Use of Spatial Patterns and Density of Inoculum of *Cylindrocladium crotalariae* During Field Evaluation of Partially Resistant Peanut Genotypes

A. K. Culbreath, M. K. Beute, and J. C. Wynne

Former graduate research assistant and professor, Department of Plant Pathology, and professor, Department of Crop Science, North Carolina State University, Raleigh 27695-7616. Current address of first author, Department of Plant Pathology, University of Georgia, Coastal Plain Experiment Station, Tifton 31793-0748.

Paper No. 12506 of the Journal Series of North Carolina State Agriculture Research Service, Raleigh 27696-7601.

Mention of a trade name or proprietary product does not constitute a guarantee or warranty of the product named, and does not imply approval to the exclusion of other products that may also be suitable.

Accepted for publication 16 July 1990 (submitted for electronic processing).

ABSTRACT

Culbreath, A. K., Beute, M. K., and Wynne, J. C. 1990. Use of spatial patterns and density of inoculum of *Cylindrocladium crotalariae* during field evaluation of partially resistant peanut genotypes. Phytopathology 80:1395-1400.

Three new peanut genotypes, NC Ac 18414, NC Ac 18416, and NC Ac 18417; susceptible cultivar, Florigiant; moderately resistant cultivar, NC 8C; and highly resistant genotypes, NC Ac 18016 and NC 3033, were evaluated in 1986 and 1987 for incidence of Cylindrocladium black rot in field experiments designed to take into account inoculum density and spatial patterns of propagules of *Cylindrocladium crotalariae* as well as genotype effects on disease incidence. Crop rotation and observation of previous black rot incidence were used to divide fields into quadrants with different average inoculum levels of *C. crotalariae*. Soil samples from each plot were assayed before planting each year to estimate inoculum density and to determine spatial patterns of inoculum. Estimates of inoculum density were used as an experimental design factor such that genotypes were assigned to replicated plots representing similar ranges of inoculum density. Final disease incidence, area under disease progress curve, and indices relating performance of each genotype to that of Florigiant were used for comparison of the genotypes. Incidence of black rot in NC Ac 18417 was not significantly higher than that of NC 8C in 1986, but was in 1987. NC Ac 18414 performed only slightly better than Florigiant. NC Ac 18417 was chosen for release as moderately resistant cultivar NC 10C. Significant correlations between initial inoculum level and final disease incidence were detected in 1986 for all genotypes except the highly resistant line NC 3033. In 1986, NC Ac 18414 and NC Ac 18417 appeared to be more sensitive to increases in inoculum density than the other resistant genotypes, although performances of NC 8C and NC Ac 18417 were comparable at low levels of inoculum. Correlation of disease incidence with initial inoculum was not detected in 1987.

Additional keywords: Arachis hypogaea.

Cylindrocladium black rot is a serious disease of peanut (*Arachis hypogaea* L.) in North Carolina and Virginia (4,22). The pathogen, *Cylindrocladium crotalariae* (Loos) Bell & Sobers (3), infects the underground parts of the peanut plant, causes necrosis and blackening of roots, pegs, and pods and often completely severs the taproot (21,22). Damage to the root system is evidenced by chlorosis, wilting, and death of peanut vines (21,22). Yield losses due to black rot are correlated to incidence of aboveground symptoms (14). Incidence of aboveground symptoms thus provides an indirect means of estimating disease severity in the roots without destructive sampling.

Current control strategies rely on the use of the moderately resistant cultivar NC 8C in conjunction with practices that reduce the inoculum density of the pathogen (1,5,7,9,19). Pod and seed size of NC 8C, however, often are inferior to those of Florigiant, a black rot susceptible cultivar that is a large-seeded, Virginia-type peanut, which makes NC 8C less desirable to shellers and processors. To address this problem, three new genotypes, NC Ac 18414, NC Ac 18416, and NC Ac 18417, were selected for a moderate level of partial resistance to *C. crotalariae* and improved seed and pod characteristics. In this study, evaluation of these genotypes was incorporated into an experiment designed to determine the effects of *C. crotalariae* at low, intermediate, and high microsclerotial densities on black rot incidence in resistant and susceptible peanut genotypes. Detailed comparisons of these genotypes with Florigiant, NC 8C, and the highly resistant breeding lines NC Ac 18016 and NC 3033 were necessary to determine which of the new genotypes would be the best candidate for release as a new black rot resistant cultivar.

In areas of high inoculum density of the pathogen, even resistant genotypes, including NC 8C, NC Ac 18016, and NC 3033 may

© 1990 The American Phytopathological Society

suffer severe damage (14,15,16). *C. crotalariae* produces microsclerotia as primary propagules of dispersal and survival (20,23). The microsclerotia are produced in roots of infected plants and are released into the soil upon decay of the tissue (23). Limited dispersal in the soil accounts for occurrence of nonrandom or clustered inoculum in the field (11). It is extremely important to compare genotypes at similar levels of inoculum density of *C. crotalariae* (14,16), but such comparisons in the field are complicated by the clustered pattern of inoculum. Ideally, field plot design should be structured such that all genotypes are exposed in equal replication to similar mean densities and ranges of inoculum.

