Comparative Analysis of Cylindrocladium Black Rot Resistance in Peanut: Greenhouse, Microplot, and Field Testing Procedures

J. K. Pataky, M. C. Black, M. K. Beute, and J. C. Wynne

Graduate research assistant, research assistant, and professor, Department of Plant Pathology, and associate professor, Department of Crop Science, respectively, North Carolina State University, Raleigh 27650. Present address of the senior author: Department of Plant Pathology, University of Illinois, Urbana 61801.

Journal Series Paper 8793 of the North Carolina Agricultural Research Service, Raleigh.

The authors wish to thank Joyce Hollowell and Kevin Jones for technical assistance.

The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service of the products named, or criticism of similar ones not mentioned.

Accepted for publication 15 June 1983.

ABSTRACT

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 73:1615-1620.

Two promising advanced generation Virginia-type peanut breeding lines, NC 18016 and NC 18229, and two commercial Virginia-type cultivars, NC 8C and Florigiant, were evaluated for Cylindrocladium black rot (CBR) resistance in greenhouse, microplot, and field trials. The overall evaluation of results indicated that NC 18016 was slightly more resistant than NC 18229; NC 8C was intermediate in resistance between the highly susceptible cultivar, Florigiant, and the resistant breeding lines, NC 18229 and NC 18016. Interpretations of results were slightly different when greenhouse, microplot, and field testing procedures were compared. In greenhouse and microplot evaluations, substantial differences in CBR resistance among cultivars Florigiant and NC 8C, and the two breeding lines were apparent, but the more subtle difference between NC 18016 and NC 18229 was not detected. In field evaluations, NC 18016 was observed to be more resistant than NC 18229 when the data were categorized by inoculum density. Results from field trials suggested that much of the variation in field evaluations of CBR resistance is due to the clustered spatial distribution of microsclerotia of *Cylindrocladium crotalariae* in naturally infested soils. Evaluations of microsclerotial production in roots of infected plants were highly variable. In general, numbers of microsclerotia per gram of root increased with root rot rating; however, it appears that reduced microsclerotia production would be an extremely difficult trait for which to select in a breeding program.

Additional key words: *Arachis hypogaea*, *Calonectria crotalariae*, groundnut.

Cylindrocladium black rot (CBR) of peanut (*Arachis hypogaea* L.) induces a peg, pod, and root rot that has been of economic importance in peanut production in North Carolina and Virginia since 1970. CBR is caused by a soilborne fungus, *Cylindrocladium crotalariae* (Loos) Bell and Sobers, for which microsclerotia are the survival structures and primary inocula. The spatial pattern of microsclerotia in naturally infested field soils is clustered (15,22). Consequently, CBR generally occurs as several foci of infection in infested fields.

Despite extensive research on CBR, no single control practice has been consistently effective. Currently, several control tactics are integrated into a CBR management program based on the strategy of reducing microsclerotial populations to inoculum densities at which commercial peanuts can be profitably grown in fields infested with *C. crotalariae*.

The use of resistant cultivars is an important part of the CBR management program. Resistance can be considered to be the ability of the host to inhibit the growth and/or reproduction of the pathogen. For a monocyclic disease, such as CBR, in which a soilborne fungus survives as microsclerotia, disease resistance can be evaluated from two practical aspects: differences in disease incidence and severity (ie, pathogen growth) and differences in microsclerotial production (ie, pathogen reproduction). While resistance to soilborne pathogens has often been measured by reduction of disease severity, pathogen production has usually not been examined. Conversely, resistance to diseases caused by soilborne nematodes has often been measured by development of nematode populations with secondary consideration given to host injury (18). Similar evaluations of pathogen reproduction for *C. crotalariae* could be useful for determining the value of genotypes in relation to the long-term CBR management objective of reducing microsclerotial populations.

Several evaluations of CBR resistance based on disease incidence or severity have been conducted in naturally infested fields (3,5–9,16,20,24) and in greenhouses (13,14,16,20), but only two evaluations have considered microsclerotial production (7,21). Three Virginia-type, one Valencia-type, and several Spanish-type peanuts have been identified as sources of CBR resistance based on disease severity (1,4,12). Heritability estimates for CBR resistance have ranged from 0.43 to 0.73 (8,9,11) with only additive genetic effects significant (8,11). However, resistance has been negatively correlated with yield and large fruit (9). These unfavorable linkages may make it difficult to develop cultivars with CBR resistance and important agronomic traits. Additionally, field evaluations of CBR resistance have sometimes resulted in large error components in the analysis of variance. Significant location × genotype interactions have also been common in field trials (3,5,6,24).

