# *Macrophomina phaseolina*: Spatio-Temporal Dynamics of Inoculum and of Disease in a Highly Susceptible Crop

J. D. Mihail

Department of Plant Pathology, University of Arizona, Tucson 85721. Present address, Department of Plant Pathology, University of Missouri, Columbia 65211.

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## ABSTRACT

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The spatio-temporal dynamics of microsclerotia of *Macrophomina phaseolina* were investigated over 2 yr in a field plot of 100 contiguous quadrats (1.52 × 3.05 m), arranged in a 10 × 10 grid. The population of microsclerotia increased from a preplant population of 8.9 microsclerotia per gram to 98.6 per gram after two successive crops of the latex-producing plant *Euphorbia lathyris*. Inoculum remained spatially aggregated over the 2 yr as measured by Morisita's index of dispersion. However, the large patch of quadrats with a similar inoculum level, demonstrated by the high-order positive autocorrelation measured by Moran's *I* statistic, was not detectable at the end of the second growing season, largely as a result of continuing cultivation operations during the first fallow period. Charcoal rot symptom development and mortality were strongly influenced by the timing of host reproduction and unaffacted by plant density. Unexpectedly, during the second epidemic, symptom expression declined following a period of cool weather. During both epidemics, a large patch of high charcoal rot mortality was visually evident and statistically confirmed by the high-order positive autocorrelation as measured by Moran's *I* statistic. Spatio-temporal autocorrelation analysis was used to find a model that would account for the observed temporal changes in mortality patterns for the two epidemics and in inoculum density patterns during the two fallow periods.

*Macrophomina phaseolina* (Tassi) Goid. is a soilborne fungus causing the charcoal rot disease of a wide variety of hosts in the arid and semiarid areas of the world (10). Disease development is generally considered to be enhanced by some combination of heat stress, soil-water deficit, light-textured soil, or the stress associated with host reproduction (10). In research with charcoal rot of sorghum (*Sorghum vulgare* Pers.), the coincidence of moisture stress, high temperatures, and seed production were found to be essential for disease development (11,12,27). Male sterile plants were found to have a high level of root infection without symptom expression (27), and one early-maturing cultivar was found to be particularly susceptible (18). In work with soybean (*Glycine max* (L.) Merr.), Wyllie and Calvert (36) demonstrated that sclerotial development in host tissues, but not infection, could be averted by flower removal, regardless of soil-moisture status. Similarly, recent studies of charcoal rot of sunflower (*Helianthus annuus* L.) have confirmed the primary importance of flowering in symptom expression (3,9,17). While various stresses favor symptom development and eventual mortality, high soil temperatures were found to inhibit early root infection of cantaloupe (*Cucumis melo* L.) by the pathogen (4). The role of plant density has been only infrequently examined. In work with sunflower (16), peanut (*Arachis hypogaea* L.) (2), and guayule (*Parthenium argentatum* A. Gray) (26), disease incidence was inversely related to plant density.

The effect of susceptible crops on inoculum levels of *M. phaseolina* and on inoculum dynamics has been demonstrated in several studies with soybeans (14,20,29). Meyer et al (20) found that soil populations were 80 or 160 microsclerotia per gram in fields that were planted in soybean monocultures for 1 or 5 yr, respectively. Similarly, Francl et al (14) found that as soybeans appeared more frequently in a 5-yr rotation, populations of microsclerotia of *M. phaseolina* were consistently higher. A similar increase in soil population after a sunflower crop has been noted (1). The spatial pattern of microsclerotia of *M. phaseolina* has been a subject of recent consideration (6,22,28). The spatial pattern in an Arizona field soil was found to be highly aggregated and to exhibit a gradient of low to high values across the experimental site (22). The existence of a gradient was demonstrated by the examination of the behavior of Moran's *I* statistic of autocorrelation in a correlogram (22,23). In studies with corn (*Zea mays* L.) and cotton (*Gossypium* sp.), aggregation of microsclerotia was found to increase after planting (6) and decrease after cultural operations (22).

*Euphorbia lathyris* L. is a latex-producing plant that has received attention during the past decade as a potential renewable source of petrochemical-like hydrocarbons (5,25). Although it was anticipated that this plant could be grown profitably in the arid southwestern United States (25), experimental plantings in California and Arizona were severely affected by charcoal rot during the hot, dry summer months (32,37,38). In one experimental planting, a population of *M. phaseolina* of less than 1 microsclerotia per gram was sufficient to cause greater than 90% mortality (38). The authors of the study also observed that the inoculum level remained stable during the growing season (1–2 microsclerotia per gram), increased to 246 microsclerotia per gram within 3 mo of incorporation of plant residue into the soil, and declined to 70 microsclerotia per gram 1 mo later (38). Recently, charcoal rot of *E. lathyris* (15) and of *E. antisyphilitica* Zucc. (33), a wax-producing species, has been reported from India.

The first objective of the present study was to examine the spatio-temporal dynamics of microsclerotia of *M. phaseolina* in the presence of a highly susceptible crop. This analysis included the use of the spatio-temporal autocorrelation programs developed and supplied by Reynolds and Madden (30). These techniques have been used to describe the spatio-temporal patterns of epidemics of leather rot of strawberry (*Fragaria* × *ananassa* Duch.) (31) and of several tobacco (*Nicotiana tabacum* L.) virus disease epidemics caused by two potyviruses (19). The second objective was to examine several factors affecting the incidence of charcoal rot of *E. lathyris* that might be manipulated to reduce mortality; these included plant density, timing of flower development, and soil temperature.

## MATERIALS AND METHODS

**Soil collection and processing.** All field work was conducted at the University of Arizona's Campbell Ave. Farm in Tucson. Field soil was a loam (51.9% sand, 33.0% silt, and 15.1% clay) with a pH of 7.25. The study plot (15.24 × 30.48 m) was divided into a 10 × 10 grid of nonoverlapping quadrats, each 1.52 × 3.05 m. On 15 September 1986 (day of year 258 = Julian day [JDay] 258), before planting, a single, centrally located soil sample was removed from each quadrat to a depth of 25 cm. The population of microsclerotia of *M. phaseolina* was determined by the assay of a single 10-g subsample from each soil sample, using the technique and semiselective medium previously described (21). During 1987 and 1988, soil samples were taken periodically from all quadrats after the incorporation of crop residue into the soil. Over the course of the study, as the population of *M. phaseolina* increased, the size of assayed subsamples was decreased so that distinct colonies could be identified on assay plates. In 1987, soil samples were taken on 10 July (JDay 191), 30 July (JDay 211), 19 August (JDay 231), and 8 September (JDay 251). Subsamples of 2 and 1 g were assayed for the first three and the last sampling dates, respectively. In 1988, soil samples were taken on 21 June (JDay 173), 12 July (JDay 194), 2 August (JDay 215), 23 August (JDay 236), 13 September (JDay 257), and 4 October (JDay 278). Subsamples of 1 and 0.5 g were assayed for the first and remaining sampling dates, respectively.