An elutriation-selective medium procedure has been developed for estimating microsclerotia populations in the soil (17). Estimates of inoculum density of *C. crotalariae* can be made with this assay before planting and used to assign treatments, in this case genotypes, to plots representing similar ranges of inoculum density. The objectives of this study were to determine the spatial patterns of inoculum of *C. crotalariae* in peanut fields in 2 yr, to use knowledge of those patterns to evaluate three new black rot resistant genotypes, and to determine the effects of inoculum density of *C. crotalariae* on black rot incidence in these and other resistant and susceptible peanut genotypes representing a range of levels of resistance to the pathogen.

MATERIALS AND METHODS

Commercial growers's fields were used to evaluate black rot development in seven peanut genotypes in 1986 and 1987. Genotypes included: Florigiant as a susceptible cultivar; NC 8C as a moderately resistant cultivar; two highly resistant breeder lines, NC Ac 18016 and NC 3033; and the three new genotypes, NC Ac 18414, NC Ac 18416, and NC Ac 18417. The new genotypes were selected for combination of high yield, large pod, and seed size, and resistance to *C. crotalariae*. They originated from the peanut breeding program at North Carolina State University, headed by Dr. J. C. Wynne. NC Ac 18414 originated from a single plant selection in the F_4 generation from a cross of NC 17922 × NC 8C. NC Ac 18416 and NC Ac 18417 originated from backcrosses of selections from NC 8C with Florigiant.

Plots were established in different fields in Martin County, NC, in 1986 and 1987. High incidences of black rot had been observed in both fields in previous years. Fields selected each year had peanut and corn grown in adjacent areas in the year preceding the test year. The four quadrants (11 × 76.8 m; Fig. 1A) were demarcated after the land was prepared. Two quadrants were situated in the area of the field planted previously to peanut, and two were situated in the area of the field previously planted to corn (Fig. 2). Each of the four quadrants was divided into three groups (11 × 25.6 m; Fig. 1B), each of which consisted of 21 contiguous plots (3.7 × 3.7 m; Fig. 1B and C). Before planting (14 April 1986 and 19 April 1987), 12 soil cores (combined volume ~1 L of soil) were taken from each of the 252 plots in a zigzag pattern similar to that recommended by Hau et al (11) (Fig. 1C). The cores were mixed separately for each plot and partitioned into subsamples for assay for microsclerotia of *C. crotalariae*. Numbers of microsclerotia per gram of soil were estimated for each plot with an elutriation-selective medium procedure developed by Phipps et al (17). The 21 plots in each group were then ranked by inoculum density estimates and were divided into three inoculum density classes, each representing seven plots of relative high, medium, and low inoculum. Each genotype was assigned at random to one plot in each inoculum density class in each group such that each genotype appeared in three plots per group, or in 36 plots across the field. A nested experimental design was used, with three replications (inoculum classes) of seven genotypes nested in the 21 plot groups which were nested in the four quadrants.

Variance-to-mean ratios (S^2/X) (18) and Lloyd's index of patchiness [$(X + S^2/X) - 1$] (12) were calculated as indices of dispersion for each quadrant in both years. For each index, values >1 indicate clustered inoculum. Inoculum density maps were constructed using microsclerotia levels of 0–2 (low), 2–5 (medium), and >5 (high) microsclerotia per gram of soil.

Ninety-five to 100 seeds were hand-planted in each plot (25 per row) at a spacing of 12–13 cm on 2 May 1986 and 7 May 1987. The seeding rate was approximately half that used for commercial production. Sparser plant stands were used to allow evaluation of individual plants. Rows were 91 cm apart. Carbofuran (2.25 kg a.i./ha) was applied in furrow at planting for nematode

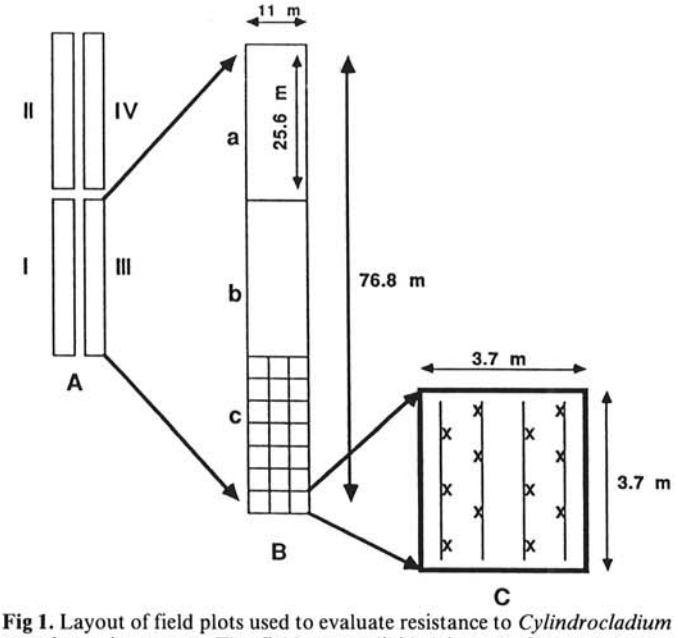

Fig 1. Layout of field plots used to evaluate resistance to *Cylindrocladium crotalariae* in peanut. The fields were divided into **A,** four quadrants; **B,** three subdivisions (a, b, and c) per quadrant, and four rows per plot. X's denote sampling pattern used in each plot.

