Inoculum Distribution and Sampling Methods for *Cylindrocladium crotalariae* in a Peanut Field

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ABSTRACT

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Inoculum density of *Cylindrocladium crotalariae* was determined by elutriation of samples from 288 quadrats (three 96-sample replicates) in a 723-m^2 peanut field in Bladen County, NC, in June 1980. Five frequency distributions (Poisson, negative binomial, Thomas double Poisson, Neyman type A, and Poisson with zeroes) were tested for goodness-of-fit to frequency class data for inoculum density. The negative binomial distribution model best described the frequency class data. Values of the "k" parameter—an index of aggregation—were 2.21, 2.77, and 2.09 for the three replicate samples and 2.31 for the combined 288 samples, which indicated a clumping or clustering of inoculum in soil. Nine simulated soil sampling methods, differing in area covered and shape of path, were analyzed for efficiency in estimating *C. crotalariae* populations from the field. Two methods utilizing diagonal paths (sample size = 16 or 32) had sample means within 5% of the population means. Random samples did not give accurate population estimates.

Additional key words: black rot of peanut, epidemiology, spatial distribution

Cylindrocladium black rot (CBR), induced by *Cylindrocladium crotalariae* (Loos) Bell & Sobers, was first reported in Georgia in 1966 as a peg, pod, and root rot of peanut, *Arachis hypogaea* L. (3). Since its appearance in North Carolina in 1970 (5), the fungus has become established in virtually all peanut growing areas of the state (14).

Microsclerotia are the primary source of inoculum and serve as survival and dispersal units of the fungus (13). Farm equipment and turbulent winds generated by combines play a role in local and regional dispersal of microsclerotia (8,13). Field and greenhouse evaluations of peanut germ plasm have identified several cultivars with varying degrees of resistance (17). Sensitivity of susceptible and resistant cultivars to inoculum density (ID) of *C. crotalariae* was characterized: at or above an ID of 0.5 and 50 ms (microsclerotia)/g of soil, susceptible cv. Florigiant and resistant cv. NC 3033, respectively, sustained moderate to severe root rot and extensive root infection (11). Accurate assessment of the ID in a peanut field is necessary to formulate control strategy for CBR.

The spatial distribution of propagules of plant pathogens, as with other natural populations of organisms, is seldom truly random or regular in nature (15). It is well recognized that, in comparison with other plant pathogens, many nematode species have a patchy or clumped distribution (1,10). Goodell and Ferris (7) reported that the distribution pattern of five plant-parasitic nematodes in an alfalfa field was described by negative binomial distribution functions, indicating clustering of the nematodes. Disease in plant populations also occurs in a nonrandom fashion (4,16).

A description of the distribution of *C. crotalariae* microsclerotia is important in development of sampling methods for accurate assessment of ID in peanut fields. In addition, monitoring the ID of *C. crotalariae* in peanut fields will be an indispensable component of integrated pest management programs for CBR. This study was initiated to determine the distribution of microsclerotia of *C. crotalariae* in a peanut field, to test the observed frequency distribution against five distribution models, and to compare the accuracy of estimation of ID by various sampling methods.

MATERIALS AND METHODS

In June 1980, a 723-m^2 peanut field with a history of CBR in Bladen County, NC, was divided into 96 contiguous quadrats measuring 2.75 × 2.75 m. Each quadrat was then subdivided into three equal subquadrats referred to as A, B, and C. The microsclerotial population in the respective subquadrats are referred to as populations A, B, and C. An 8.26-cm-diameter bucket sampling auger (Art's Machine Shop, American Falls, ID 83211) was used to remove two soil cores from each subquadrat in the 96 quadrats to a depth of 15 cm. A total of 288 samples was taken. Each core was placed in a polyethylene bag labeled with grid coordinates and transported to the laboratory, where all field samples were stored at room temperature (25–28 C) and assayed within 3 wk.

In the laboratory, each soil sample was mixed thoroughly by hand and screened through an opening of 0.25 cm^2. Soil moisture content was determined for each sample, and the density of microsclerotia of *C. crotalariae* was assayed using a semiautomatic elutriator (12). To test the efficiency and consistency of the elutriation method, four random soil samples were divided into four subsamples, and each subsample was assayed as described above.