**Disease assessment.** During the two growing seasons (1986-1987, 1987-1988), *E. lathyris* was planted in the fall and charcoal rot development was assessed during the following spring and summer. On 2 October (JDay 275) of both years (1986 and 1987), seeds were hand-planted on raised beds 31 m long, on 1.02-m centers. On 11 March 1987 (JDay 70), the number of plants in each quadrat was recorded and the first disease assessment was made. On 17 April 1987 (JDay 107), all plants flowering at that time were flagged as the "early flowering" cohort. Disease assessments for all plants in the plot were also made on 31 March (JDay 90), 15 April (JDay 105), 29 April (JDay 119), 13 May (JDay 133), 28 May (JDay 148), 10 June (JDay 161), and 25 June (JDay 176). On each assessment date, the number of symptomatic and dead plants (both in the early flowering cohort and in the general population) were noted for each quadrat. By the last assessment date, average mortality was 65%, and on 10 July 1987 (JDay 191), all plants were incorporated into the soil by disking. Normal cultivation operations for weed control were continued during the summer and late fall throughout the entire field containing the study plot, such that plant residue might be moved outside the study site, and soil from bordering areas might be brought in. During this fallow period, soil samples were periodically removed from quadrat centers, as described above.

During the 1987-1988 growing season, the experimental design was modified to explore more closely the effects of plant density and flowering status on disease development. On 25 February 1988 (JDay 56), alternating quadrats (both within and across rows) were thinned to 13.1 plants per meter (high density) or 6.6 plants per meter (low density), such that a checkerboard pattern of alternating high- and low-density quadrats was produced. These densities were selected to represent the 66th and 12th percentiles of plant density, respectively, during the 1986-1987 growing season. The first flowering was observed on 14 March 1988 (JDay 74), and the first flowering cohort was tagged 3 wk later on 4 April (JDay 95). This cohort consisted of all plants that had flowered between JDay 74 and JDay 95 in 60 contiguous quadrats (30 high-density and 30 low-density). The second and third flowering cohorts flagged comprised those plants that flowered from 5 April (JDay 96) to 25 April (JDay 116), and from 26 April (JDay 117) to 16 May (JDay 137), respectively. Throughout the text of this article, the term "late-flowering/nonflowering cohort" of the 1987-1988 growing season refers to those plants in the 60 quadrats that had not specifically been tagged as part of a flowering cohort on the date of reference. The plants in the 40 quadrats not used in this portion of the study were included in those portions of the study not specifically dealing with flowering status. Disease assessments were made on 9 March (JDay 69), 24 March (JDay 84), 5 April (JDay 96), 11 April (JDay 102), 26 April (JDay 117), 6 May (JDay 127), 16 May (JDay 137), 25 May (JDay 146), 6 June (JDay 158), and 15 June (JDay 167). On each assessment date, the number of symptomatic and dead plants was recorded for each flowering cohort (where applicable) in each quadrat. Charcoal rot of *E. lathyris*, beginning as chlorosis of lower leaves, progresses acropetally, eventually resulting in plant death. By the last assessment date, average mortality was 57%, and all plants were incorporated into the soil by disking. Normal cultivation operations and soil sampling continued through the summer and fall of 1988 as described for 1987 above.

During the 1986-1987 and 1987-1988 growing seasons, soil temperatures were measured at 5 and 20 cm, using a thermistor connected to a Campbell Scientific CR21 micrologger (Campbell Scientific, Inc., Logan, UT).

**Data analysis.** Standard statistical tests such as chi-square, Student's *t*-test, linear regression, and correlation analysis were performed using the SPSS/PC+ software package (SPSS Inc., Chicago, IL). Inoculum aggregation was assessed by the degree to which the value of Morisita's index of dispersion exceeded a value of one (13,22,24). Moran's *I* statistic of autocorrelation was used to describe the spatial patterns of inoculum and of mortality (7,8,22,23). Initially, the statistic was calculated for quadrats with shared sides and weighted proportionally to the length of the shared sides. In this case, positive values of *I* indicated adjacent quadrats with similar variate values (positive autocorrelation). To further characterize these spatial patterns, variate values (population of microsclerotia, mortality) in each quadrat were compared with variate values of quadrats increasingly distant (22,34,35). In this case, quadrats were considered joined if the distance between their centers fell within the particular distance class. In this analysis, binary weights were used. The *I* statistics were then plotted against distance, and the shape of the resulting correlogram was used to describe the spatial pattern.

The changes in inoculum density patterns and in charcoal rot-induced mortality patterns were characterized by the spatio-temporal autocorrelation techniques described by Reynolds and Madden (30). The goal of this effort was to identify models to account for the observed temporal changes in spatial patterns. Such models have been termed spatio-temporal transfer functions (STFs) (30). The computer programs used were those developed and supplied by Reynolds and Madden (30). For the analyses of inoculum density patterns, the rook's definition of spatial proximity was used on natural-log-transformed data for each fallow period (1987 and 1988). This data transformation markedly decreased variance heterogeneity. For all analyses of inoculum density data, binary weights were used. Then, spatial, temporal, or simultaneous spatial and temporal differencing were applied to transformed inoculum density data using the rook's definition of spatial proximity. Three levels of spatial lag were used for all analyses, and two and three levels of temporal lag were used for inoculum density data for 1987 and 1988, respectively. For the analyses of mortality patterns, both the rook's and queen's definitions of spatial proximity were used. For each proximity assumption, the analysis was performed for nontransformed mortality data and for arcsine transformed data. For all analyses, binary weights were used. Finally, simultaneous spatial and temporal differencing was applied to nontransformed mortality data, using the rook's definition of spatial proximity. All analyses of mortality data used three levels of spatial and temporal lag. For a more comprehensive explanation of these assumptions and STFs, the reader is directed to the discussion of Reynolds and Madden (30).

