Inoculum Pattern, Inoculum Density-Disease Incidence Relationships, and Population Fluctuations of *Cylindrocladium crotalariae* Microsclerotia in Peanut Field Soil

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ABSTRACT

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The horizontal pattern (distribution) of *Cylindrocladium crotalariae* microsclerotial inoculum in soil was determined in a 1975 field plot established to study inoculum density-disease relationships and the influence of crop rotation on pathogen populations. The microsclerotial-inoculum pattern fitted the negative binomial distribution, indicating the microsclerotial-inoculum pattern was not random but was clumped or clustered. From 1975 to 1977, appreciable incidence of Cylindrocladium black rot of peanut was found only in 1976 (mean = 18.0%). For that year, first-order regression equations gave the best fit in arithmetic plots of inoculum density vs disease incidence (transformed to $\log_e [1/1-y]$). Log $_{10}$-log$_{10}$ regression line slopes of the same variables were low for both a corn-peanut sequence ($b = 0.45$) and a peanut-peanut sequence ($b = 0.30$). Inoculum clumping appeared to contribute to the low slope values. Physical environmental factors had a greater influence on the quantity of microsclerotia in soil than did crop sequence. A drought period in June 1975 was associated with a reduction in microsclerotial populations in the upper (0 to 12.7 cm) soil layer, compared to populations in the 12.7- to 25.4-cm soil layer. The extremely cold winter of 1976–1977 was associated with a large decrease in germinable microsclerotia in all field-plot soils between 1976 and 1977. Microsclerotial populations remained low following a cold winter in 1977-1978.

Additional key words: inoculum potential.

Baker and colleagues (2–5) have laid the foundation for the development of mathematical relationships between inoculum density and disease incidence for root-infecting fungi. Most modeling studies by these workers and others have been conducted under relatively controlled conditions with greenhouse soil-temperature tanks or growth chambers, and with inoculum that has been thoroughly mixed into soil. If disease management systems are to be developed for root-infecting fungi, attempts at disease modeling in the field will be needed. Baker (3) has indicated that development of mathematical relationships between inoculum and disease incidence is possible in the field when capacity (eg, environmental) factors among fields are relatively constant and when inoculum is randomly distributed in soils. In the field, variation in capacity factors from field to field can be minimized by studying inoculum density-disease incidence relationships in one area of a single field. Virtually nothing is known about inoculum pattern (distribution) in cultivated field soils. Nash and Snyder (15) reported that the pattern of *Fusarium solani* f. sp. *phaseoli* inoculum in cultivated bean field soil was remarkably uniform, but as developed later in this paper, there is a strong possibility that the pattern of this fungus in soil is neither uniform nor random. Roth and Griffin (21) found that the inoculum pattern of *Cylindrocladium* in a Virginia forest nursery fitted the Neyman Type A distribution; this indicates that the inoculum pattern was not random, but was clumped or clustered.

Populations of soilborne pathogens may be influenced by cropping practices and by physical and chemical soil factors (1). In the Virginia peanut-growing region, corn and soybean are commonly grown as rotation crops, and the former crop typically is incorporated into a crop rotation scheme with peanut. Also, minimum-tillage soybean cropping is gaining acceptance in Virginia. Both peanut and soybean are susceptible to *Cylindrocladium crotalariae* (Loos) Bell and Sobers, whereas corn is not (13,23). Recently, Hadi (10) found that green manuring with sorghum-sudangrass reduced the population of *C. floridanum* in forest nursery soils. This paper presents information on: the influence of different crop sequences on microsclerotial populations of *C. crotalariae* in field soil from 1975 to 1978, the horizontal and vertical microsclerotial pattern of *C. crotalariae* in peanut field soil, the relationship between microsclerotial inoculum density and Cylindrocladium black rot (CBR) incidence on peanut in field soil, and the association of drought and low winter temperature with reduced microsclerotial populations in field soil. Due to the strict statistical connotation of the word distribution (19), the term inoculum pattern will be used instead of inoculum distribution throughout the remainder of this paper.

MATERIALS AND METHODS

Establishment of field plots. Two field plots were established in 1975 on a farm in Southampton County in the state of Virginia. One plot (crop rotation plot) was used to investigate the influence of corn (C), soybean (S), and peanut (P) rotations on *C. crotalariae* microsclerotial population densities and the other plot (minimum-tillage and green-manured plot) was used to investigate the influence of a soybean rotation, with and without minimum tillage, and green manuring with sorghum-sudangrass on *C. crotalariae* microsclerotial populations. Both plots were situated in an area of the field in which nearly 100% of the peanut plants were killed by CBR in 1974, as indicated by aerial infrared imagery and ground observations (20). The plots were based on a completely

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randomized block design of five treatments (crop sequences) and four replications. Replicates were composed of five, four-row subplots and were 9.1 × 19.7 m. Three alleyways, 6.1-m wide, separated the four replicates. Crop sequences from 1975 to 1977 for the crop rotation plot were: Coker 16 corn, Florigiant peanut and Essex soybean (C-P-S); continuous corn (C-C-C); soybean, corn, and peanut (S-C-P); continuous peanut (P-P-P); and continuous fallow (F-F-F). In November, ryegrass (cultivar Western) was planted as a cover crop and constituted an additional crop to the above. Crop sequences in both 1976 and 1977 for the minimum-tillage and green-manured plot were: winter wheat (W) (cultivar Arthur) followed by minimum-tillage soybean (W/S-W/S); winter wheat followed by fallow (W/F-W/F); winter fallow followed by conventionally tilled soybeans (F/S-F/S); winter fallow followed by sorghum-sudangrass, 'FF-66' (F/SS); and continuous fallow (F-F). Paraquat dichloride was used to treat winter wheat minimum-tillage subplots at planting time.