Five statistical probability distribution models were tested for goodness-of-fit to data on the distribution of microsclerotia in the peanut field, using a FORTRAN program developed by Gates and Ethridge (6). Models included were Poisson, negative binomial, Thomas

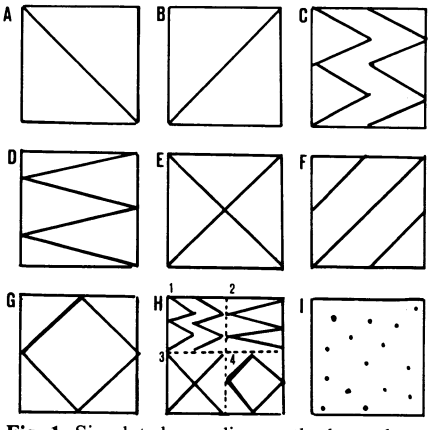

Fig. 1. Simulated sampling methods used to draw samples from the predetermined inoculum density in quadrats in the peanut field: **(A)** Left-to-right diagonal path. **(B)** Right-to-left diagonal path. **(C)** Z-shaped path (two- or four-row). **(D)** W-shaped path. **(E)** X-shaped path. **(F)** Three-diagonal path. **(G)** Diamond-shaped path. **(H)** 1 = demarcated Z, 2 = demarcated W, 3 = demarcated X, 4 = demarcated diamond-shaped path. **(I)** Random samples.

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double Poisson, Neyman type A, and Poisson with zeroes. Goodness-of-fit was determined by chi-square analysis.

Nine sampling methods differing in field area covered and shape of path were simulated to draw samples from the field (Fig. 1). Demarcated sampling refers to sampling only one quarter of the field with the method indicated. Sample mean, variance, and coefficient of variation (CV) were calculated for each of the sampling methods. All four microsclerotial populations (A, B, C, and combined) were analyzed using each sampling procedure.

RESULTS

In general, the technique of assaying microsclerotia for elutriation gave reasonably precise results. Four samples, chosen at random, were separated into four subsamples each, and the subsamples were elutriated individually. Coefficients of variation of the samples were 21, 12, 18, and 31%.

The ID ranged from 0.0 to 45.5 ms/g, with a mean ID of 7.88 ms/g. In all four populations, the variance exceeded the mean (Table 1), indicating a clumped distribution of inoculum (15). A three-dimensional map plotted for each of the sample populations (Fig. 2A–C) shows that the cluster effect was evident in the populations. Curves of the frequency distribution (Fig. 2D–F) were positively skewed, which also indicates a clumped distribution (15).

Among the five distribution models tested by chi-square analysis, only the negative binomial distribution consistently described the observed frequency distributions of microsclerotia of *C. crotalariae* (Table 2). The negative

Table 1. General statistics of the four populations of microsclerotia of *Cylindrocladium crotalariae*

Population	Sample size	Microsclerotia per gram				
		Mean	Minimum value	Maximum value	Variance	CV (%)
A	96	7.7	0.1	45.5	55.9	96.8
B	96	8.0	0.0	26.0	30.3	68.8
C	96	7.9	0.1	38.8	48.4	87.7
Combined	288	7.9	0.0	45.5	44.6	84.6

Table 2. Goodness-of-fit of distribution functions and observed frequencies of microsclerotia of *Cylindrocladium crotalariae* against five theoretical frequency models

Models	Populations							
	A		B		C		Combined	
	χ^2	P[z]	χ^2	P	χ^2	P	χ^2	P
Poisson	186.42	0.00	183.53	0.00	314.99	0.00	1,254.71	0.00
Poisson with zeroes	330.98	0.00	219.33	0.00	306.95	0.00	1,031.51	0.00
Negative binomial	21.86	0.53	24.85	0.36	18.47	0.78	22.09	0.57
Thomas double Poisson	···	0.00	···	0.00	···	0.00	···	0.00
Neyman type A	30.07	0.05	25.49	0.18	38.55	0.01	65.37	0.00

[z]Probability of exceeding calculated chi-square value.