## RESULTS

**Dynamics of populations of microsclerotia of *M. phaseolina*.** The average population of microsclerotia of *M. phaseolina* increased from 8.9 microsclerotia per gram (JDay 258, 1986) at

the beginning of the study to 42.2 microsclerotia per gram (JDay 251) at the end of the 1987 fallow period (Fig. 1B). During the 1988 fallow period, the population fluctuated, with the highest average population recorded for JDay 194 (98.6 microsclerotia per gram), an increase of one order of magnitude over the initial population (Fig. 1B). Morisita's index of dispersion (13,24), calculated to assess the degree of aggregation in the population of microsclerotia, varied between 1.18 and 1.50 and was statistically greater than one for each sampling date ($P \leq 0.01$), indicating significant aggregation of inoculum. Moran's index of autocorrelation ($I$) was 0.6 ($P \leq 0.001$) for adjacent quadrats at the beginning of the study (JDay 258, 1986), indicating similar inoculum densities in adjacent quadrats (Fig. 1A). During the 1987 fallow period, a decrease in the autocorrelation between adjacent quadrats was reflected in declining values of Moran's

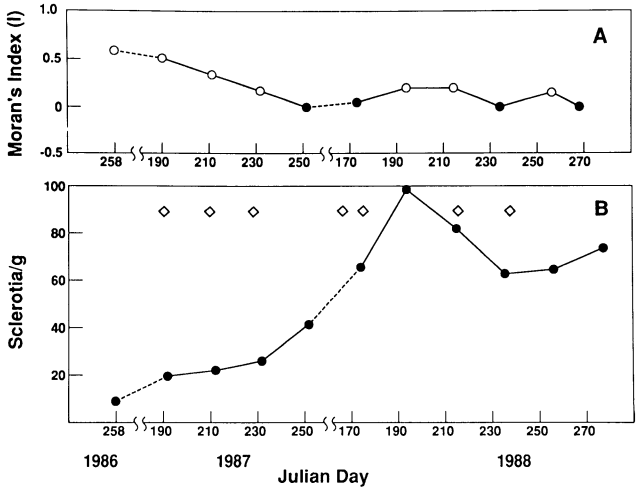


**Fig. 1.** Dynamics of the population and pattern of microsclerotia of *Macrophomina phaseolina*. **A,** Temporal fluctuation in Moran's index of autocorrelation ($I$) over 2 yr for adjacent quadrats. Open symbols indicate index values statistically different from zero ($P \leq 0.05$). **B,** Average population of microsclerotia for 100 quadrats. Broken lines denote growing season for *Euphorbia lathyris* during which no soil samples were taken. Open diamonds indicate cultivation operations across study site. Julian Day is equivalent to day of year.

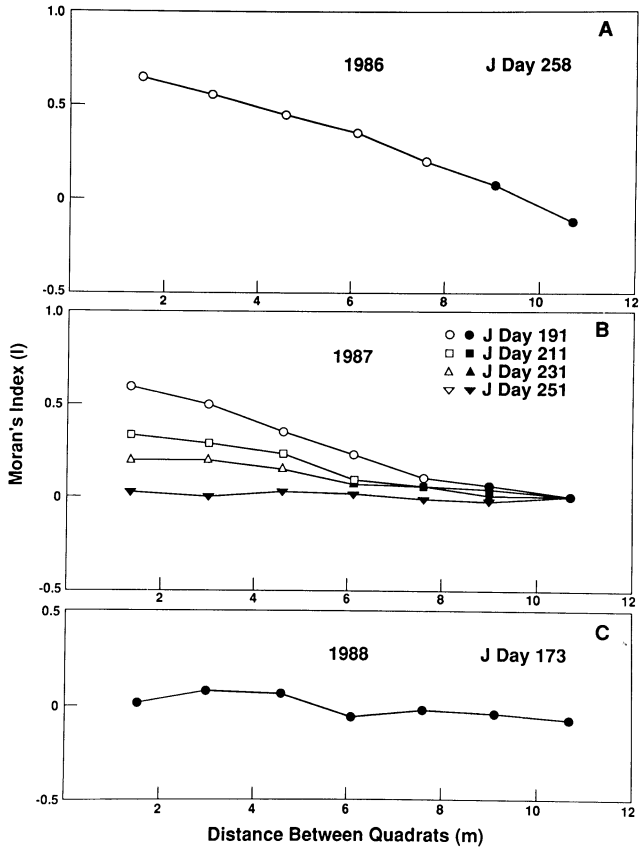


**Fig. 2.** Temporal change in the spatial pattern of microsclerotia of *Macrophomina phaseolina* as measured by Moran's index of autocorrelation ($I$) for quadrats increasingly distant. Open symbols indicate values of $I$ statistically different from zero ($P \leq 0.05$). Day of year is JDay (Julian Day).