Pesticides (alachlor, carbaryl, and Spreader-sticker 268) were utilized in both plots to control weeds, insects, and animal pests, and chlorothalonil was used to control Cercospora leaf spot on peanut in the crop rotation plot. All pesticides were used at commercial rates. In addition to herbicide usage, hand cultivation was required to control late-season grasses. Animal damage necessitated hand replanting peanut, corn, and soybean seed until middle June in 1975 and late May in 1976. Other chemicals (calcium nitrate for corn and calcium sulfate for peanut) were applied to appropriate treatments in subplots at recommended commercial rates. Plant residues in the crop rotation plot were disked into the soil in November before planting of the cover crop. The sorghum-sudangrass was disked into the soil in September. The crop rotation plot and all the minimum-tillage and green-manured subplots, except those with winter wheat, were plowed 25 cm deep in April with a moldboard plow and disked twice before planting.

Soil sampling, assays for germinable microsclerotia of *C. crotalariae*, and disease ratings. For the horizontal inoculum pattern determinations, soil samples (widely spaced quadrats) were taken on June 2, 1975 with a standard soil probe (2 cm in diameter and 25 cm deep) in a manner consistent with the peanut plant pattern in the plot. Soil samples were obtained every 61 cm of row, near the center of the third row for each of three randomly located peanut-replicate subplots (replicates 1,2, and 3 of crop sequence P-P-P). Sixteen soil samples were obtained in each replicate row and 48 individual samples were obtained in all. For the vertical inoculum determinations, the samples were obtained near the center of the same rows, equidistant from both row ends, and to a depth of 30.5 cm. The soil core from each row was divided into four 7.6-cm sections. For inoculum density-disease incidence studies and other population studies, the entire crop rotation plot was sampled on 20 June 1975, 3 July 1976, 4 July 1977 and 30 May 1978 by obtaining 2-cm × 25.4-cm soil cores, every 30.5 cm of row, from each of the middle two rows of all four-row subplots. The soil core was divided into two sections, 0–12.7 cm and 12.7–25 cm. A similar procedure was used for the minimum-tillage and green-manured plot in 1976 and 1977, except that the soil core was not divided into two portions. In 1978, the soil cores were divided into two 12.7-cm sections. All soil cores from each row layer were pooled. Soil samples were thoroughly hand-mixed in a plastic bag for 5 min before being transported to the laboratory in an insulated container. Air holes in plastic bags allowed air exchange during transport and during storage at 28 C.

Populations of germinable microsclerotia of *C. crotalariae* were determined for 44-g soil subsamples from the thoroughly mixed samples of each row with the wet-sieving and selective-plating method of Krigsvold and Griffin (14). Due to a different source of oxgall, the concentration of this material was increased in the selective medium for 1976, 1977, and 1978 to 4 g/L. Ten petri plates were prepared for each soil sample.

Nematode populations, soil fertility, and soil texture were determined also for the two thoroughly mixed row soil samples from all subplots in 1975. The latter assays indicated that population densities of *Tylenchorhynchus, Belonalaimus,* and *Helicotylenchus* species of 5–225, 0–7, and 0–3 per 250 cc of soil, respectively, were present in the crop rotation plot. Soil pH ranged from 5.3 to 5.9; Ca, Mg, P, and K ranged 60–110, 9–13, >50, and 10–117 kg/ha, respectively, for both plot areas. Soil texture ranged from sandy loam to loamy sand-sand, and the soil types present were Emporia, Goldsboro, and Kenansville. Meterological data were obtained during the study at Boykins, VA, 12.5 km from the field plots.

Shoot symptom ratings on peanut in the crop rotation plot were made on 12, 18, and 20 September in 1975, 1976, and 1977, respectively. The presence or absence of perithecia on peanut and soybean plants and the number of peanut plants displaying chlorotic, wilted or dead branches in each of the two middle rows for each replicate was counted. Percentage of diseased peanut plants per row was based on the number of plants per 30.5 cm of row with a plant spacing of approximately 7.6 cm. Root disease severity ratings were made for peanut and soybean during harvest and were based upon a random selection of 20 plants per subplot. The following scale was used: 0 = nonnecrotic tissue, 1 = slight rot, 2 = moderate rot, 3 = moderate-to-severe rot, 4 = severe rot, and 5 = completely destroyed taproot. Representative plants were transported to the laboratory where root tissue was surface-sterilized for 30 sec in 0.5% NaClO and rinsed in sterile distilled water before being plated on sucrose-TBZ agar medium (14).