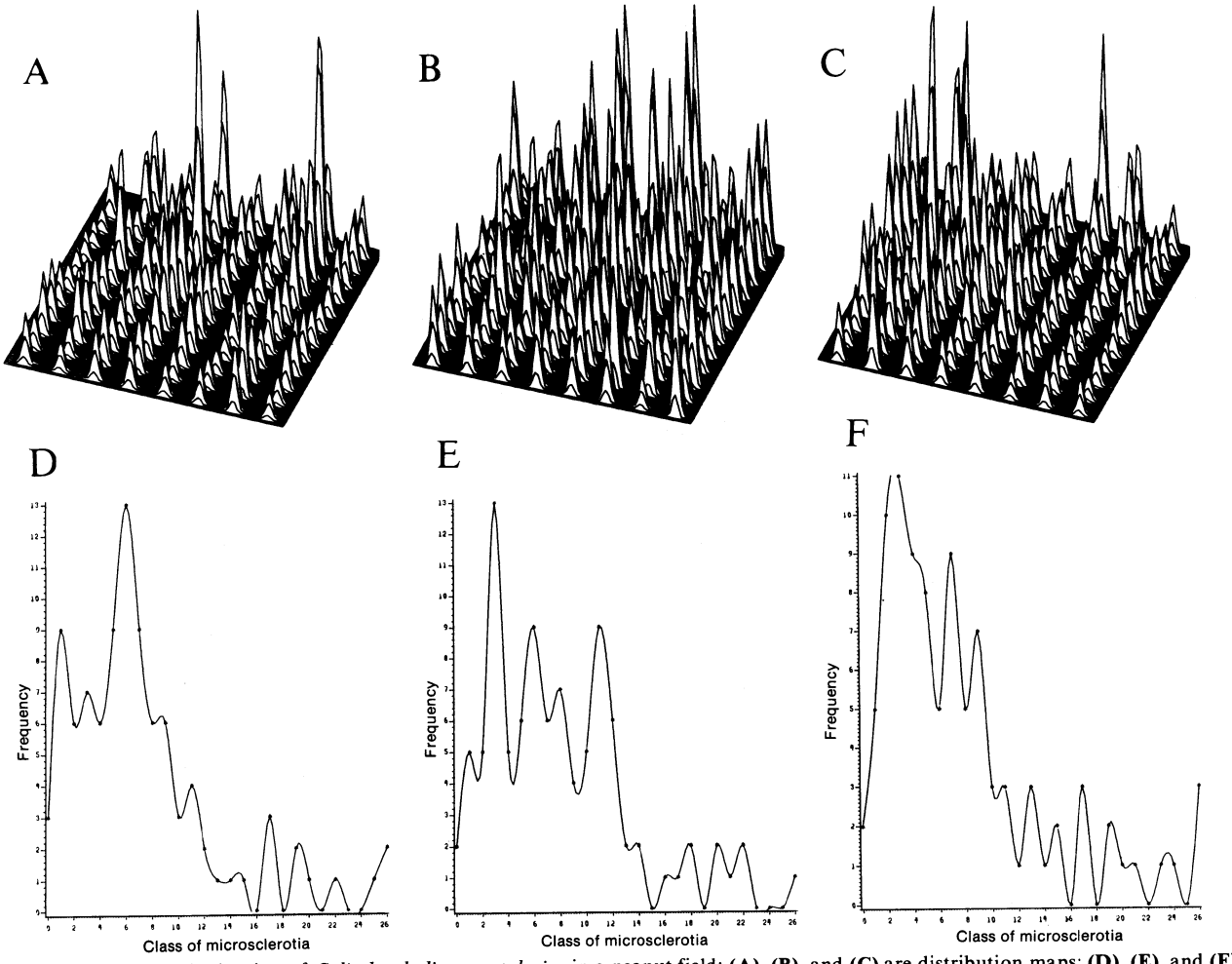

Fig. 2. Microsclerotial distribution of *Cylindrocladium crotalariae* in a peanut field: **(A)**, **(B)**, and **(C)** are distribution maps; **(D)**, **(E)**, and **(F)** are frequency distributions for populations A, B, and C, respectively. Individual class of microsclerotia represents the number of microsclerotia (ms) per gram of soil (eg, class 1 = 1 ms/g).