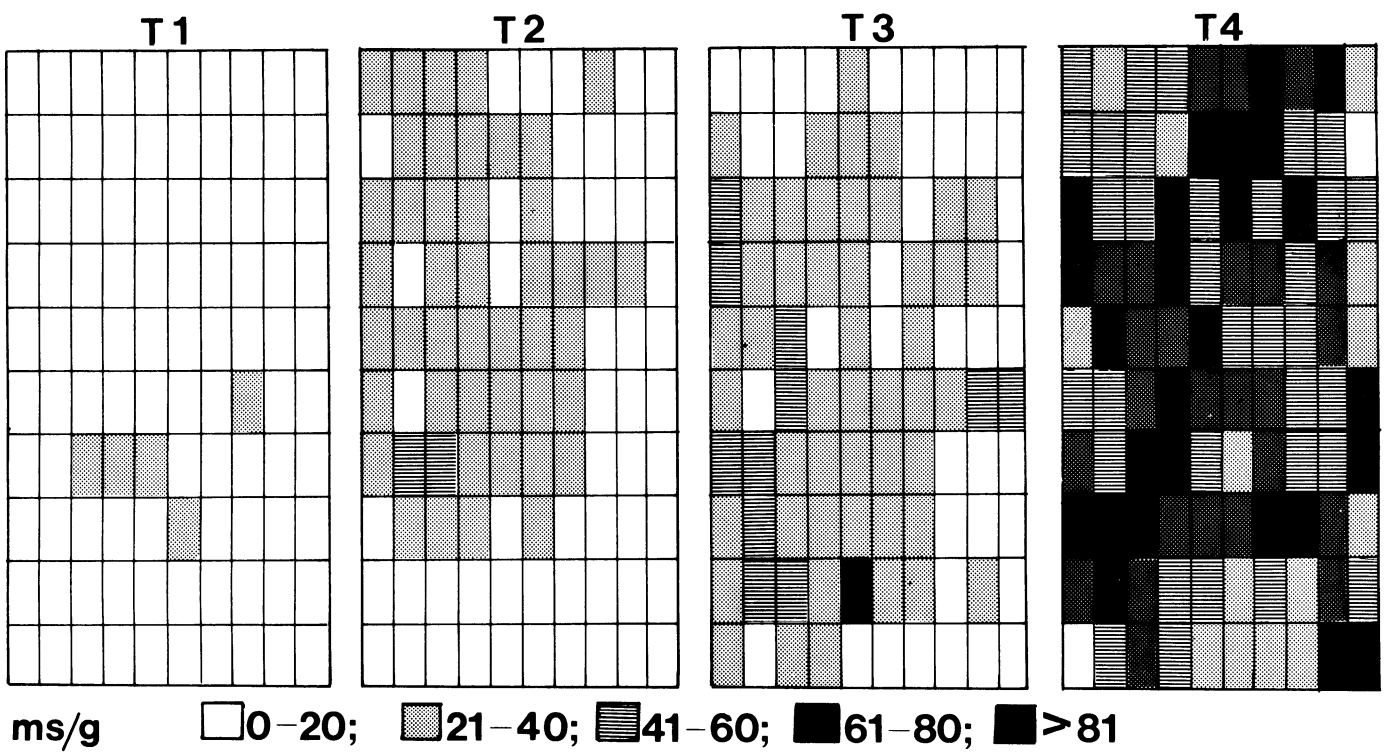


**Fig. 3.** Spatial pattern of microsclerotia of *Macrophomina phaseolina* on four sampling dates. T1 = Day 258, 1986; T2 = Day 191, 1987; T3 = Day 231, 1987; T4 = Day 173, 1988. ms/g = microsclerotia per gram of soil.

*I*. In contrast, during the 1988 fallow period, there was only a low degree of autocorrelation between adjacent quadrats, as illustrated by significantly positive *I* values on three of the six sampling dates (Fig. 1A). The spatial pattern of microsclerotia of *M. phaseolina* was further characterized by comparing quadrats increasingly distant, using Moran's index (Fig. 2). At the beginning of the study (Fig. 2A), Moran's *I* was significantly positive for quadrats up to 8 m distant (high order positive autocorrelation), indicating a large patch of similar values (34,35). The decrease in patch size during the 1987 fallow period is illustrated by the decreasing distance over which Moran's *I* was statistically different from zero (Fig. 2B). The correlogram for the first soil sampling date in 1988 (Fig. 2C) illustrates the typical lack of autocorrelation among quadrats during this second fallow period, which indicates that quadrats with low and high populations of microsclerotia of *Macrophomina* were randomly proximal. This change in the spatial pattern of the microsclerotia of *M. phaseolina* is illustrated by the spatial pattern maps for selected sampling dates (Fig. 3).

Spatio-temporal autocorrelations and partial autocorrelations for nondifferenced inoculum density data are summarized in Table 1. The slow decline of autocorrelations with increasing spatial and temporal lag suggested a nonstationarity of level in these data (30). That is, the expected average inoculum density would not be the same for each combination of spatial and temporal lag order. When the analyses were redone using spatial, temporal, or simultaneous spatial and temporal differencing, the simplest

TABLE 1. Estimated spatio-temporal autocorrelations for inoculum density of *Macrophomina phaseolina*, using binary weights, natural log transformation, and the rook's definition of spatial proximity

| Year | Temporal lag order | Autocorrelations[a] for spatial lag order | | | | Partial autocorrelations[a] for spatial lag order | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 0 | 1 | 2 | 3 |
| 1987 | 1 | 0.37** | 0.44*** | 0.35** | 0.27* | 0.08 | 0.19** | 0.07 | 0.10 |
| | 2 | 0.28* | 0.30* | 0.21 | 0.17 | 0.02 | 0.02 | −0.11 | −0.04 |
| 1988 | 1 | 0.26** | 0.29** | 0.16 | 0.08 | 0.10 | 0.16** | 0.08 | 0.09 |
| | 2 | 0.25* | 0.16 | −0.01 | −0.06 | 0.16** | −0.02 | −0.12* | −0.15** |
| | 3 | 0.26* | 0.19 | 0.07 | −0.02 | 0.17** | 0.03 | 0.03 | −0.05 |

[a]*, **, and *** indicate that the coefficient is different from 0 at $P \leq 0.05$, 0.01, and 0.001, respectively.

TABLE 2. Estimated spatio-temporal autocorrelations for inoculum density of *Macrophomina phaseolina* using binary weights, natural log transformation, the rook's definition of spatial proximity, and spatial differencing

| Year | Temporal lag order | Autocorrelations[a] for spatial lag order | | | | Partial autocorrelations[a] for spatial lag order | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 0 | 1 | 2 | 3 |
| 1987 | 1 | −0.02 | 0.16 | −0.02 | 0.06 | 0.09 | 0.18* | 0.05 | 0.09 |
| | 2 | 0.05 | 0.02 | −0.14 | −0.02 | 0.01 | 0.00 | −0.14* | −0.05 |
| 1988 | 1 | 0.01 | 0.11 | 0.01 | −0.02 | 0.08 | 0.12* | 0.01 | −0.02 |
| | 2 | 0.14 | 0.02 | −0.02 | −0.03 | 0.15* | 0.06 | −0.07 | −0.09 |
| | 3 | 0.12 | 0.01 | 0.02 | 0.05 | 0.11 | 0.06 | 0.03 | 0.03 |

[a]* indicates that the coefficient is different from 0 at $P \leq 0.05$.