RESULTS

Inoculum pattern. Analyses of the data for the horizontal pattern of *C. crotalariae* microsclerotia indicated that the pattern was not random; there was a poor fit of the observed frequencies of *C. crotalariae* microsclerotial populations to the expected values indicated by the Poisson distribution (Fig. 1). Also, the variance-to-mean ratio was greater than one ($s^2/\bar{Y} = 7.82$). However, a good fit of the observed frequencies to expected values was obtained for the negative binomial or Poisson-logarithmic (12,19) distribution (Fig. 1). The Chi-square and probability values obtained for the Poisson and negative binomial distributions were $P(\chi^2 > 57.73) = 0.00$ and $P(\chi^2 > 3.615) = 0.607$, respectively. Values obtained for the Neyman Type A(= Poisson-Poisson) distribution, another contagious distribution, were $P(\chi^2 > 80.382) = 0.00$, indicating a poor fit of the observed to expected frequencies. The negative binomial is a contagious distribution that describes clumped, patchy, or clustered patterns. It suggests that the number of microsclerotia within each clump has a logarithmic distribution and that the clumps are dispersed randomly in the field. This distribution is given by:

$$\Pr[X = K] = \binom{N + K - 1}{N - 1} (P/Q)^K (1 - P/Q)^N \quad (K = 0,1,2..)$$

in which Pr is the probability of the number of units (sample locations) in a frequency class (microsclerotia per 5 g of soil), K; where the product of the parameters, NP, is the mean of the distribution; N is the index of clumping; and $Q = 1 + P$ (12). A mean population ($\bar{Y}$) of 1.3 microsclerotia per gram soil was obtained for all samples. A value of 0.96 was obtained for $\hat{N}$ (or k in other textbooks) in the negative binomial distribution, in which small values of $\hat{N}$ indicate a high degree of aggregation or clumping.

Analysis of the data for the vertical *C. crotalariae* microsclerotial populations among the four depths that were sampled indicated that the highest mean population (2.5 microslerotia per gram of soil) was found at the 7.6–15.2 cm depth. However, this population differed significantly only from the population at the 0–7.6 cm (0.5 microsclerotia per gram of soil) depth, according to Duncan's multiple range test ($P = 0.10$). The mean populations at the 15.2–22.9- and 22.9–30.5-cm depths were 1.2 and 0.8 microsclerotia per gram of soil, respectively. An overall mean population of 1.3 microsclerotia per gram of soil was obtained for the vertical pattern assays.

Inoculum density-disease incidence relationships. Mean CBR incidences on peanut obtained for 1975, 1976, and 1977 were 2.6, 18.0, and 7.1%, respectively. No evidence of Sclerotinia blight or

stem rot of peanut were found in the symptomatic plants assayed. Only in 1976 was the incidence of CBR sufficiently high and the range of incidences sufficiently wide to permit adequate analyses of the relationship between microsclerotial population and disease incidence. In 1976, the variance-to-mean ratios of microsclerotial densities for the thoroughly mixed, pooled (composited) soil samples of eight rows were 4.2 for the C-P crop sequence and 1.8 for the P-P crop sequence. An arithmetic plot of inoculum density vs disease incidence for the eight peanut rows assayed for each crop sequence is shown in Fig. 2. Arithmetic and $\log_{10}$–$\log_{10}$ plots of inoculum density vs the estimated number of infections per peanut plant, obtained by transforming the proportion of symptomatic plants with the multiple-infection correction (3,24), $\log_e (1/1-y)$, are indicated in Figs. 3 and 4, respectively. The data in the arithmetic plot of inoculum density vs the estimated number of infections for the P-P crop sequence may be regarded by visual examination as fitting a straight line or a curved line.

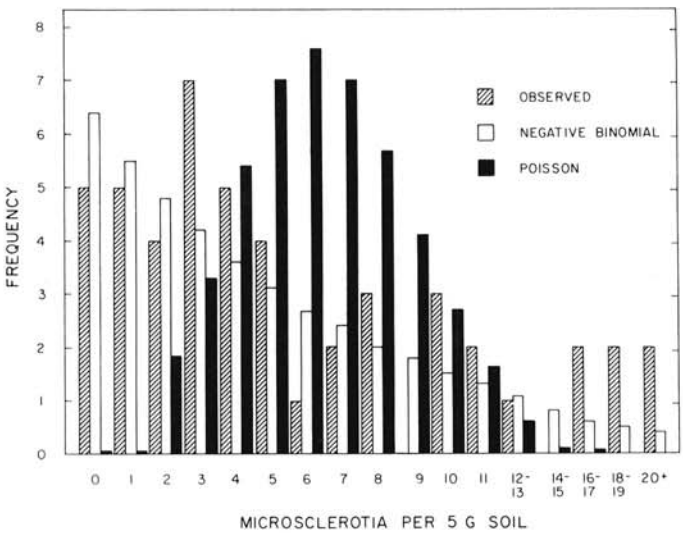

Fig. 1. Observed and expected frequencies for the Poisson distribution and the negative binomial distribution of the number of microsclerotia of *Cylindrocladium crotalariae* per 5-g of soil for 48 locations in the crop rotation field plot for 1975 $P(\chi^2 > 3.615) = 0.607$ for the negative binomial distribution and $P(\chi^2 > 57.73) = 0.00$ for the Poisson distribution.