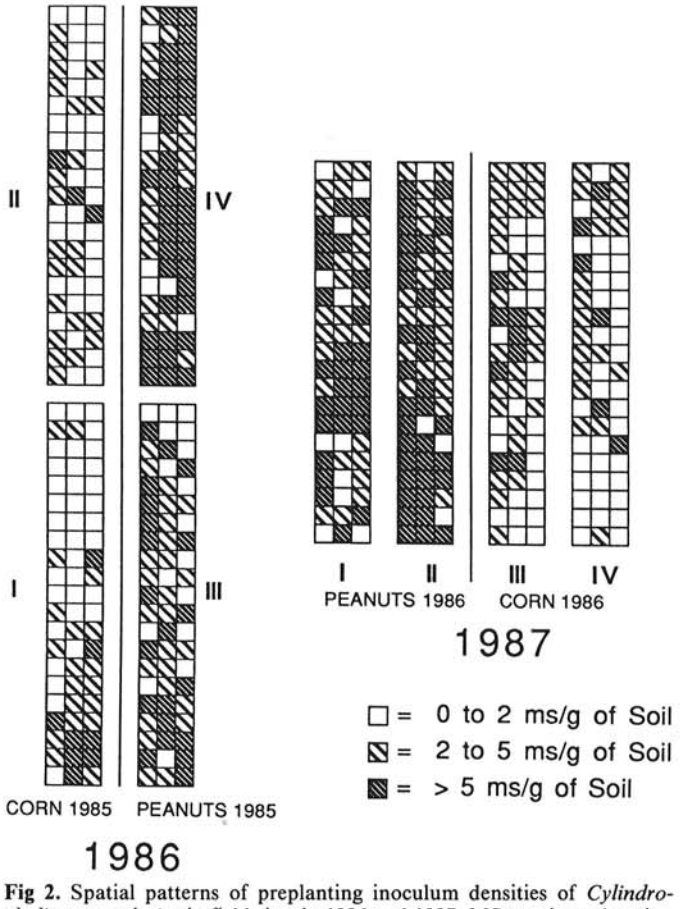

Fig 2. Spatial patterns of preplanting inoculum densities of *Cylindrocladium crotalariae* in field sites in 1986 and 1987. MS = microsclerotia.

and insect control. The plants were maintained and other pests controlled as recommended for commercial peanut production (27).

Assessments of black rot incidence were made weekly from observation of first symptoms until harvest by counting the number of dead and wilted plants per plot. Plants were marked each week with colored surveyors's flags, and the number and position of symptomatic plants were recorded for each row each week. Percent disease incidence was calculated by dividing number of dead and wilted plants by the total plant stand per plot.

Area under the disease progress curve (AUDPC) was calculated for each plot according to the formula:

$$\text{AUDPC} = \sum_{i=0}^{n-1}[(L_{i+1} + L_i)/2][(t_{i+1} - t_i)]$$

where t = time in days, $i = 0...n$, L_i = apparent disease incidence on day i (24). Nine and eight weeks of disease incidence observations were used in AUDPC calculations for the epidemics in 1986 and 1987, respectively. Final black rot incidence was recorded 20 wk after planting in both years.

Indices expressing both final disease incidence and AUDPC as percentage of that of Florigiant were calculated for each of the other genotypes for each quadrant as well as for the entire field in both years. Data (including initial inoculum of the pathogen, final disease incidence, AUDPC, final incidence indices, and AUDPC indices) were analyzed with analysis of variance (ANOVA). Statistical significance of genotype effects was evaluated by single-degree-of-freedom contrast statements and by Fisher's least significant difference (LSD) (26). Differences referred to in the text were significant at the 0.05 level of probability unless otherwise stated. Pearson's correlation coefficients were calculated for the relationship between final disease incidence and initial inoculum density of the pathogen. Linear regression was used to describe the response in final incidence to increasing inoculum density for each genotype (26). Slopes of inoculum density-disease incidence lines were tested for homogeneity using Student's t tests (25). LD_{50} values were estimated for each genotype in which significant correlations between final incidence and inoculum density were detected. These represent initial inoculum density level that caused wilting or death of half of the plant population.

RESULTS

In both years, quadrants of the field planted to peanuts the previous year had inoculum levels more than twice those of the quadrants planted to corn the previous year (Table 1). Inoculum was clustered in all quadrants in both years as evidenced by maps of the inoculum density classes and indices of dispersion (Fig. 2, Table 1). In all quadrants, values for S^2/X and Lloyd's index of patchiness were greater than one, indicating clustered or nonrandom inoculum patterns. Inoculum densities ranged from below the detection limit of the assay (~ 0.2 microsclerotia per gram of soil) to 25 and 36 microsclerotia per gram of soil for 1986 and 1987, respectively.

Initial inoculum density values were similar for all genotypes in 1986 (Table 2). No significant differences were detected in inoculum density for genotypes in either individual quadrants or across the entire field. In 1987, inoculum density values were similar for most genotypes. Significant differences were detected in three quadrants. In quadrants 1 and 2, only genotypes representing the highest and lowest inoculum density levels were different. In quadrant 3, NC Ac 18016 had an initial inoculum density that was significantly higher than those of both NC Ac 18417 and NC 3033, although differences were 1.37 microsclerotia per gram of soil or less. Only NC Ac 18416 had inoculum density estimates that were significantly higher than those of other genotypes across the entire field.