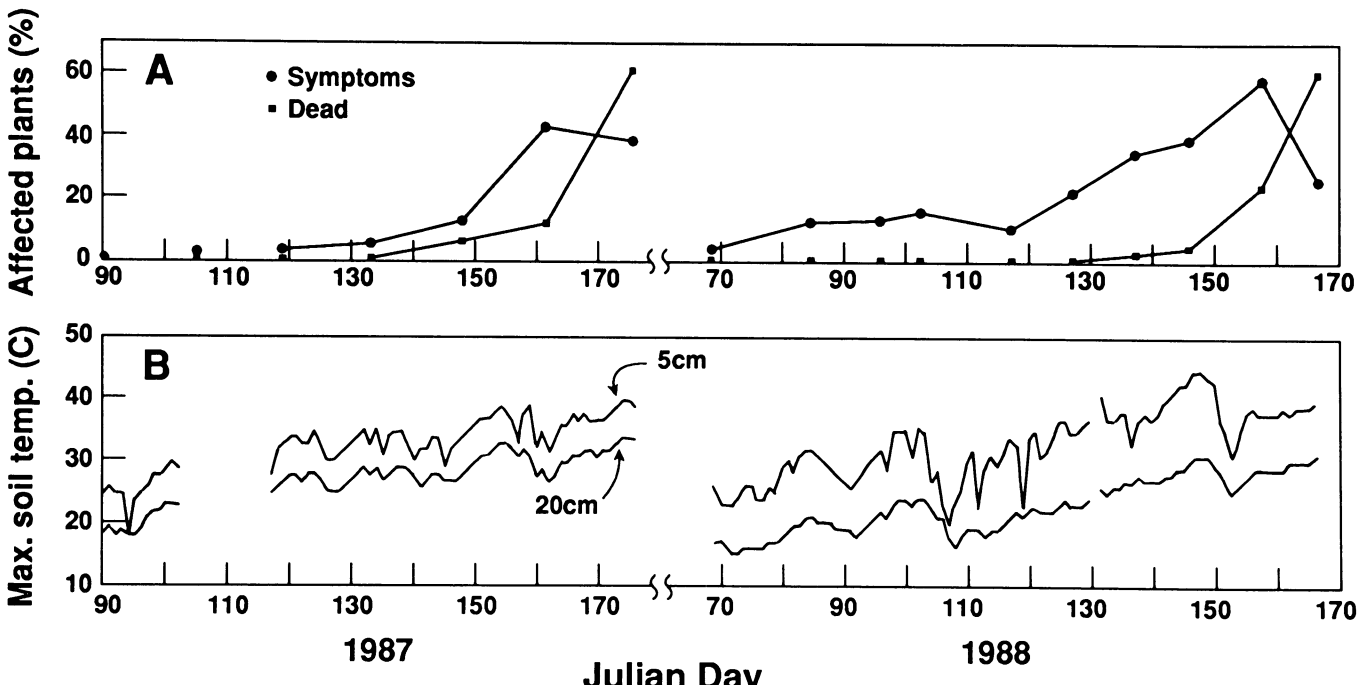


**Fig. 4. A,** Charcoal rot symptoms and mortality of *Euphorbia lathyris* over two growing seasons. Symptoms and dead are expressed as the percentage of living and total plants, respectively. Average of 100 quadrats. **B,** Maximum daily soil temperatures at 5- and 20-cm. Within each year, breaks in the two lines indicate missing data owing to equipment failure. Julian Day is equivalent to day of year.

pattern of autocorrelations and partial-autocorrelations was obtained with spatial differencing (Table 2). In this case, for both data sets, only the partial-autocorrelation coefficients were significant, suggesting that a pure moving-average STF would be an appropriate model for these two data sets. When temporal or simultaneous spatial and temporal differencing were used, both autocorrelation coefficients and partial-autocorrelation coefficients were significant.

**Factors related to disease development.** Average symptom expression and mortality (Fig. 4A) increased with increasing soil temperature (Fig. 4B) during both growing seasons. Symptom expression (percentage of living plants with symptoms) declined at the end of each growing season, suggesting that there was a subset of plants responding differently than the majority to those stresses that trigger symptom development. During both years, mortality increased markedly after the soil temperature at 5 cm reached the range of 28–30 C. During 1988, a late spring cold spell (JDay 102–117) was followed by a noticeable reduction in symptom expression (Fig. 4).

Throughout the 1986-1987 growing season, the percentage of living plants expressing symptoms was significantly higher for those plants in the early-flowering cohort, whereas no difference in mortality was observed between the early- and late-flowering groups (Table 3). This phenomenon was more critically explored during 1987-1988, as the plants in 60 contiguous quadrats (30 low-density, 30 high-density) were partitioned into four groups based on timing of flowering as described above. Throughout 1988, symptom expression was higher in the early-flowering cohorts (1 and 2), irrespective of plant density (Table 4). For example, at the end of the study (JDay 168), among low-density quadrats, 80.5% of the plants in flowering cohort 1 were dead, and virtually all the rest were expressing symptoms (Table 4). In contrast, for the late-flowering/nonflowering cohort ("Other", Table 4) only 23.1% of the plants were dead and of the remainder,

TABLE 3. Charcoal rot symptom development and mortality influenced by flowering status, 1987

| JDay[a] | Flowering[b] | N[c] | Symptoms[d] | | | Mortality[e] | | |
|---|---|---|---|---|---|---|---|---|
| | | | % | $\chi^2$ | $P$ | % | $\chi^2$ | $P$ |
| 119 | Early | 190 | 12.6 | 287.4 | 0.0 | 0.0 | 0.0 | 1.0 |
| | Other | 5,211 | 0.5 | | | 0.1 | | |
| 133 | Early | 190 | 27.4 | 209.5 | 0.0 | 0.5 | 0.0 | 1.0 |
| | Other | 5,209 | 4.1 | | | 0.6 | | |
| 148 | Early | 190 | 65.3 | 736.3 | 0.0 | 0.5 | 4.0 | 0.03 |
| | Other | 5,210 | 7.1 | | | 4.0 | | |
| 161 | Early | 190 | 76.8 | 69.8 | 0.0 | 9.5 | 0.1 | 0.79 |
| | Other | 5,209 | 45.7 | | | 10.3 | | |

[a] Julian Day (day of year).
[b] Early and other refer to those plants flowering before and after JDay 107, respectively.
[c] Number of plants in each category summed over 100 quadrats.
[d] Percentage of living plants expressing symptoms. Chi-square test with one degree of freedom.
[e] Percentage of all plants dead on the sampling date. Chi-square test with one degree of freedom.