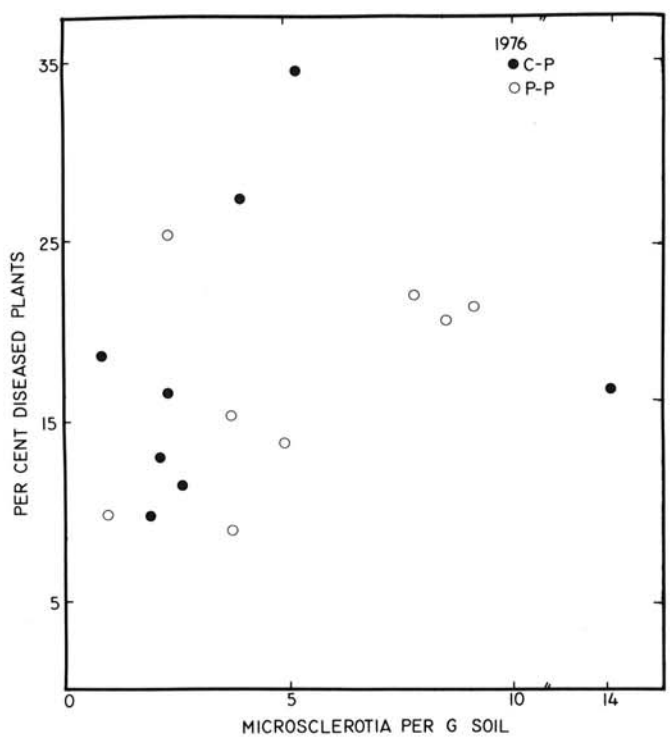

Fig. 2. Scatter diagram of the relation between the percentage peanut plants with Cylindrocladium black rot symptoms and inoculum density of *Cylindrocladium crotalariae* microsclerotia for the corn-peanut (1975–1976) and the peanut-peanut (1975–1976) crop sequences in 1976.

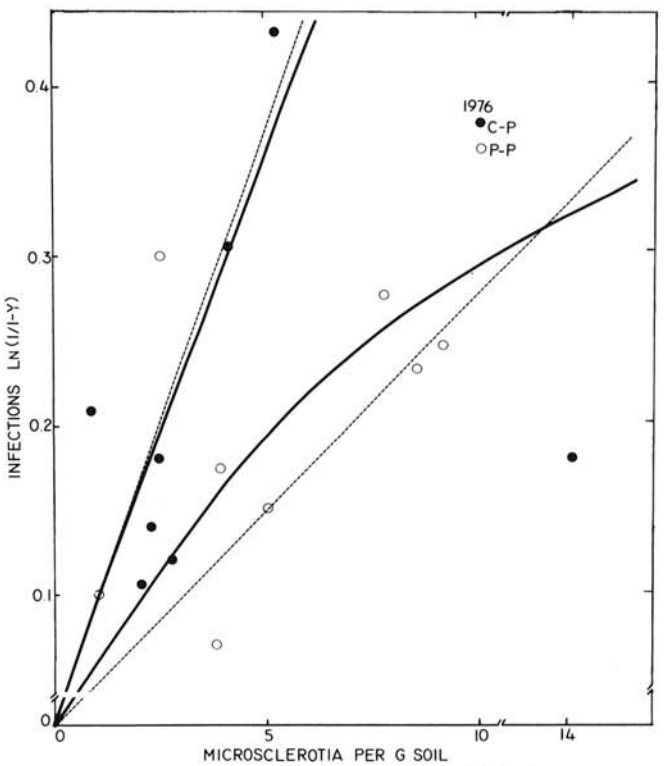

Fig. 3. Arithmetic plot of the estimated number of infections caused by *Cylindrocladium crotalariae* per peanut plant [$\log_e (1/1-y)$, in which y = proportion of symptomatic plants] vs microsclerotial inoculum density for the corn-peanut (C-P, 1975–1976) and peanut-peanut (P-P, 1975–1976) crop sequences in 1976. First-order (-----) and second order (——) regression lines, forced through the origin, are shown. For the corn-peanut sequence, values for r were 0.96 and 0.96 for the first- and second-order equations, respectively. For the peanut-peanut sequence, values for r were 0.91 and 0.94, respectively.

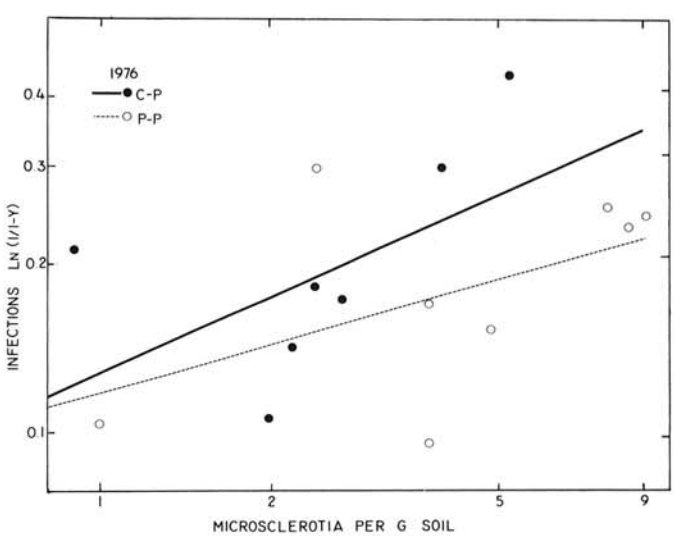

Fig. 4. Log_{10}-$\log_{10}$ plot of the estimated number of infections caused by *Cylindrocladium crotalariae* per peanut plant ($\log_e [1/1-y]$, in which y = proportion of symptomatic plants) vs microsclerotial inoculum density for the corn-peanut (C-P) (1975–1976) and peanut-peanut (P-P) (1975–1976) crop sequences in 1976. Slopes for the C-P and P-P regression lines were 0.45 and 0.30, respectively. Values for r were 0.50 and 0.53, respectively.