Overall disease incidence was generally less in 1986 than in 1987 (Table 3), but final incidence values and AUDPCs of resistant genotypes were less than those of Florigiant in both years (Table 3). The new genotypes were ranked the same by black rot incidence and AUDPC in both years (Table 3). Quadrant, groups nested in quadrants, inoculum density classes nested in quadrants, quadrant by groups, genotype, and quadrant by genotype interaction effects on AUDPC were highly significant ($P = 0.01$) in 1986. All of these except inoculum density class, which was significant ($P = 0.05$), were highly significant for final incidence also. No significant group by genotype interaction was detected for either variable. AUDPC of NC Ac 18416 ranked lowest among the new genotype, in 1986, although the difference between NC Ac 18416 and NC Ac 18417 was not significant in 1987. Among the three new genotypes, AUDPC of NC Ac 18414 ranked highest in both years when averaged over the four quadrants. NC Ac 18416 performed similarly to NC 8C in both years. NC Ac 18417 was comparable to NC 8C in 1986 with AUDPC values that were not significantly different. In 1987, quadrant, groups within quadrants and genotype effects were significant for AUDPC and final incidence. No other effect was significant for either variable. In 1987, AUDPC for NC Ac 18417 was significantly greater than for NC 8C. Highly resistant genotypes NC Ac 18016 and NC 3033 ranked lowest in AUDPC values in both years and did not differ from each other (Table 3). In 1986, differences in final black rot incidence associated with genotypes were similar to

TABLE 1. Inoculum densities of *Cylindrocladium crotalariae* and their indices of dispersion for four quadrants at March County, North Carolina, field sites

Quadrant	Crop previous year	Mean inoculum density[a]	Variance to mean ratio[b]	Loyd's index of patchiness[c]
1986				
I	Corn	2.26	2.26	1.56
II	Corn	2.11	1.30	1.14
III	Peanut	4.63	2.98	1.43
IV	Peanut	7.29	3.18	1.30
1987				
I	Peanut	5.17	4.86	1.75
II	Peanut	6.03	1.78	1.13
III	Corn	2.42	1.37	1.15
IV	Corn	1.99	1.49	1.25

[a] Microsclerotia per gram of dry soil.
[b] Values greater than 1 indicate clustered patterns of inoculum.
[c] Values greater than 1 indicate clustered patterns of inoculum.

TABLE 2. Mean inoculum density estimates for genotypes in Cylindrocladium black rot evaluation experiments in 1986 and 1987

Genotype	Inoculum density[a] Quadrant 1	Quadrant 2	Quadrant 3	Quadrant 4	Combined
1986					
Florigiant	1.90	2.17	4.14	7.96	4.04
NC 8C	2.38	2.08	4.83	7.06	4.09
NC Ac 18417	2.09	2.34	4.52	7.65	4.15
NC Ac 18414	2.0	2.03	5.16	7.55	4.18
NC Ac 18416	2.92	2.15	3.88	6.99	3.99
NC Ac 18016	2.24	1.95	5.24	7.77	4.31
NC 3033	2.27	2.06	4.67	6.04	3.76
LSD ($P=0.05$)	1.20	0.63	2.15	2.21	0.82
1987					
Florigant	3.60	6.03	2.59	2.11	3.58
NC 8C	4.78	5.64	2.40	1.69	3.63
NC Ac 18417	5.55	5.68	2.11	1.77	3.80
NC Ac 18414	4.77	5.79	2.23	2.25	3.76
NC Ac 18416	8.06	6.63	2.36	2.20	4.81
NC Ac 18016	4.59	6.85	3.34	1.95	4.17
NC 3033	4.91	5.51	1.97	1.93	3.58
LSD ($P=0.05$)	3.79	1.22	1.13	0.94	1.09

[a] Microsclerotia per gram of dry soil.

differences in AUDPC except in NC Ac 18416, where final incidence was greater that of NC Ac 18016, whereas AUDPCs of these genotypes were not different. Ranking of the genotypes was the same for both variables (Table 3).

Performances of most of the genotypes in proportion to the performance of Florigiant were consistent over the 2 yr (Table 4). Indices for final incidence and AUDPC were similar, although the final incidence index tended to be slightly higher than the AUDPC index for most genotypes in both years.

In 1986, final incidence and AUDPC increased with increasing initial inoculum density as indicated by the significant correlations between final incidence or AUDPC and inoculum density for all genotypes except NC 3033 (Table 5). In regressions of final incidence on inoculum density, slopes were significantly greater than zero for all genotypes except NC 3033 (Fig. 3). Estimated slope parameters were similar in Florigiant, NC Ac 18414, and NC Ac 18417, although Florigiant had higher intercept values. Lower slopes indicated that disease in NC 8C, NC Ac 18416, and NC Ac 18016 was not as sensitive to increased initial inoculum density as the other moderately resistant and susceptible genotypes. Response to inoculum density in Nc Ac 18016 was small, and response to increases in inoculum density was not detected in NC 3033 in the inoculum density range represented in this field. Estimates of initial inoculum density required to cause 50% dead and wilted plants were 16.6, 9.1, 6.0, and 27.3 for moderately resistant genotypes, NC 8C, NC Ac 18417, NC Ac 18414, and NC Ac 18416, respectively, in comparison to 3.4 for Florigiant. LD_{50} was 82.8 for NC Ac 18016. In 1987, no significant correlations were detected between final incidence or AUDPC and inoculum density in any genotype (Table 5).