TABLE 4. Charcoal rot symptom development and mortality influenced by flowering status, 1988

| JDay[a] | Flowering[b] cohort | Low plant density[c] | | | | | | | High plant density[d] | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | N | Symptoms | | | Mortality | | | N | Symptoms | | | Mortality | | |
| | | | % | $\chi^2$ | $P$ | % | $\chi^2$ | $P$ | | % | $\chi^2$ | $P$ | % | $\chi^2$ | $P$ |
| 96 | 1 | 353 | 20.7 | 57.8 | 0.0 | 0.0 | 5.0 | 0.03 | 601 | 26.0 | 171.3 | 0.0 | 0.0 | 3.0 | 0.08 |
| | Other | 547 | 4.3 | | | 1.8 | | | 1,095 | 4.2 | | | 0.7 | | |
| 102 | 1 | 353 | 29.7 | 96.8 | 0.0 | 0.0 | 5.0 | 0.03 | 601 | 33.3 | 228.7 | 0.0 | 0.0 | 2.5 | 0.12 |
| | Other | 547 | 5.4 | | | 1.8 | | | 1,093 | 5.4 | | | 0.6 | | |
| 117 | 1 | 353 | 21.0 | 82.0 | 0.0 | 0.3 | 7.6 | 0.02 | 601 | 21.1 | 163.2 | 0.0 | 0.5 | 5.2 | 0.07 |
| | 2 | 124 | 12.9 | | | 0.0 | | | 250 | 9.2 | | | 0.0 | | |
| | Other | 424 | 1.0 | | | 2.1 | | | 843 | 1.1 | | | 1.3 | | |
| 127 | 1 | 351 | 47.9 | 217.0 | 0.0 | 0.6 | 5.6 | 0.06 | 599 | 44.3 | 399.9 | 0.0 | 0.0 | 6.9 | 0.03 |
| | 2 | 124 | 38.7 | | | 0.0 | | | 250 | 30.8 | | | 0.5 | | |
| | Other | 423 | 2.7 | | | 2.1 | | | 843 | 1.4 | | | 1.5 | | |
| 137 | 1 | 352 | 71.7 | 353.8 | 0.0 | 0.6 | 10.1 | 0.02 | 601 | 66.9 | 649.2 | 0.0 | 1.5 | 4.7 | 0.20 |
| | 2 | 124 | 54.8 | | | 0.0 | | | 250 | 56.9 | | | 1.6 | | |
| | 3 | 25 | 28.0 | | | 0.0 | | | 77 | 26.0 | | | 0.0 | | |
| | Other | 398 | 5.4 | | | 3.0 | | | 766 | 3.2 | | | 2.7 | | |
| 146 | 1 | 353 | 76.6 | 403.9 | 0.0 | 4.5 | 4.8 | 0.19 | 601 | 78.4 | 824.4 | 0.0 | 6.0 | 7.6 | 0.06 |
| | 2 | 124 | 63.4 | | | 0.8 | | | 250 | 65.6 | | | 3.6 | | |
| | 3 | 25 | 32.0 | | | 0.0 | | | 77 | 31.2 | | | 0.0 | | |
| | Other | 398 | 5.0 | | | 4.3 | | | 766 | 3.4 | | | 4.0 | | |
| 158 | 1 | 353 | 98.6 | 186.3 | 0.0 | 38.0 | 129.6 | 0.0 | 601 | 99.4 | 207.5 | 0.0 | 45.8 | 319.7 | 0.0 |
| | 2 | 124 | 92.3 | | | 26.6 | | | 250 | 95.6 | | | 36.0 | | |
| | 3 | 25 | 75.0 | | | 4.0 | | | 77 | 81.4 | | | 9.1 | | |
| | Other | 398 | 49.5 | | | 5.0 | | | 766 | 62.9 | | | 5.7 | | |
| 167 | 1 | 353 | 97.1 | 46.6 | 0.0 | 80.5 | 258.1 | 0.0 | 601 | 97.5 | 86.8 | 0.0 | 86.7 | 374.3 | 0.0 |
| | 2 | 124 | 81.0 | | | 66.1 | | | 250 | 97.7 | | | 82.4 | | |
| | 3 | 25 | 64.3 | | | 44.0 | | | 77 | 80.6 | | | 59.7 | | |
| | Other | 398 | 56.2 | | | 23.1 | | | 773 | 53.4 | | | 39.2 | | |

[a] Julian Day (day of year).
[b] 1 = plants flowering between JDay 74 and JDay 95. 2 = plants flowering between JDay 96 and JDay 116. 3 = plants flowering between JDay 117 and JDay 137. Other = plants not flowering or flowering after date of reference.
[c] Low density = 6.6 plants per meter. N = number of plants in each category summed over 30 quadrats. Symptoms is the percentage of living plants expressing symptoms. Mortality is the percentage of all plants dead on the sampling date. Chi-square analysis with 1, 2, or 3 degrees of freedom when there are two, three, or four flowering cohort categories, respectively.
[d] High density = 13.1 plants per meter. N, Symptoms, Mortality as for low density. Degrees of freedom for chi-square analysis as for low density.

56.2% expressed charcoal rot symptoms. These results suggest a significant group of later-flowering plants that may escape infection or may be insensitive to the factors that induce active disease development. For both plant densities, mortality, like symptom expression, was significantly less among later-flowering plants. During the 1986-1987 and 1987-1988 growing seasons, a large patch of high disease incidence could be visually discerned in southern quadrats compared with low levels in northern quadrats. Upon visual inspection in 1986-1987, disease incidence appeared to be inversely associated with plant density. Indeed, on the last two assessment dates (JDay 161 and JDay 176), Pearson's correlation coefficients for the relationship between plant density and mortality were −0.21 and −0.48, respectively ($P \leq 0.02$). During the 1987-1988 growing season, high- and low-density quadrats alternated in a checkerboard pattern over the plot. Mortality was found to be virtually identical in the two plot types until the last sampling date (JDay 168, Fig. 5A) when mortality was statistically higher in the high-density quadrats than in the low-density quadrats. In contrast, symptom expression on JDay 168 was statistically lower in high-density quadrats than in the low-density quadrats (Fig. 5B).

The relationship between the preplant population of microsclerotia of *M. phaseolina* and mortality through each growing season was assessed with Pearson's correlation coefficient. In 1987, the relationship was significantly positive ($P \leq 0.05$) on JDay 133, 148, and 176 ($r = 0.21$, 0.17, and 0.30, respectively) and nonsignificant otherwise (JDay 70, 90, 105, 119, and 161). The positive correlation coefficients suggested a dose-response relationship between preplant inoculum level and mortality. For JDay 176, this relationship could be expressed by the linear regression equation $Y = 0.017X + 0.5$, where $Y$ is the proportion dead and $X$ is the preplant population of *Macrophomina* expressed as microsclerotia per gram ($r = 0.30$, [$F = 9.49$, $P = 0.003$]). For data from the 1987-1988 growing season, similar analyses were largely nonsignificant at both plant densities, indicating no significant dose-response relationship in this second epidemic.