However, regression analysis for the arithmetic plot indicated that the coefficient of the quadratic term (X^2) in the second-order regression equation, which prescribes a curved line, was not significant ($P = 0.05$) for either crop sequence; both coefficients of the X term in first-order equations were significant. The straight and curved regression lines (Fig. 3) for the arithmetic plots were forced through the origin as, without this, the lines intersected the y axis slightly above the x axis (0.04 and 0.13 for C-P and P-P, respectively, for first-order equations). Such a situation is not possible biologically; it indicates that disease occurred in the absence of inoculum. For forced first-order equations, r values of 0.96 and 0.91 were obtained for the C-P and P-P sequences, respectively. For forced second-order equations, r values were 0.96 and 0.94, respectively. For the $\log_{10}$–$\log_{10}$ plot, regression line slope values of 0.45 ($r = 0.50$) and 0.30 ($r = 0.53$) were obtained for the two crop sequences, C-P and P-P, respectively. The slope values for the C-P sequence was determined without the outlying 14 microsclerotia per gram soil data point, which appeared to be an extreme case of microsclerotial inoculum clumping in the crop rotation field plot, and which lowered the slope value. Also, the 0.9 microsclerotia per gram of soil data point for the same crop sequence strongly influenced (lowered) the slope of the regression line.

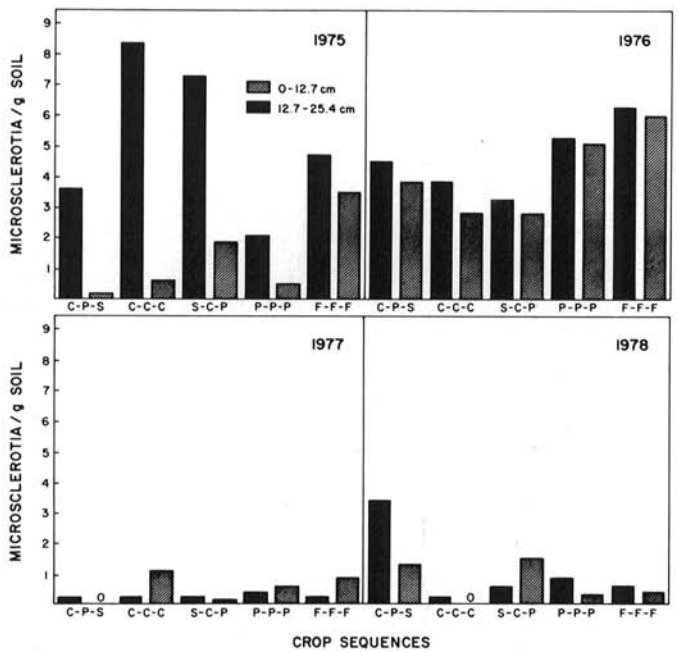

Fig. 5. Microsclerotial populations of *Cylindrocladium crotalariae* from 1975 through 1978 at two soil depths and for various crop sequences in the crop rotation field plot. Crop sequences from 1975 through 1977 are indicated; C = corn, P = peanut, S = soybean, and F = fallow.

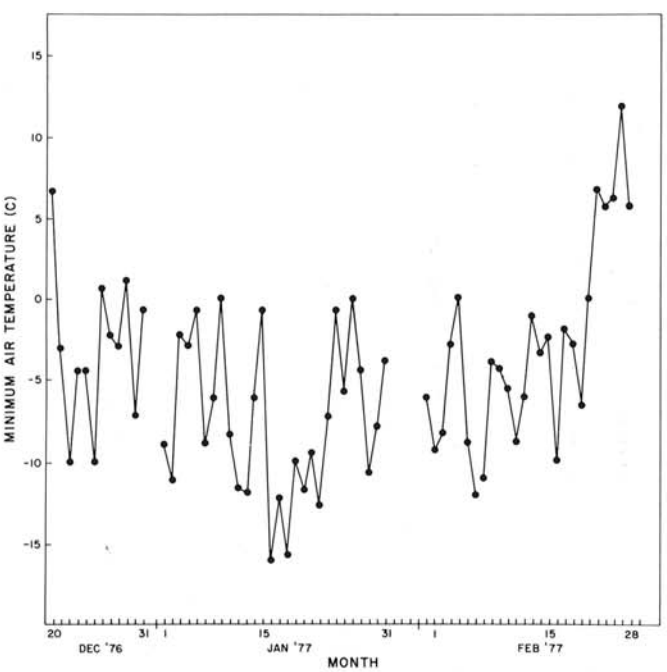

Fig. 6. Minimum air temperatures recorded during December, January, and February 1976–1977 at Boykins, VA, near the crop rotation field plot.

ED_{50} values (microsclerotia per gram of soil required for 50% disease) that were within the range of typical natural microsclerotial populations of plowed and disked peanut fields were found only for the arithmetic plots (Fig. 3). This required extrapolation beyond the range of the experimental data; disease incidences in this year were not high enough to interpolate ED_{50} values. This reduces the reliability of the estimates. ED_{50} values of 9.4 and 22.0 microsclerotia per gram of soil were found for the first-order equations of the C-P and P-P crop, respectively. The corresponding ED_{50} values for the $\log_{10}$–$\log_{10}$ plots were 39.8 and 352.6 microsclerotia per gram of soil, respectively.