DISCUSSION

Nonrandom inoculum patterns generally complicate field investigations involving most soilborne pathogens (8) and may cause erroneous conclusions in evaluation of treatment effects or disease losses. In several disease systems, assays are available for estimation of initial inoculum density of the pathogen before

TABLE 3. Effect of peanut genotype on Cylindrocladium black rot final disease incidence and area under the disease progress curves for 1986 and 1987 field evaluations

	Quadrant 1		Quadrant 2		Quadrant 3		Quadrant 4		Combined	
Genotype	FI[a]	AUDC[b]	FI	AUDC	FI	AUDC	FI	AUDC	FI	AUDC
1986										
Florigiant	34.5	493.0	35.2	622.7	68.5	1,129.3	72.2	1,370.4	52.6	903.9
NC 8C	7.2	68.5	9.1	120.5	40.3	384.0	41.3	673.6	24.5	311.7
NC Ac 18417	12.7	143.5	18.5	303.7	38.1	469.9	52.7	839.4	30.5	439.1
NC Ac 18414	29.4	457.6	27.4	415.6	61.3	920.9	53.1	834.3	42.8	658.1
NC Ac 18416	9.6	129.2	13.4	259.1	17.0	217.9	21.6	394.7	15.4	250.2
Nc Ac 18016	1.6	30.6	3.8	110.6	5.1	81.9	8.2	120.0	4.6	85.8
NC 3033	1.0	4.6	1.2	19.9	2.3	34.0	3.6	56.8	2.0	28.8
LSD ($P = 0.05$)	8.0	126.5	9.2	207.9	14.1	286.4	22.3	442.5	7.1	141.5
1987										
Florigiant	75.2	1,058.6	66.0	1,078.9	77.4	1,509.5	63.0	824.2	70.4	1,115.3
NC 8C	29.6	264.8	19.0	176.7	53.8	617.4	32.0	324.4	33.6	345.8
NC Ac 18417	40.8	445.9	35.9	400.7	7.2	687.0	54.3	548.2	47.1	520.5
NC Ac 18414	59.5	695.3	46.7	478.7	53.8	599.8	55.7	674.4	53.9	612.1
NC Ac 18416	21.0	221.4	29.1	405.9	39.6	602.1	29.4	369.0	29.8	399.6
NC Ac 18016	4.2	52.5	7.1	75.1	16.7	242.1	11.0	109.0	9.8	119.7
NC 3033	2.7	28.4	2.3	28.8	7.9	95.6	5.2	61.2	4.5	53.5
LSD ($P = 0.05$)	15.2	250.7	13.7	291.5	20.9	359.3	20.2	235.8	8.3	134.6

[a] Final black rot incidence 140 days after planting.
[b] Area under disease curves for 9 wk of epidemic, 1986, and for 8 wk of epidemic, 1987.

TABLE 4. Disease index values for final Cylindrocladium black rot disease incidence and area under the disease progress curves for 1986 and 1987 field evaluations

	Quadrant 1		Quadrant 2		Quadrant 3		Quadrant 4		Combined	
Genotype	FI[a]	AUDC[b]	FI	AUDC	FI	AUDC	FI	AUDC	FI	AUDC
1986										
Florigiant	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
NC 8C	0.20	0.14	0.19	0.26	0.59	0.34	0.57	0.49	0.41	0.29
NC Ac 18417	0.36	0.29	0.49	0.52	0.57	0.42	0.73	0.61	0.54	0.45
NC Ac 18414	0.85	0.93	0.67	0.78	0.90	0.82	0.73	0.61	0.82	0.76
NC Ac 18416	0.28	0.26	0.41	0.38	0.25	0.19	0.30	0.29	0.30	0.29
NC Ac 18016	0.03	0.06	0.17	0.11	0.07	0.07	0.11	0.09	0.08	0.09
NC 3033	0.03	0.01	0.03	0.04	0.03	0.03	0.05	0.04	0.04	0.03
LSD ($P = 0.05$)	0.23	0.26	0.26	0.26	0.21	0.25	0.31	0.32	0.14	0.13
1987										
Florigiant	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
NC 8C	0.39	0.25	0.29	0.16	0.69	0.41	0.51	0.39	0.47	0.30
NC Ac 18417	0.54	0.43	0.54	0.37	0.74	0.45	0.86	0.66	0.67	0.48
NC Ac 18414	0.79	0.66	0.71	0.44	0.51	0.40	0.88	0.81	0.76	0.58
NC Ac 18416	0.28	0.21	0.44	0.37	0.51	0.40	0.46	0.45	0.42	0.35
NC Ac 18016	0.06	0.05	0.11	0.07	0.22	0.16	0.18	0.13	0.14	0.10
NC 3033	0.04	0.03	0.04	0.03	0.11	0.06	0.08	0.07	0.06	0.05
LSD ($P = 0.05$)	0.04	0.03	0.04	0.03	0.11	0.06	0.08	0.07	0.06	0.05

[a] Calculated as incidence divided by average incidence for Florigiant in that group.
[b] Calculated as area under disease progress curve divided by average area under disease progress curve for Florigiant for that group.

planting (8). Such assays allow consideration of inoculum level in designing the experiment such that effects of clustered inoculum may be overcome. Although inoculum of *C. crotalariae* typically is clustered in the field, it lends itself to assay via an elutriation-selective medium procedure (17). With estimates of the inoculum density in each plot, inoculum patterns may be determined and treatments (in this case genotypes) can be assigned to plots based on inoculum grouping rather than spatial proximity of the plots. This not only helps reduce the chances of erroneous conclusions regarding treatment effects due to unknown inoculum effects, but also allows evaluation of the inoculum effects themselves.