The objectives of this study were to compare greenhouse, microplot, and field methods of evaluating CBR resistance while quantitatively characterizing CBR resistance for two promising advanced generation CBR breeding lines and two Virginia-type commercial cultivars.

MATERIALS AND METHODS

Peanuts evaluated. Cultivars Florigiant and NC 8C, and two promising advanced-generation CBR breeding lines, NC 18016 and NC 18229, were evaluated in all studies. NC 3033, a source of CBR resistance (1), was included in greenhouse studies as a check. Florigiant, a widely grown commercial Virginia-type cultivar that is very susceptible to CBR, is a composite of seven sibling lines. NC 8C was recently released as a commercial Virginia-type cultivar with moderate CBR resistance (23). The two breeding lines and NC 8C

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

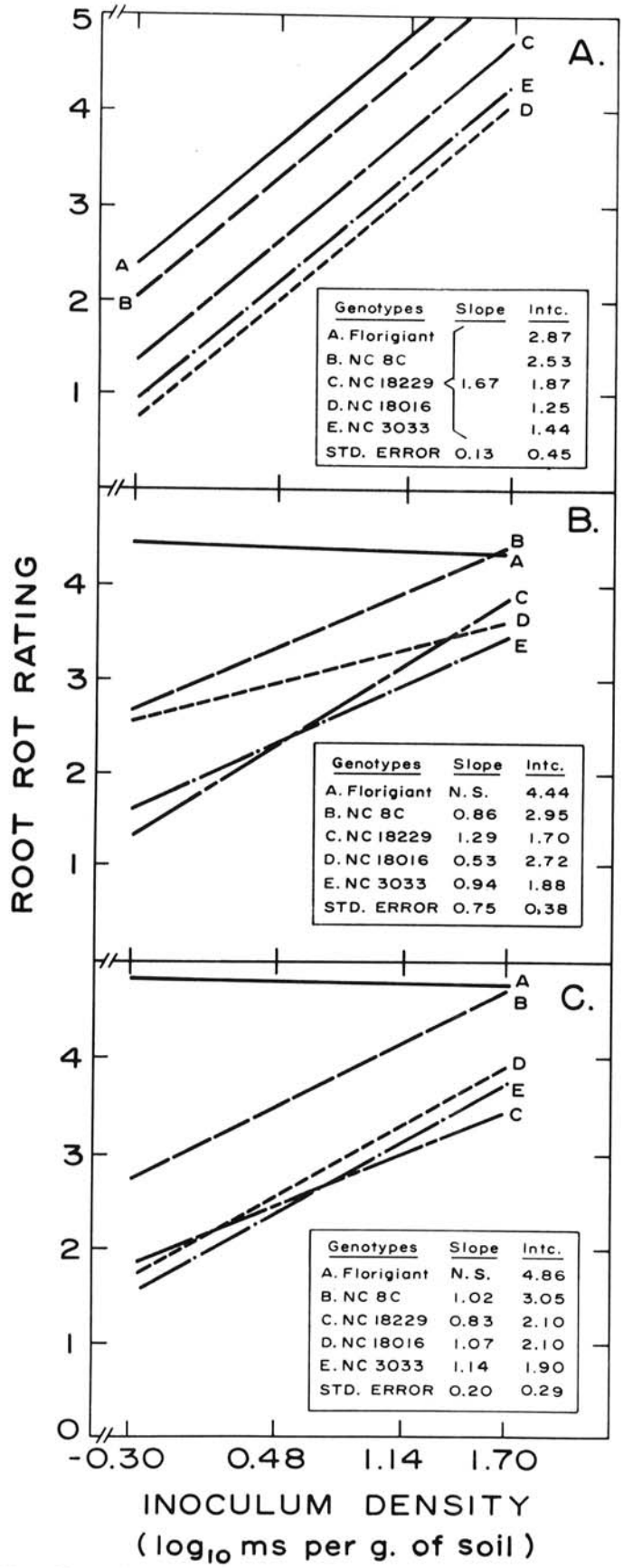

Fig. 1. Regressions of root rot rating on $\log_{10}$ inoculum density for peanut cultivars Florigiant, NC 8C, and NC 3033, and two peanut breeding lines evaluated for Cylindrocladium black rot resistance in three greenhouse trials: **A**, trial 1; **B**, trial 2; and **C**, trial 3.