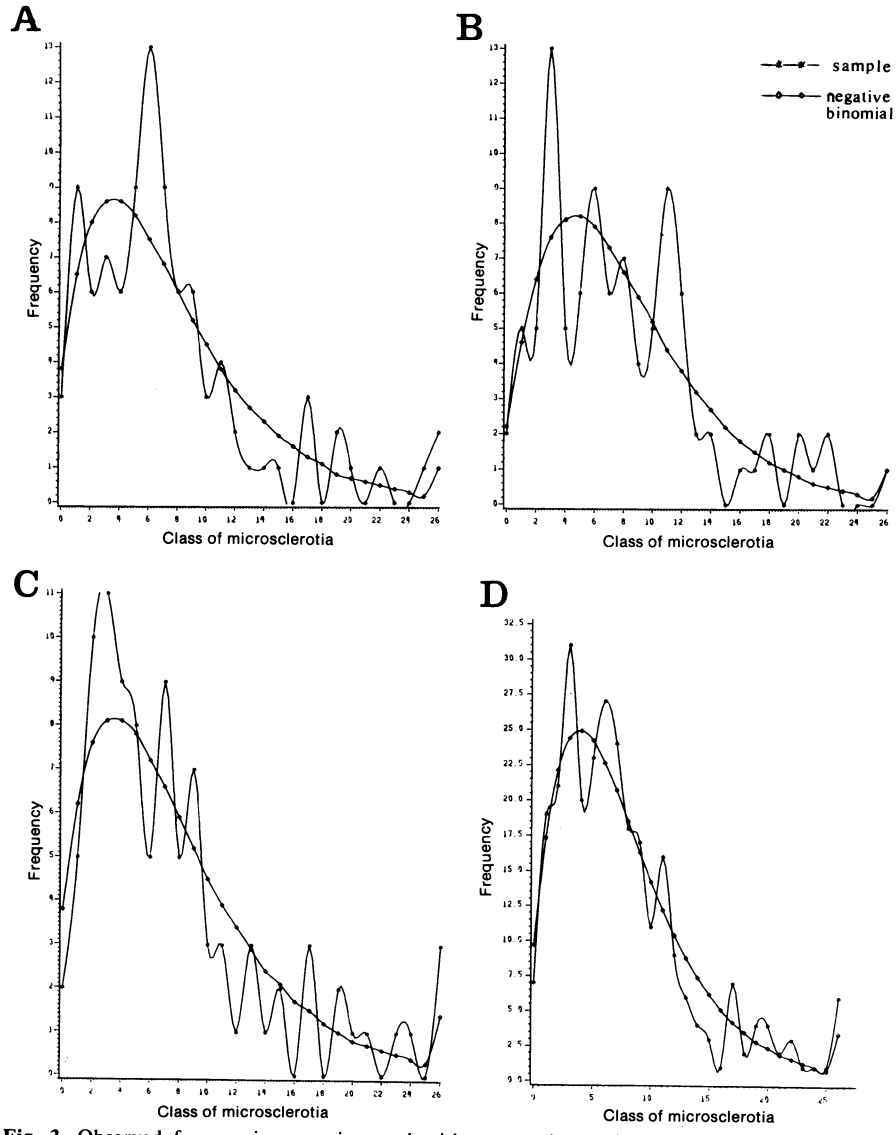

Fig. 3. Observed frequencies superimposed with expected negative binomial frequencies of microsclerotia of *Cylindrocladium crotalariae*: **(A)**, **(B)**, and **(C)** are the three 96-sample replicates; **(D)** is frequency distribution of the combined sample.

binomial distribution is described by two parameters, the mean and the k value, which is often referred to as dispersion parameter (15). Observed frequencies and expected frequencies of negative binomial distribution were both positively skewed, as shown in Figure 3. Values of k obtained in the present study were 2.21, 2.71, 2.09, and 2.31 for A, B, C, and combined sample, respectively.

Nine simulated sampling methods were used to determine accuracy in predicting population densities in the field (Fig. 1). Only the three-diagonal and diamond-shaped path methods (with either sample size of 16 or 32) had sample means within 5% of the population means. The Z-shaped path had a sample mean within 10% of all the population means (Table 3). The remainder of the sampling methods did not give consistent, accurate estimates of the population means; however, an improvement was noted in the accuracy of estimates as random sample size increased from 10 to 30 samples (Table 3). Increase in sample size did not necessarily increase accuracy of estimates or decrease CV. However, an increased number of "arms" (variable path directions) reduced CV (Table 3). For example, method A (1 arm, 16 samples) had a CV equal to 89.84%, and methods D, E, F, and G (4 arms, 16 samples) had CV percentages below 80.

