increase in inoculum density in the soil of one order of magnitude or even more after one (31) or two growing seasons (22). The temporal dynamics of microsclerotia in fields in which soybean (21, 29) or soybean and other crops (9) were grown showed less extreme fluctuations in inoculum density and were comparable to those found in this study (i.e., the highest densities were approximately two to three times higher than the lowest).

The temporal dynamics of the microsclerotia in soil did not show characteristic seasonal fluctuations, nor did they show a general increase or decrease over the 4 and 5 yr of sampling in C-1 and C-2, respectively. In one field (W), a small but significant decrease was observed (Fig. 1). On the basis of these observations and of the relative low inoculum density, we conclude that conditions were not conducive to the formation of microsclerotia. Charcoal rot caused by *M. phaseolina* was generally of minor importance in 1983–1987 according to crop loss reports in North Carolina (16–20).

**Spatial dynamics of propagule density.** Microsclerotia of *M. phaseolina* were aggregated at each sampling date. Spatial aggregation of propagules of *M. phaseolina* has been found by others (2,22,23,25) and is generally found for propagules of most soilborne pathogens (13). It should, however, be emphasized that the degree of aggregation detected may depend on the size of the quadrat. Olanya and Campbell (26), for example, found a nearly random pattern for propagules of *M. phaseolina* with relatively small quadrats of $15 \times 15$ and $18 \times 18$ cm. Mihail (22) and Mihail and Alcorn (23) found aggregation of propagules with quadrats of $1.5 \times 3.0$ m and $1.02 \times 0.3$ m, respectively. Olanya and Campbell (25) observed a low to moderate degree

![Fig. 4. Map of the density of microsclerotia of *Macrosporina phaseolina* in Weaver on three sampling dates. Because the density is lower in Weaver than in Coolmore 1 and 2 (Fig. 3), a different scale for propagule density is used for Weaver.](image)
of aggregation with 1 × 1 m quadrats at eight locations. In our study, we also found consistent aggregation of propagules with quadrats of 4.6 × 4.6 m (W) and 6.1 × 6.1 m (C-1 and C-2). The concept of geographic scale and degree of aggregation of propagules of microsclerotia-forming pathogens should be further addressed in future research.

The spatial pattern of microsclerotia in the soil remained aggregated; however, fluctuations in degree of clustering indicated the occurrence of factors with apparently opposing effects on the level of aggregation. In C-1, LIP decreased after spring tillage operations (disking, levelling, and chisel plowing) in 1984 and 1985 (Fig. 1B). In W, high levels of clustering (LIP > 3) clearly decreased after tillage operations (disking and chisel plowing) (Fig. 1F). These observations indicated dispersal of microsclerotia by tillage of the soil, as was suggested by Mihail (22) and Mihail and Alcorn (23) and demonstrated by Olanya and Campbell (25). An increase in LIP was generally associated with a decrease in inoculum density in W (Figs. 1E and 2F). Because LIP is not affected by random events (27), we suggest the occurrence of nonrandom death of microsclerotia in C-2, the opposite was observed; i.e., a relatively large increase in LIP was associated with an increase in inoculum density in 1987 (Fig. 1C and D). Thus, the nonrandom death or increase of microsclerotia in the field appeared to counteract the process of randomization by the mixture of inoculum through the soil by tillage. The indications of the role of nonrandom death of inoculum support the conclusion of Mihail (22) that spatiotemporal changes in inoculum density of M. phaseolina can be viewed as an autoregressive process; i.e., changes in inoculum density within a quadrat are dependent spatiotemporally on factors within that quadrat. (Changes in inoculum density pattern were described as a pure moving average spatiotemporal transfer function by Mihail (22) on the basis of nonsignificant autocorrelation and significant partial autocorrelation coefficients; however, this unintentional error has been corrected subsequently.)

Correlograms and density maps identified the appearance of new foci in C-1 (Figs. 2A and 3A). New foci arose near old ones; they probably developed through a combination of the spread of microsclerotia from existing foci and the local differences in increase and/or decrease of inoculum. In C-2, inoculum was more evenly distributed than in C-1 at the initiation of the study, and no new foci were detected during the 4 yr of sampling. The pattern of significantly correlated spatial lags in C-2 on the first assessment date (July 1984) showed a positively correlated element near the lower left corner, which was not detected by SCA for the other 11 assessment dates (Fig. 2B). An extremely high value of the estimated number of propagules (298 microsclerotia per 10 g of soil) in the quadrat in the lower left corner of the field accounted for this pattern. The positively correlated element near the lower left corner consisting of seven spatial lags (Fig. 2B) disappeared when this high value was replaced by a value (which ranged from 12 to 44) of the same order as in the same quadrat on the other 11 assessment dates.

The spatial correlation model of Modjeska and Rawlings (24) assumes that all pairs of observations that have the same spatial relationship have the same correlation. However, this assumption should not be expected to hold true for most plant diseases or patterns of inoculum. On the basis of the computed autocorrelation coefficient, the mean correlation between points with a certain spatial relationship and, therefore, estimates of the spatial correlation coefficient ($\rho_L$) are sensitive to extreme values in individual quadrats especially over large lag distances (large $I$ and $k$). An extreme value in only one quadrat has significant consequences for the magnitude of the autocorrelation coefficients for several spatial lags. Moran's $I$ statistic of autocorrelation, which is quite similar to the Modjeska and Rawlings correlation coefficient, also is sensitive to extreme values (14). The large differences in correlograms computed for W among sampling dates (data not shown) can be ascribed to the sensitivity of SCA to extreme values as well. The estimated number of propagules in some quadrats fluctuated greatly between assessment dates. Thus, in W, SCA did not contribute to the description of the spatial pattern and spatial dynamics.

The calculation of the average location of inoculum is a new approach in the examination of the spatial attributes of soilborne pathogens, which, in conjunction with density maps, was useful in the analysis of the spatial pattern of M. phaseolina. In W, the calculation of the centroid of propagule occurrence and the maps illustrated clearly that the clusters of inoculum were not distributed equally over the field but rather were concentrated at one side of the field on all sampling dates. Tillage operations performed predominantly in one direction, i.e., parallel to the plant rows (Fig. 4), probably contributed to this situation. In C-1 and C-2, where the centroid of propagule occurrence was consistently near the field center, the change in tillage direction between along plant rows and perpendicular to plant rows probably contributed to the fact that the centroid location was maintained. Mihail and Alcorn (23) explained a gradient in microsclerotia in a field by irregular deposition of soil and debris by flooding and subsequent movement of the soil by disk and leveling. However, in cases when conditions are very favorable for an increase in propagule density of M. phaseolina, i.e., when even low inoculum densities can cause high incidence of disease and a subsequent large increase in inoculum density, movement of the soil by tillage may be of less importance in the enlargement of old foci and the development of new ones than in cases that are less favorable for inoculum increase.

Except for the index for Mn, when a small negative correlation was found in each field, no consistent correlations between number of microsclerotia of M. phaseolina and physical and chemical properties of soil (soil bulk density $[\%]$, soil particle size, humic matter $[\%]$, weight/volume, CEC, base saturation $[\%]$), acid [meq/100 cm$^3$], pH, Ca and Mg as percentages of CEC, and indices of P, K, Zn, and Cu) were found in the three fields we studied. Thus, in our study, spatial variation in chemical and physical properties of soil could not explain the observed patterns of propagules. In each field, the observed pattern of microsclerotia of M. phaseolina was likely a result of unintentional contamination of the soil by microsclerotia or by infested plant debris and subsequent cultivation.

**LITERATURE CITED**