were selections from an accelerated breeding program that used the modified pedigree selection method. Their parentages are: NC 8C = (NC 3139 × Florigiant), NC 18016 = (NC 9088 × NC 3033), and NC 18229 = (NC 3033 × NC 2).

Greenhouse evaluations. Greenhouse studies consisted of three trials of a five by five factorial experiment. The two breeding lines, NC 8C and Florigiant, and NC 3033 were grown in all combinations of five inoculum densities: 0, 0.5, 3, 13, and 50 microsclerotia per gram of soil. A randomized complete block with four replications was used for each trial.

In each trial, inoculum was prepared from approximately 10 isolates of *C. crotalariae* grown on potato-dextrose agar. Isolates used in the first trial had been maintained in culture for ~1 yr. In the second and third trials, isolates were obtained from peanut field soil and were cultured for <2 mo. Inoculum and *Rhizobium* spp. (cowpea group) were added to 2,200 g of 3:2 steamed sand:soil in 100 polyethylene bags. The infested soil was thoroughly mixed by shaking the soil in bags for 2 min. Soil was placed in 15-cm-diameter clay pots. Two 3-day-old peanut seedlings were transplanted into each pot. Plants were harvested after growing for 8 wk in a greenhouse at ~25 C. Root rot ratings were made using a 0 to 5 scale (19). Fresh and oven-dry root and shoot weights were measured. Data were analyzed by regression analysis ($P < 0.05$) with inoculum density as a quantitative variable and cultivars and breeding lines as a qualitative variable. Correlations of root rot ratings and plant growth variables were evaluated.

Microplot evaluation. Microplot evaluations were conducted at the Central Crops Research Station, Clayton, NC, in 1982. Four levels of inoculum density were achieved in microplots by hand-mixing various proportions of soil infested with *C. crotalariae* from the top 50 cm of 80 microplots (76-cm diameter) that had been previously cropped to peanuts (2). Florigiant, NC 8C, and the two breeding lines were grown at all combinations of inoculum densities in a four by four factorial treatment design with five replications arranged in a randomized complete block experimental design. Ten seeds were planted per microplot on 14 May. After emergence, plots were thinned to six plants. Plants were harvested 27 October.

Two weeks after planting, 10 soil samples were collected from each microplot and microsclerotial populations were determined by an elutriation-semiselective medium assay procedure (17). Aboveground symptoms of CBR were measured throughout the growing season as the number of dead and wilted plants per plot. At harvest, root systems were collected from each plot. Taproots were split in half longitudinally and the entire root system was rated for root rot according to a 0 to 5 scale. To assay roots for microsclerotia, all six root systems from a microplot were weighed, blended in a Waring blender for 3 min (1.5 min at low speed and 1.5 min at high speed) and washed through a set of 420-μm and 38-μm nested sieves. Root fragments and microsclerotia collected on the 38-μm sieve were washed for 1 min in 0.25% sodium hypochlorite, thoroughly rinsed, and suspended in 1 L of water. A 10-ml sample of the suspension was assayed on the semiselective medium previously described. Data were analyzed by analysis of variance ($P < 0.05$).

Field evaluations. Field trials were conducted in Bladen County, NC, in 1981 and 1982 and in Martin County, NC, in 1981. Twenty plots each of the two breeding lines, Florigiant, and NC 8C were planted at each location in mid-May in fields that were known to be naturally infested with *C. crotalariae*. Plots were four rows wide (0.91-m row width) and ~6.1 m long with ~45 plants per row. The middle two rows of each plot were evaluated. The plots were arranged as a randomized complete block with 20 replications; however, inoculum density varied within as well as among blocks.