If genotypes are to be compared in fields that are infested with soilborne pathogens with clustered inoculum, random assignment of genotypes to plots does not ensure that the ranges in which genotypes are represented will be similar. By assigning each genotype to a particular range of inoculum, we helped to ensure that differences observed were due to levels of resistance, and that we could examine appearance and progression of symptoms at different inoculum levels. In addition, we demonstrated that when environmental conditions permit, inoculum of *C. crotalariae* can be related to black rot incidence in these genotypes and that the peanut genotype may determine the magnitude of the inoculum density effects on disease.

The experimental design used in this investigation allowed us to represent the genotypes in similar inoculum density ranges. Although inoculum density of the pathogen was clustered in both fields used, significant differences in inoculum density for different genotypes were not detected in 1986 and were detected only for one genotype in each of three quadrants in 1987. With one exception in quadrant 1, these differences were still very small considering the range of inoculum density possible in fields with clustered inoculum (11). Only one genotype had initial inoculum density estimates that were higher than any other across all quadrants. This serves as reminder that, given the clustered patterns of inoculum density of *C. crotalariae* in a field, it is difficult, if not impossible, to establish identical inoculum density ranges for several different genotypes. However, the lack of differences in any genotype in 1986 and most of the genotypes in 1987 demonstrates that similar ranges can be established in fields with clustered inoculum.

In the black rot system, increase in root rot severity resulting from increase in inoculum density has been demonstrated for both susceptible and resistant peanut genotypes in greenhouse, microplot, and field experiments (6,15,17). Correlation of final black rot incidence with initial inoculum density of *C. crotalariae* in the black rot-susceptible cultivar Florigiant has been reported (28). In 1986, we detected a relationship between inoculum density and final disease incidence in six of the seven genotypes, representing both resistant and susceptible genotypes. Black rot incidence was not correlated with inoculum density in NC 3033, which is highly resistant, and usually requires >50 microsclerotia per gram of soil for severe black rot damage (16). In the other genotypes, significant correlations between disease incidence and inoculum density, and slopes in regressions of incidence on inoculum density indicated that incidence of black rot was related to inoculum density. Increases in incidence with increasing inoculum density in Florigiant, NC Ac 18414, and NC Ac 18417 were similar. The estimated *y*-axis intercept for Florigiant was greater than zero and may indicate that it is sensitive to inoculum density levels below the detection limit of the inoculum assay. Although NC Ac 18417 and NC 8C had similar *y*-axis intercepts, response of plants of NC Ac 18417 to increases in inoculum density was greater than that of NC 8C. Predicted LD_{50} gives an indication of combined intercept and slope components for the inoculum density-incidence relationship in each genotype. LD_{50} estimates for Florigiant, NC Ac 18414, and NC Ac 18417 were well within the range of inoculum encountered in the experiment. Predicted LD_{50} value for NC 8C was in the range of inoculum density in the field. However, very few plots occurred in that range. Although LD_{50} estimates for NC Ac 18416 and NC Ac 18016 are beyond the range of inoculum that was found, these estimates still serve as indices to consider in the characterization of the responses of the genotypes.

In 1987, our inability to detect a relationship between inoculum density and disease incidence may have been caused by environmental and biotic effects. Black rot severity depends on many environmental factors (4,25); the combination of drought stress on the plants during much of the early 1987 season with increased moisture and conditions conducive for black rot development during the latter part of the season may have enhanced black rot severity and rapid disease progress in areas with even low inoculum density. In addition, levels and species of root-knot nematode (*Meloidogyne* spp.) present were different for the two years (A. K. Culbreath, *unpublished data*). In 1987, late-season populations of root-knot nematode juveniles were higher in quadrants planted to corn the previous year than in those previously planted to peanut. The opposite trend was observed in 1986. Root-knot nematodes have been shown to enhance black rot severity (10), and high populations may have enhanced the efficiency of the inoculum of the fungal pathogen. High incidence was observed in all susceptible and moderately resistant genotypes in even the low (<2 microsclerotia per gram of soil) inoculum density class. In both years, the choice of fields with high black rot incidence

TABLE 5. Pearson's correlation coefficients for relationship between disease incidence and area under disease progress curves in peanut and inoculum density of *Cylindrocladium crotalariae* in 1986 and 1987 field experiments

	1986		1987	
Peanut genotype	AUDC	Final incidence	AUDC	Final incidence
Florigiant	0.55**[a]	0.59**	NS	NS
NC 8C	0.36*	0.35*	NS	NS
NC Ac 18417	0.68**	0.67**	NS	NS
NC Ac 18414	0.58**	0.60**	NS	NS
NC Ac 18416	0.57**	0.60**	NS	NS
Nc Ac 18016	0.38*	0.51**	NS	NS
NC 3033	NS	NS	NS	NS
All genotypes	0.37**	0.37**	NS	NS

[a] * and ** indicate significantly different from 0 at $P = 0.05$ and 0.01, respectively.