In 1976, the mean root rot index obtained for peanut in both crop sequences was 1.8 at harvest. Perithecia were observed on 2% of the plants. In both field plots, 5% of the soybean plants had perithecia, and the typical red discoloration of hypocotyls was usually observed in association with the presence of perithecia. Little or no root rot of soybean was observed in either plot, but the pathogen was isolated from 25.6% of the asymptomatic soybean root segments plated.

Microsclerotial population fluctuations and the influence of crop sequence on microsclerotial populations. Assay of 20 June 1975 soil samples (initial populations) indicated there were differences in microsclerotial population densities among crop sequences of the crop rotation plot, but none were significant ($P = 0.05$). However, appreciable differences in microsclerotial population densities occurred in soils for all crop sequences between the two soil depths (Fig. 5). All crop-sequence soils containing crop plants had significantly lower population densities in the top soil zone than in the bottom soil zone ($P = 0.001$). The fallow treatment, however, had a smaller difference in microsclerotial population density between the two soil depths than the treatments with crop plants. During the 21 days previous to soil sampling, less than 5 mm of rainfall was recorded at the Boykins weather station located near the field plots. Normal rainfall for the month of June is approximately 130 mm. In 1976, only small differences in microsclerotial populations were noted among the different crop sequences or between soil depths, and none were significant ($P = 0.05$). In 1977, large, significant decreases in microsclerotial populations were observed for all crop-sequence soils and at both soil depths, compared to 1976

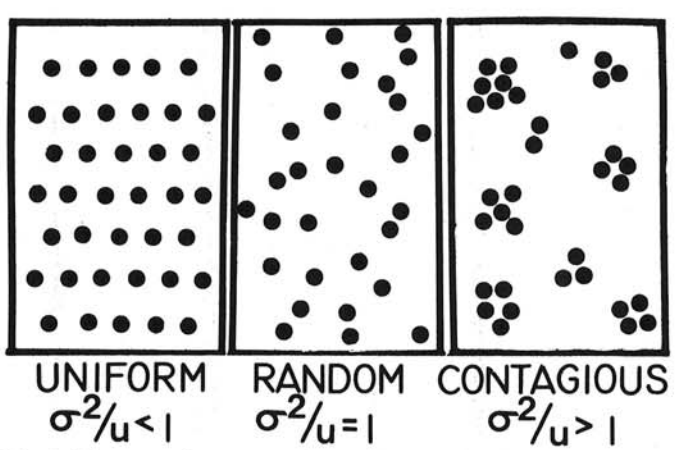

Fig. 7. Diagrammatic representations of hypothetical inoculum patterns that are uniform, random or contagious (clumped). Expected variance-to-mean ratios are indicated. Modified from Balaam (6).

populations ($P = 0.05$). The 1976–1977 winter was unusually cold as minimum air temperatures were below 0 C for most of the winter months (Fig. 6). In the peanut-growing area of Virginia, the plow layer was frozen much of this time. In 1978, the microsclerotial populations generally remained low, and no significant differences in populations were noted among crop sequences.

In the minimum-tillage and green manured plot, no significant differences were noted for 1976 microsclerotial populations among the different treatments. Population densities ranged from 4.0 to 6.5 microsclerotia per gram of soil (mean = 5.5 microsclerotia per gram of soil). In 1977, low microsclerotial populations were found for all soils (mean and range = 0.5 and 0.2 to 1.0 microsclerotia per gram of soil, respectively), as found for the crop rotation plot. Similar low populations were observed in 1978 (mean and range = 0.8 and 0.2 to 1.4 microsclerotia per gram of soil, respectively).

DISCUSSION

Analyses of the data on the horizontal pattern of *C. crotalariae* microsclerotia in the crop rotation field plot in 1975 indicated that the pathogen had a clumped or clustered pattern, as there was a good fit of the observed frequencies to expected frequencies calculated from the negative binomial (Poisson-logarithmic) distribution. To our knowledge, this is the first direct evidence that a root-infecting fungus has such an inoculum pattern in soil. Roth and Griffin (21) provided evidence recently that the inoculum pattern of *Cylindrocladium* in a forest nursery soil fitted the Neyman Type A (Poisson-Poisson) distribution, another contagious distribution that describes patterns that are aggregated or clumped. A poor fit to the Neyman Type A distribution was found in this study. The mechanical action of moldboard plowing and disking, used in this study, apparently was insufficient to thoroughly mix *C. crotalariae* microsclerotia in soil and yield a random pattern. According to the Poisson-logarithmic nature of the negative binomial distribution (19), it is possible that the clumps of inoculum were originally microsclerotia in individual colonized peanut root and pod systems, and that the pattern of microsclerotia within this volume follows a logarithmic distribution. The results suggest also that the clumps have a random pattern in the field.