Sixteen soil samples (2-cm diameter and 16-cm depth) were taken from the middle two rows of each plot 1–2 wk after planting. Samples were collected ~16 cm from the center of each row by using the three-diagonal sampling pattern suggested by Hau et al (15). Inoculum density was determined by the elutriation-semiselective medium procedure. Incidence of CBR aboveground symptoms was evaluated throughout the growing season.

Incidence of CBR (presented as proportions from 0 to 1) was determined by dividing the number of dead and wilted plants per plot by stand counts. Plots were dug and harvested in late September or early October. After digging, roots were collected from 20 plants selected at random from each plot. Roots were rated for root rot as previously described. Roots of each breeding line, NC 8C, and Florigiant were grouped according to root rot rating and assayed for microsclerotia as previously described. Distributions of number of plants per root rot rating were compared by chi-square analyses. Correlations of CBR incidence and root rot rating were evaluated ($P < 0.05$). Microsclerotial production was analyzed by analysis of variance ($P < 0.05$).

RESULTS

Greenhouse evaluations. In the combined ANOVA of root rot ratings, all first-order interactions involving trials were significant. Consequently, data were sorted and analyzed by trial.

Inoculum density and genotype main effects were significant, but the interaction term was not significant in the first trial. Therefore, the positions of the regressions of root rot rating on inoculum density differed among Florigiant, NC 8C, and the three breeding lines, but the slope did not differ (Fig. 1A). A slope of 1.67 was the best estimate of the response of all genotypes in trial 1. Based on *t*-tests, intercepts of NC 3033, NC 18016, and NC 18229 were significantly lower than NC 8C and Florigiant.

Inoculum density, genotype, and inoculum density × genotype interaction term were significant in the second and third trials. For Florigiant, the slope was less and the intercept was greater than those of the other four genotypes in both trials (Fig. 1B and C). In trial 3, the slopes of NC 8C and the three breeding lines did not differ significantly, but the intercept of NC 8C was greater than those of the breeding lines. In trial 2, slopes of NC 8C and NC 18229 were similar, but their intercepts differed. Likewise, slopes of NC 8C, NC 18016, and NC 3033 did not differ significantly, but the intercept of NC 3033 was less than that of NC 8C.

Analyses of plant growth measurements were similar to those for root rot rating. Correlations of root rot rating and plant growth variables were high and negative: fresh root weight ($r = -0.88$), fresh shoot weight ($r = -0.80$), dry root weight ($r = -0.56$), and dry shoot weight ($r = -0.82$).

Microplot evaluation. Four different inoculum levels were achieved by mixing infested soil from previously established microplots. Mean microsclerotial populations per gram of soil were 9.3, 4.0, 2.9, and 1.7 for the very high, high, medium, and low treatments, respectively. The number of dead and wilted plants per plot differed among inoculum densities only at the last two ratings, 23 September and 15 October. Aboveground symptoms of CBR at those two ratings and root rot ratings were less for the low inoculum density than for the other three treatments.

Differences among Florigiant, NC 8C, and the two breeding lines were significant for number of dead and wilted plants at all rating dates, for root rot ratings, and for microsclerotia per gram of root (Table 1). The most discernible differences among genotypes in CBR aboveground symptoms occurred on the 23 September rating. The number of dead and wilted plants at that rating was greater for Florigiant than for NC 8C and the two breeding lines. Aboveground symptoms on 23 September were also greater for NC 8C than for the two breeding lines. Root rot rating was higher for Florigiant than for the other genotypes; however, no differences occurred among NC 8C and the two lines. Microsclerotia per gram of root was greater for Florigiant than for NC 8C or NC 18229, but no differences occurred between Florigiant and NC 18016 or among NC 8C and the two breeding lines.

Field evaluations of CBR severity. Inoculum density in field plots at the three locations ranged from 0 to 10.3 microsclerotia per gram of soil. Since CBR resistance is inoculum density-dependent (16), data were categorized by inoculum density. Inoculum density was considered to be low, medium, or high if the microsclerotial population per gram of soil in a plot was estimated to be less than one, from one to three, or greater than three microsclerotia per gram of soil, respectively.