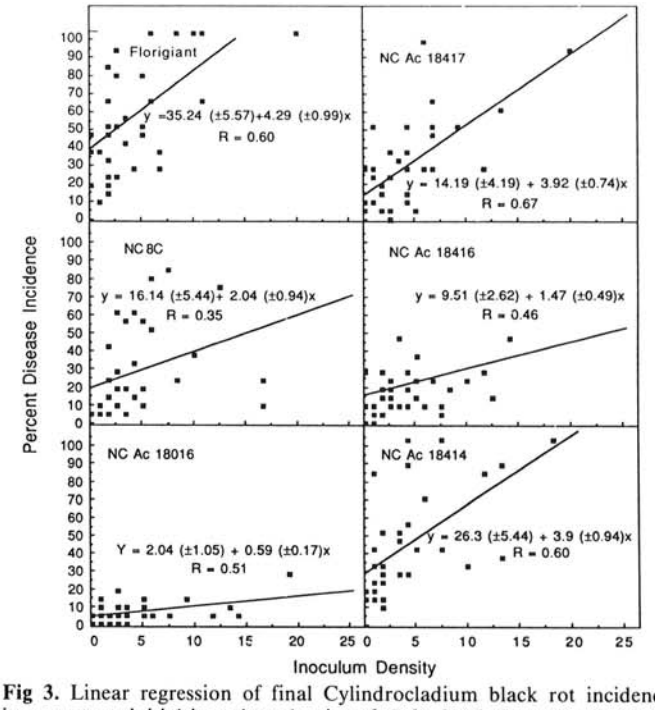

Fig 3. Linear regression of final Cylindrocladium black rot incidence in peanut on initial inoculum density of *Cylindrocladium crotalariae* in 1986.

in past years, early planting dates (25), and unbedded rows (25) were used to promote high amounts of disease. In 1987, extremely high disease pressure in plots, with even low inoculum density, may have masked effects of the inoculum density on disease incidence. Whereas Florigiant, NC Ac 18414, and NC Ac 18016 tended to have higher average final incidence and AUDPC values in medium and high inoculum density classes, the increase was not sufficient to allow detection for a significant correlation. This emphasizes that inoculum density is not the sole factor that determines apparent incidence of black rot in any of these genotypes.

Although the relationship between inoculum density and disease incidence was inconsistent between the 2 yr, rankings of the individual genotypes were consistent. Given the relationship between incidence and inoculum density in 1986, conclusions about one or more of the moderately resistant genotypes could have been quite different, had they been represented in plots with inoculum density lower or higher than that of the other genotypes. By accounting for spatial patterns and density of inoculum, we also can have greater confidence that differences in performance genotypes were not due to large differences in inoculum in plots in which the genotypes were evaluated in either year.

NC Ac 18417 is being released as cultivar NC 10C. Although NC Ac 18416 ranked best in disease resistance in our experiments both years and ranked slightly better than NC 8C in 1986, further evaluation of pod and seed characteristics of NC Ac 18416 showed too little improvement in comparison with NC 8C to justify its release. NC Ac 18417 is comparable to Florigiant in quality and yield (13), and although not as resistant as NC 8C, is more resistant than Florigiant.

Knowledge of the effects of inoculum density on black rot incidence and severity in NC Ac 18417 (NC 10C) should be valuable in deploying this genotype in black rot control programs. Although, overall, NC Ac 18417 is less resistant to *C. crotalariae* than NC 8C, improvements in size of the pods and seed as compared to NC 8C, may compensate for somewhat higher disease losses. In 1986, when inoculum density effects were detected, disease incidence and progress curves for the two genotypes were similar in low (fewer than two microsclerotia per gram of soil) and medium (two to five microsclerotia per gram of soil) inoculum density classes, although disease incidence in the two genotypes was different in the high inoculum class. This indicates, as do regression parameters, that NC Ac 18417 responds more drastically than the other genotypes to inoculum density increases, but also indicates that performance of NC 8C and NC Ac 18417 should be similar if low inoculum density is maintained. This relationship is supported by first-year results of an experiment combining the use of NC 10C (NC Ac 18417) and NC 8C with preplant fumigations using metam sodium to reduce inoculum of *C. crotalariae* (2). Final black rot incidence in NC Ac 18417 was as high as that in a susceptible check (NC 7) in untreated plots, but response of NC Ac 18417 to fumigant application was comparable to that of NC 8C (2). Based on 1986 results and preliminary fumigation results, NC Ac 18417 should perform well in this inoculum density range. Adequate black rot control with NC Ac 18417 depends on maintenance of low levels of inoculum of *C. crotalariae* through the use of treatments such as fumigants and crop rotation.