Nash and Snyder (15) considered the pattern of *F. solani* f. sp. *phaseoli* in soil was uniform or even. As depicted in Fig. 7, uniform spatial patterns are characterized by a variance-to-mean ratio of less than one, random patterns are characterized by a variance-to-mean ratio equal to one, while contagious (clumped) patterns have a variance-to-mean ratio greater than one (6). Computations by us from the data given by Nash and Snyder (15) in Table 3 of their paper, which are based on 37 individual, approximately equally spaced soil-core samples, indicated the variance-to-mean ratios for two sampling times in a single field were 24.5 and 30.5. These ratios, much greater than one, suggest *F. solani* f. sp. *phaseoli* has a clumped pattern in the field. That no differences, at the 95% confidence level, were found among *F. solani* f. sp. *phaseoli* population densities at the various locations in the field may be due to the high variances obtained. Variance-to-mean ratios for fields based on composite soil samples (Table 4 and data given in the text on page 570) were similar or somewhat higher (32.1, 143.6, 48.7, and 30.7) to those indicated above. Indirect evidence, based on lesion pattern, that *Rhizoctonia solani* may have a clumped inoculum pattern in bean field soil has been presented recently (8).

As experimental conditions were not optimal, it was not the purpose of this study to examine directly the models of Baker et al (5). This has been done in a separate greenhouse study (Tomimatsu and Griffin, *unpublished*). However, some important considerations for root disease modeling in the field were found in regression analyses. The clumped microsclerotial pattern appeared to contribute, in part, to the low slope values and to the relatively low r values obtained in the $\log_{10}$-$\log_{10}$ plot of inoculum density vs estimated infections. This may be due to the high sample variances generated by such a pattern. However, the regression line slopes were similar to those found by Hanounik et al (11) for *C. crotalariae* lesion incidence on peanut in a greenhouse study which utilized microsclerotia that were thoroughly mixed into soil. The latter probably precluded inoculum clumping. The $\log_{10}$-$\log_{10}$ slopes obtained by Phipps et al (18), with the same host-pathogen system used here, for four North Carolina peanut fields and in the same year (1976), were also low. ED_{50} values obtained by them for October from $\log_{10}$-$\log_{10}$ plots were lower than found here for the $\log_{10}$-$\log_{10}$ plot, but were higher than found here for the arithmetic plot. With some exceptions (7), differences in capacity factors among peanut fields may be no more critical to ED_{50} values and regression line slopes within years than differences in inoculum clumping. However, as low soil water potential and high soil temperature critically reduce microsclerotium germinability (9, and Graham and Griffin, *unpublished*) and disease development (16), ED_{50} values may not have as much reliability between or among years as within years. In 1979, for example, an epiphytotic (88.7% disease) developed in the area of the field used for the present study when the inoculum density was approximately 1.1 microsclerotia per gram of soil (Tomimatsu and Griffin, *unpublished*). It does not appear that ascospores contributed greatly to disease incidence in our study, because the ED_{50} values for microsclerotia alone were high, and only a low percentage of the plants at harvest in 1975 or 1976 had perithecia.

Lloyd's index of patchiness (19) or the $\hat{N}$ parameter (index of aggregation) of the negative binomial distribution may be helpful in characterizing the degree of inoculum clumping and refining inoculum density-disease relationships in field studies. On this basis, a greater degree of aggregation or patchiness (higher index of patchiness value) was observed for *Cylindrocladium* inoculum in a Virginia forest nursery than for *C. crotalariae* in the peanut field examined here. Lloyd's index of patchiness is indicated by Y'/Y, where $Y' = \overline{Y} + (s^2 / \overline{Y} - 1)$. Patchiness values of 10.2 and 2.0 were obtained for *Cylindrocladium* in the nursery (21) and *C. crotalariae* in a peanut field, respectively. These values should not be affected by random deaths in the population *Cylindrocladium* (19). Index of patchiness values obtained by us for the *F. solani* f. sp. *phaseoli* populations, referred to above, were low (1.02 to 1.38). Together with the mean population, Lloyd's index of patchiness may find application in epidemiology of root diseases by providing a relative index of the probability that a plant root system or hypocotyl would contact inoculum, assuming a clumped inoculum pattern. Because mean fungal pathogen populations may vary over a wide range, from 10^{-3} to 10^{3} or 10^{4} propagules per gram of soil, depending on the species, it is not clear how useful $Y'/\overline{Y}$ will be for comparing the relative aggregation of soilborne fungal pathogens as a group.

Physical environmental factors appeared to have a much greater influence on microsclerotial populations than crop sequence. The low amount of rainfall during June 1975 was associated with a decrease in the number of germinable microsclerotia in the upper zone of the plow layer. In laboratory tests, Griffin et al (9) and Graham and Griffin (*unpublished*) found that drying of soil to low water potentials (−224 to −2,000 bars) in the field decreased the germinability of *C. crotalariae* microsclerotia. The presence of transpiring crop plants in all subplots, except the fallow, may be responsible for a greater apparent drought effect in those subplots. Phipps and Beute (17) did not observe a significant decrease in *C. crotalariae* microsclerotium populations in microplots planted to peanut or soybean between April and October of the same year (1975), but observed significant decreases in fallow soil, and soil planted to tobacco, cotton and corn. Their microplots were irrigated twice during the month of June (P. M. Phipps, *personal communication*). A drought in August 1975 appeared to combine with the effects of the June drought to suppress CBR development in 1975. In contrast to 1975, similar microsclerotium populations were found in both the upper and lower zone of the plow layer during 1976. The inverting and mixing effect of moldboard plowing and the mixing effect of disking may be responsible, in part, for this. Also, Griffin et al (9) found that some microsclerotia may recover from the detrimental effects of drying if soils are remoistened following exposure to drought.