**Disease dynamics.** In 1986-1987, the spatial pattern of symptoms and mortality showed increasing autocorrelation over the growing season, as reflected in increasing values of Moran's $I$ (Fig. 6A), indicating that adjacent quadrats had similar levels of symptom expression and mortality. By the end of the growing season, significantly positive autocorrelation was observed between quadrats more than 10 m distant (Fig. 6B) confirming the visual observation of a large patch of uniformly high mortality. Analysis of disease data for 1988 gave very similar results, with Moran's $I$ for mortality in adjacent quadrats reaching a maximum of 0.43 ($P \leq 0.001$) at the end of the growing season (JDay 168). The correlograms for the two seasons were very similar.

Spatio-temporal autocorrelations and partial autocorrelations for mortality data are summarized in Table 5 for three levels of spatial and temporal lag. When the estimates in Table 5 were made using the queen's definition of spatial proximity or using the arcsine transformation for either definition of spatial proximity, the results were virtually identical. The high degree of autocorrelation over three spatial lag orders (temporal lag 1) in both years suggests a nonstationary spatial process (30). Further, it is clear from Figure 5 that the data were not temporally stationary. These coefficients were then recalculated for the rook's definition of spatial proximity and binary weights using nontrans-

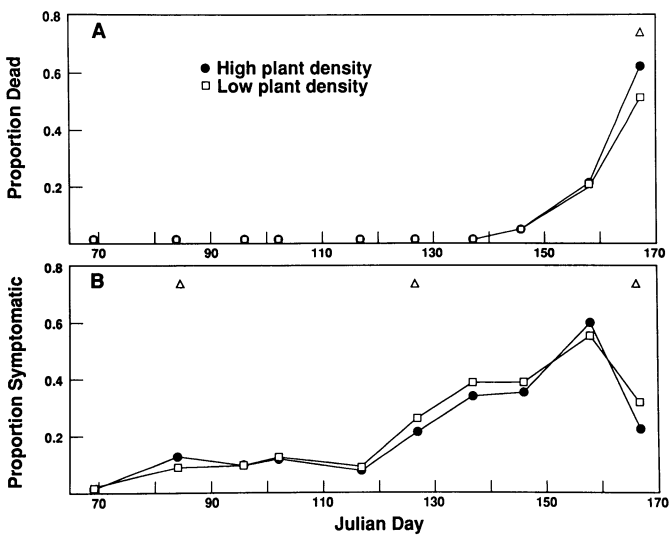


**Fig. 5. A,** Charcoal rot mortality and **B,** symptoms of *Euphorbia lathyris* during 1988 for high and low plant densities. Open triangles indicate that the paired means are statistically different ($P \leq 0.05$) using a Student's $t$-test. Symptoms and dead are expressed as the percentage of total plants. Julian Day is equivalent to day of year.

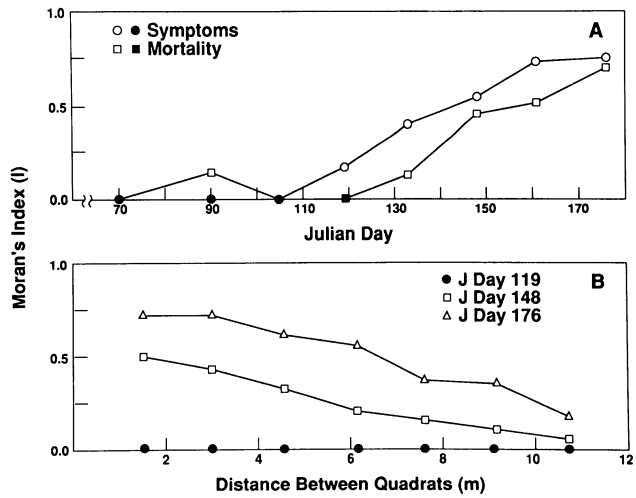


**Fig. 6. A,** Temporal fluctuation in charcoal rot symptoms and mortality of *Euphorbia lathyris* during 1987 as measured by Moran's index of autocorrelation ($I$) for adjacent quadrats. **B,** Temporal change in spatial pattern of *E. lathyris* mortality during 1987 as measured by Moran's index for quadrats increasingly distant for selected sampling dates. Day of year is JDay (= Julian Day). For A and B, open symbols indicate index values statistically different from zero ($P \leq 0.05$).

TABLE 5. Estimated spatio-temporal autocorrelations for charcoal rot-induced mortality of *Euphorbia lathyris*, using binary weights and the rook's definition of spatial proximity

| Year | Temporal lag order | Autocorrelations[a] for spatial lag order | | | | Partial autocorrelations[a] for spatial lag order | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 0 | 1 | 2 | 3 |
| 1987 | 1 | 0.38*** | 0.37*** | 0.35*** | 0.31*** | 0.19*** | 0.26*** | 0.11** | 0.23*** |
| | 2 | −0.01 | −0.02 | −0.02 | −0.05 | −0.04 | −0.01 | 0.00 | 0.07 |
| | 3 | −0.17* | −0.17* | −0.17* | −0.18* | −0.00 | −0.11* | −0.08 | −0.08 |
| 1988 | 1 | 0.67*** | 0.67*** | 0.63*** | 0.61*** | 0.40*** | 0.27*** | 0.10* | 0.16*** |
| | 2 | −0.06 | −0.07 | −0.08 | −0.08 | −0.07 | −0.01 | 0.07 | −0.08* |
| | 3 | −0.16* | −0.17* | −0.17* | −0.17* | 0.03 | −0.06 | −0.07 | 0.04 |

[a] *, **, and *** indicate that the coefficient is statistically different from 0 at $P \leq 0.05$, 0.01, and 0.001, respectively.

formed data, but including simultaneous spatial and temporal differencing (Table 6). In this case, at the zero-order spatial lag, the autocorrelations were significantly negative for the first-order temporal lag in both epidemics and for the second-order temporal lag in the 1987-1988 epidemic (Table 6). Beyond this, there is little similarity between the two epidemics in the patterns of the autocorrelograms and partial autocorrelograms.