LITERATURE CITED

1. Bailey, J. E. 1983. Use of fumigants for black root rot (CBR) control. Virginia-Carolina Peanut News 29(1):14.
2. Bailey, J. E. 1987. Applied research on peanuts, cotton and wheat. N. C. State Univ. Ext. Rep. 58 pp.
3. Bell, D. K., and Sobers, E. K. 1966. A peg, pod, and root necrosis of peanut caused by a species of *Calonectria*. Phytopathology 56:1361-1364.
4. Beute, M. K. 1980. Cylindrocladium Black Rot (CBR) disease of peanuts (*Arachis hypogaea*). Pages 171-176 in: Proceedings of International Workshop on Groundnuts. ICRISAT, Patancheru, P.O. Andra Pradesh 502 324 India. 324 pp.
5. Black, M. C., and Beute, M. K. 1984. Effects of rotations with susceptible and resistant peanuts, soybeans and corn on inoculum efficiency of *Cylindrocladium crotalariae* on peanuts. Plant Dis. 68:401-405.
6. Black, M. C., and Beute, M. K. 1984. Relationships among inoculum density microsclerotium size, and inoculum efficiency of *Cylindrocladium crotalariae* causing root rot on peanuts. Phytopathology 74:1128-1132.
7. Black, M. C., Pataky, J. K., Beute, M. K., and Wynne, J. C. 1984. Management tactics that compliment host resistance for control of Cylindrocladium black rot of peanuts. Peanut Sci. 11:70-73.
8. Campbell, C. L., and Noe, J. P. 1985. The spatial analysis of soilborne pathogens and root diseases. Annu. Rev. Phytopathol. 23:129-148.
9. Cline, W. O., and Beute, M. K. 1986. Effect of metam-sodium, peanut genotype and inoculum density on incidence of Cylindrocladium black rot. Peanut Sci. 13:41-45.
10. Diomande, M., and Beute, M. K. 1981. Effects of *Meloidogyne hapla* and *Macroposthonia ornata* on Cylindrocladium black rot of peanut. Phytopathology 65:491-496.
11. Hau, F. C., Campbell, C. L., and Beute, M. K. 1982. Inoculum distribution and sampling methods for *Cylindrocladium crotalariae* in a peanut field. Plant Dis. 66:568-571.
12. Lloyd, M. 1967. Mean crowding. J. Anim. Ecol. 36:1-30.
13. Mozingo, R. W. 1986. Peanut variety and quality evaluation results. V. Polytech. Inst. State Univ. Info. Ser. 164. 35 pp.
14. Pataky, J. K., and Beute, M. K. 1983. Peanut yield, market quality, and value reductions due to Cylindrocladium black rot. Peanut Sci. 10:62-66.
15. Pataky, J. K., Black, M. C., Beute, M. K., and Wynne, J. C. 1983. Comparative analysis of Cylindrocladium black rot resistance in peanut: Greenhouse, microplot, and field testing procedures. Phytopathology 73:1615-1612.
16. Phipps, P. M., and Beute, M. K. 1977. Sensitivity of susceptible and resistant peanut cultivars to inoculum densities of *Cylindrocladium crotalariae* microsclerotia in soil. Plant Dis. Rep. 57:1035-1039.
17. Phipps, P. M., Beute, M. K., and Barker, K. R. 1976. An elutriation method for quantitative isolation of *Cylindrocladium crotalariae* microsclerotia from peanut field soil. Phytopathology 66:1255-1259.
18. Pielou, E. C. 1969. An Introduction to Mathematical Ecology. John Wiley & Sons, New York. 286 pp.
19. Rowe, R. C., and Beute, M. K. 1973. Susceptibility of peanut rotational crops (tobacco, cotton, and corn) to *Cylindrocladium crotalariae*. Plant Dis. Rep. 57:1035-1039.
20. Rowe, R. C., and Beute, M. K. 1975. Ascospore formation and discharge by *Calonectria crotalariae*. Phytopathology 65:393-398.
21. Rowe, R. C., Beute, M. K., and Wells, J. C. 1973. Cylindrocladium black rot of peanuts in North Carolina—1972. Plant Dis. Rep. 57:387-389.
22. Rowe, R. C., Beute, M. K., Wells, J. C., and Wynne, J. C. 1973. Incidence and control of Cylindrocladium black rot of peanuts in North Carolina during 1973. Plant Dis. Rep. 58:348-352.
23. Rowe, R. C., Johnson, S. A., and Beute, M. K. 1974. Formation and dispersal of *Cylindrocladium crotalariae* microsclerotia in infected peanut roots. Phytopathology 64:1294-1297.
24. Shaner, G., and Finney, P. E. 1977. The effect of nitrogen fertilizer on expression of slow mildewing resistance in Knox wheat. Phytopathology 67:1051-1056.
25. Sidebottom, J. R., and Beute, M. K. 1989. Control of Cylindrocladium black rot of peanut with cultural practices that modify soil temperature. Plant Dis. 73:672-676.
26. Steel, R. G. D., and Torrie, J. D. 1960. Principles of Statistics. McGraw-Hill Book Co., New York. 481 pp.
27. Sullivan, G. A., ed. 1984. Peanuts. N. C. Agric. Ext. Bull. AG-33. 67 pp.
28. Taylor, J. D., Griffin, G. J., and Garren, K. H. 1981. Inoculum pattern, inoculum density-disease incidence relationships, and populations of *Cylindrocladium crotalariae* microsclerotia in peanut field soil. Phytopathology 71:1297-1302.