DISCUSSION

The pattern of distribution of microsclerotia of *C. crotalariae*, which are survival units and serve as the primary inoculum, is not random or uniform, but rather is clumped or clustered. This pattern is best described by the negative binomial distribution. Values of the k-parameter of the negative binomial distribution ranged from 2.09 to 2.71 in

Table 3. Sample mean ($\bar{x}$) and coefficient of variation of various sampling patterns for microsclerotia of *Cylindrocladium crotalariae* drawn from predetermined inoculum densities in quadrats in peanut field with history of Cylindrocladium black rot

Sampling pattern[y]	Sample size	Population A		Population B		Population C		Combined population	
		$\bar{x}$[z]	CV (%)	$\bar{x}$	CV (%)	$\bar{x}$	CV (%)	$\bar{x}$	CV (%)
A	16	11.8	89.8	9.4	47.1	7.0	72.9	8.6*	57.9
B	16	7.5**	76.8	8.2**	61.4	8.9	96.4	10.1	89.5
C	24	7.8**	121.2	7.5*	81.5	7.6**	77.8	8.4	84.5
C	48	6.5	72.7	8.0**	75.6	8.0**	77.9	6.9	83.4
D	16	5.9	60.7	9.8	58.5	8.0**	69.5	10.2	71.5
D	32	6.3	64.3	9.1	66.2	8.5*	75.1	10.1	73.1
E	16	8.3*	78.8	10.0	40.6	7.8**	96.3	10.1	69.7
E	32	9.0	95.5	8.8	54.3	8.0**	89.40	9.7	71.5
F	16	7.5**	71.9	9.6	55.5	8.3*	86.7	7.9**	70.6
F	32	7.4**	69.9	8.1**	72.8	8.1**	88.7	6.8	79.9
G	16	7.3**	74.1	7.5*	82.9	8.5*	101.1	6.6	56.6
G	32	6.8	83.4	7.9**	89.2	7.8**	95.0	7.5**	101.1
H	12	8.7	57.9	11.2	44.7	7.7**	81.3	9.6	76.4
H	16	8.5	61.3	11.4	43.3	5.1	54.8	9.3	65.4
H	16	11.0	94.2	11.2	52.3	5.8	60.4	8.5*	64.7
H	16	9.9	98.3	10.8	55.2	7.1	85.6	10.5	96.5
I	10	11.8	65.3	8.8	67.4	5.1	58.9	8.9	58.4
I	20	9.7	78.7	8.5*	67.4	6.7	78.9	9.2	59.9
I	30	8.8	79.6	8.6*	61.0	8.2**	94.1	8.9	64.6

[y] The sampling patterns are described in Figure 1.

[z] ** and * represent sample means within 5 and 10% of population means, respectively. Population mean of A = 7.72, B = 8.00, C = 7.94, and combined = 7.89.

this study; if the k values become larger than 2, the distribution approaches and eventually is identical with that of Poisson (random), whereas k values less than 2 lead into logarithmic series (extreme clustering) (15).

The clustering of microsclerotia in the peanut field sampled illustrates the clumping of CBR-diseased plant populations frequently observed in CBR-infested peanut fields. When groups of diseased plants and root fragments containing a large quantity of microsclerotia are expelled from combines, the microsclerotia will be dispersed within peanut fields. Distribution, however, will continue to be a reflection of the distribution of infested peanut debris.

A comparison of different sampling methods for surveying alfalfa foliar diseases indicated that the precision of sampling method was of the same magnitude for all methods under random disease conditions; however, it differed considerably for clustered distribution (2). An X- or W-shaped sampling path covering the whole field was best for clustered distribution (2). Our results showed that X- and W-shaped sampling paths gave reasonable estimates of population means, but method F (three-diagonal path) and G (diamond-shaped path) consistently gave better estimates, within 5% of the population means.

Recently, Lin et al (9) examined five sampling methods under random and clustered disease distribution using simulation. They reported that when diseased plants were distributed randomly, sampling size was more important than sampling method; however, when diseased plants were clustered, sampling method became more important than sample size. Results of the present study agree with the findings of Lin et al (9) that sampling method is of major importance in a clustered situation. In our study, increasing sample size did not necessarily reduce CV, but increased arms in a sampling method did.

In light of the present results, the following practical approach for determining ID of *C. crotalariae* is suggested: Divide a 0.4-ha (1-acre) peanut field into four large quadrats and collect soil samples on either a three-diagonal or diamond-shaped path. (The number of samples required varies with size of the field sampled; 32 samples are adequate for a 0.4-ha field.) Place samples into a plastic bag and hand mix thoroughly before assay. The hand-mixing process is critical because it can improve efficiency and reproducibility of the assay. This sampling procedure should give a reasonable estimate of the ID of *C. crotalariae* in a peanut field.

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