## DISCUSSION

Over the 2 yr of this study, the population of microsclerotia of *M. phaseolina* increased one order of magnitude (from 8.9 to 98.6 microsclerotia per gram) before declining. This is less than the increase of two orders of magnitude observed by Young and Alcorn (38), although the final population near 70 microsclerotia per gram was very similar in both studies (Fig. 1). These two studies and those with other hosts of *M. phaseolina* (1,14,20,29) suggest that the soil population of microsclerotia increases as host debris incorporated into soil decomposes. Further, the present study and that of Young and Alcorn (38) point to the existence of factors that impose a limit on the increase of the population of microsclerotia of *Macrophomina.* One avenue for further investigation should be those factors (biotic and abiotic) that limit this increase. During the course of this study, the spatial arrangement of the microsclerotia of *M. phaseolina* was found to be aggregated, as measured by Morisita's index of dispersion (24). In contrast, Olanya and Campbell (28) noted a decrease in the aggregation of populations of microsclerotia with continuing cultivation, which may be attributable to the lower inoculum levels recorded by these authors. However, the large patch of uniform population present at the beginning of the study (Fig. 2A), decreased in size during the first fallow period (1987, Fig. 2B), as indicated by the reduction in Moran's index of autocorrelation. These observations demonstrated that although the population of microsclerotia varied greatly among quadrats, continuing cultivation redistributed inoculum so that quadrats of close spatial proximity were less likely to have similar populations (Fig. 3).

Spatio-temporal autocorrelation analysis of inoculum density patterns in both fallow periods resulted in nonsignificant autocorrelation coefficients and significant partial autocorrelations (Table 2), suggesting a pure moving-average STF as an appropriate model for observed changes in pattern. In a purely moving-average process, variate values depend only on external inputs (30). In this study, the redistribution of inoculum by disking is the external factor most likely to have governed the pattern of significant partial autocorrelations summarized in Table 2.

In examining the relationship between disease progress and soil temperature (Fig. 4), it was particularly unexpected to see a reduction in symptom expression following a brief period of cool weather during 1988. Although not conclusive, this observation strongly suggests some degree of plant recovery after the commencement of active disease development. If these observations are borne out by subsequent experimental work, there is a possibility that the effects of charcoal rot may be minimized through cultural manipulation after the first observation of symptoms.

The results of the current investigations demonstrate the importance of flowering in the development of charcoal rot symptoms (Tables 3 and 4). During the second epidemic (Table 4), although plants in the third-flowering cohort developed mature flowers and fruit, mortality was significantly lower than that of plants in the first- or second-flowering cohorts. Thus, the timing of flowering appears to be quite important, with early-flowering individuals more likely to succumb to charcoal rot than later-flowering plants. These results confirm the suggestions from the early work with charcoal rot of sorghum (18,27) and should serve as a guideline for breeding efforts where the agronomic viability of a particular crop is not seriously compromised by altering the timing of reproduction.

In studies with several crops, charcoal rot was found to be inversely related to plant density (2,16,26). This does not seem to be the case for charcoal rot of *E. lathyris* under the conditions tested (Fig. 5).

During the 1986-1987 growing season, a positive relationship generally existed between the preplant population of microsclerotia of *M. phaseolina* and mortality of *E. lathyris* after mortality exceeded 1% (JDay 133, Fig. 4A). During the 1987-1988 growing season, the absence of a similar dose-response relationship is probably due to the higher inoculum levels during the intervening fallow period. The correlogram for preplant inoculum level in 1986 (Fig. 2A) and mortality in 1987 (Fig. 6B) were very similar, showing a high-order positive autocorrelation, which indicates a large patch of similar variate values. Indeed, a visual examination of the maps of inoculum level and mortality indicated that these patches largely overlapped. However, there was no large patch of similar inoculum populations at the end of the 1987 fallow period (Fig. 2B), while a large patch of high-mortality values was again evident in 1988. This result suggests strongly some extrinsic factor other than plant density or host flowering status exerting a strong influence on disease development. Edaphic factors would seem to be probable candidates and deserve more critical examination.

Spatio-temporal autocorrelation analysis was used in an attempt to identify a model that would account for observed temporal shifts in mortality patterns. The first analyses (Table 5) suggested that observed patterns were related to spatial nonstationarity. The increase in mortality during both epidemics (Fig. 5) indicated temporal nonstationarity as well. When spatio-temporal autocorrelation analyses were redone with simultaneous spatial and temporal differencing (Table 6), much of the significant autocorrelation was removed as expected (30). However, the resulting patterns of significant autocorrelation coefficients (and partial autocorrelations) are not immediately suggestive of a simple STF model, although an STF model for charcoal rot mortality would probably be mixed, having both autoregressive and moving-average components. Further, the patterns in Table 6 are not similar for the two epidemics, despite field observations of very similar mortality patterns for the two epidemics. Before any further attempts are made to find an appropriate STF model for charcoal rot epidemics, additional epidemics must be monitored, and the patterns of the autocorrelograms (and partial autocorrelograms) compared with those presented here. They

TABLE 6. Estimated spatio-temporal autocorrelations for charcoal rot-induced mortality of *Euphorbia lathyris*, using binary weights, the rook's definition of spatial proximity, and simultaneous spatial and temporal differencing

| Year | Temporal lag order | Autocorrelations[a] for spatial lag order | | | | Partial autocorrelations[a] for spatial lag order | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 0 | 1 | 2 | 3 |
| 1987 | 1 | −0.18* | 0.20* | −0.05 | 0.12 | −0.05 | 0.13** | 0.05 | 0.10* |
| | 2 | −0.12 | 0.05 | 0.04 | −0.06 | −0.15** | −0.01 | 0.02 | −0.02 |
| | 3 | −0.06 | 0.06 | −0.10 | 0.13 | −0.08 | −0.01 | −0.02 | 0.08 |
| 1988 | 1 | −0.18* | 0.14 | −0.01 | −0.06 | −0.13** | 0.00 | −0.02 | −0.01 |
| | 2 | −0.20** | 0.23** | 0.03 | −0.06 | −0.03 | 0.16*** | 0.13** | −0.02 |
| | 3 | 0.01 | −0.05 | 0.16* | −0.13 | −0.01 | 0.00 | 0.10* | −0.06 |

[a] *, **, and *** indicate that the coefficient is different from 0 at $P \leq 0.05$, 0.01, and 0.001, respectively.

should be examined to identify those components that are common to all the epidemics and that might serve in the construction of a usable STF model.

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