The microsclerotial population in both field plots were greatly reduced in 1977, compared to 1976, following the cold winter of

1976–1977. This obscured any population density increases on susceptible crops during the 1976 growing season. Phipps and Beute (17) observed a similar association of the 1976–1977 winter temperatures with reduced microsclerotial populations in microplot experiments in North Carolina. In laboratory experiments, Roth et al (22) demonstrated that the germinability of *C. crotalariae* microsclerotia was directly related to minimum soil temperature. Axenic culture tests indicated that the reduction in microsclerotium germinability, following low temperature exposure (<6 C), probably resulted from injury to microsclerotia. The 1977–1978 winter was cold also, and microsclerotial populations remained low in field plot soils during 1978. CBR development in the Virginia peanut-growing area showed a corresponding drastic decline during the 1977 and 1978 growing seasons.

LITERATURE CITED

1. Baker, K. F., and Cook, R. J. 1974. Biological Control of Plant Pathogens. W. H. Freeman, San Francisco. 433 pp.
2. Baker, R. 1971. Analyses involving inoculum density of soilborne plant pathogens in epidemiology. Phytopathology 61:1280-1292.
3. Baker, R. 1978. Inoculum potential. Pages 137–157 in: J. G. Horsfall and E. B. Cowling, eds. Plant Disease, An Advanced Treatise. Vol. 2. Academic Press, New York, San Francisco and London. 436 pp.
4. Baker, R., and Drury, R. 1981. Inoculum potential and soilborne pathogens: The essence of every model is within the frame. Phytopathology 71:363-372.
5. Baker, R., Maurer, C. L., and Maurer, R. A. 1967. Ecology of plant pathogens in soil. VII. Mathematical models and inoculum density. Phytopathology 57:662-666.
6. Balaam, L. N. 1972. Fundamentals of Biometry. George Allen and Unwin Ltd., London 259 pp.
7. Beute, M. K. 1980. Relations of nematode populations to Cylindrocladium black rot (CBR) in peanut fields. (Abstr.). Proc. American Peanut Research and Education Soc. 12:78.
8. Campbell, C. L., and Pennypacker, S. P. 1980. Distribution of hypocotyl rot caused in snapbean by *Rhizoctonia solani*. Phytopathology 70:521-525.
9. Griffin, G. J., Roth, D. A., and Powell, N. L. 1978. Physical factors that influence the recovery of microsclerotium populations of *Cylindrocladium crotalariae* from naturally infested soils. Phytopathology 68:887-891.
10. Hadi, S. 1974. Epidemiology and control of Cylindrocladium stem canker and root rot of conifers in nurseries. Ph.D. Dissertation, University of Wisconsin. 103 pp.
11. Hanounik, S. B., Pirie, W. R. and Osborne, W. W. 1977. Influence of soil chemical treatment and host genotypes or the inoculum density-disease relationships of Cylindrocladium black rot of peanut. Plant Dis. Rep. 61:431-435.
12. Johnson, N. L., and Kotz, S. 1969. Discrete Distributions. Houghton Mifflin Co., Boston. 328 pp.
13. Krigsvold, D. T., Garren, K. H., 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.
14. Krigsvold, D. T., and Griffin, G. J. 1975. Quantitative isolation of *Cylindrocladium crotalariae* microsclerotia from naturally infested peanut and soybean field soils. Plant Dis. Rep. 59:543-546.
15. Nash, S. M., and Snyder, W. C. 1962. Quantitative estimation by plate counts of propagules of the bean root rot of *Fusarium* in soil. Phytopathology 52:567-572.
16. Phipps, P. M., and Beute, M. K. 1977. Influence of soil temperature and moisture on the severity of Cylindrocladium black rot of peanut. Phytopathology 67:1104-1107.
17. Phipps, P. M., and Beute, M. K. 1979. Population dynamics of *Cylindrocladium crotalariae* microsclerotia in naturally infested soil. Phytopathology 69:240-243.
18. Phipps, P. M., Beute, M. K., and Hadley, B. A. 1977. Relationship of inoculum density and time to incidence of Cylindrocladium black rot or peanut in naturally infested fields. (Abstr.). Proc. Am. Peanut Res. Educ. Assn. 9:51.
19. Pielou, E. C. 1969. An Introduction to Mathematical Ecology. John Wiley & Sons, New York. 286 pp.
20. Powell, N. L., Griffin, G. J., Garren, K. H., and Pettry, D. E. 1976. Use of aerial photography to detect diseases in peanut fields. II. Cylindrocladium black rot. Peanut Sci. 3:25-29.
21. Roth, D. A., and Griffin, G. J. 1981. Cylindrocladium root rot of black walnut seedlings and inoculum pattern in nursery soil. Can. J. Plant Pathology 3:1-8.
22. Roth, D. A., Griffin, G. J., and Graham, P. J. 1979. Low temperature induces decreased germinability of *Cylindrocladium* microsclerotia. Can. J. Microbiol. 25:157-162.
23. Rowe, R. C., and Beute, M. K. 1973. Susceptibility of peanut rotation crops (tobacco, cotton and corn) to *Cylindrocladium crotalariae*. Plant Dis. Rep. 57:1035-1039.
24. Vanderplank, J. E. 1975. Principles of Plant Infection. Academic Press, New York. 216 pp.