Frequency distributions of plants per root rot rating differed among the two cultivars and breeding lines within and among inoculum classes (Fig. 2). Mean root rot rating was greatest for Florigiant within an inoculum class (Table 2); however, when all of the 66 possible pairwise comparisons of distributions of plants per root rot rating were evaluated by chi-square analyses ($P > 0.05$), the null hypothesis of homogeneity of distributions failed to be rejected for nine comparisons (Table 3). For example, the distributions for NC 18229 at medium and high inoculum densities were homogeneous to that of Florigiant at the low inoculum density.

Evaluations of CBR incidence were similar to those for root rot rating. In each inoculum class CBR incidence was greatest for Florigiant and least for NC 18016 (Table 2). Correlations of CBR incidence ~1 wk before digging and mean root rot rating per plot were high and positive except for NC 18229 at the Bladen County location in 1981 (Table 4).

Field evaluations of microsclerotia production. Evaluations

TABLE 1. Cylindrocladium black rot (CBR) above ground symptoms, root rot rating, and microsclerotia per gram of root for peanut cultivars Florigiant and NC 8C, and two peanut breeding lines evaluated in microplots

Cultivars and lines	CBR symptoms[a] August 20	September 10	September 23	October 15	RRR[b]	ms[c]
Florigiant	1.5	4.3	5.4	5.8	4.32	3,566
NC 8C	0.6	1.3	3.7	5.1	3.52	1,921
NC 18229	0.1	0.8	2.2	3.9	3.29	2,091
NC 18016	0.2	0.7	1.7	3.8	3.38	2,515
FSLD ($P = 0.05$)	0.6	0.8	0.9	0.9	0.43	1,222

[a] CBR symptoms = number of dead and wilted plants per plot (six plants per microplot).
[b] RRR = root rot rating on a scale of 0 to 5, in which 0 = no visible disease symptoms and 5 = completely decayed (19).
[c] ms = microsclerotia of *Cylindrocladium crotalariae* per gram of root.

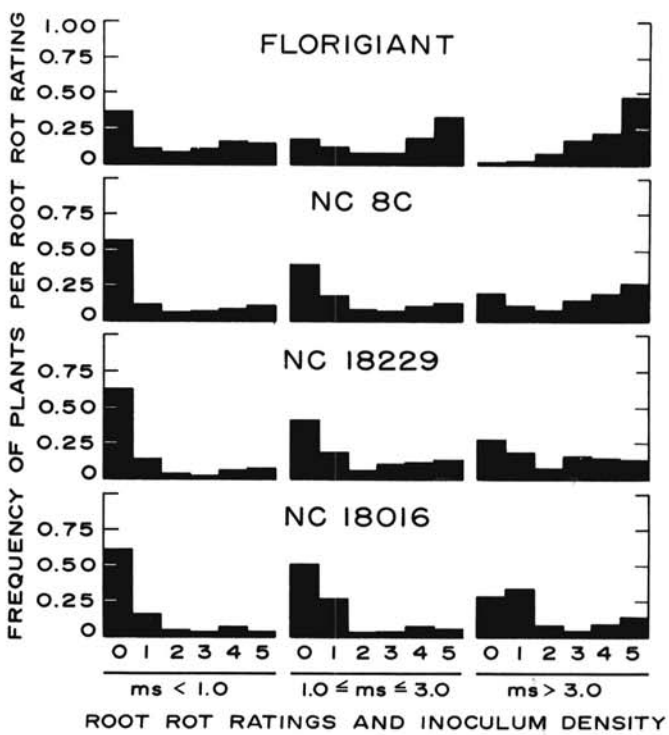

Fig. 2. Frequency distributions of plants per root rot rating class for cultivars Florigiant and NC 8C, and two peanut breeding lines evaluated in field plots with low, medium, or high inoculum density (microsclerotia [ms] per gram of soil). Root rot rating scale: 0 = no visible disease and 5 = completely decayed root.

showed that microsclerotial production in infected roots collected from field trials was highly variable. In general, the number of microsclerotia per gram of root increased with root rot rating but differences among the cultivars and breeding lines varied. Root rot rating × genotype and root rot rating × genotype × location interactions were significant. Therefore, microsclerotial production was compared among cultivars and lines within trials and root rot rating (Table 5).

At the Bladen County location in 1981, a greater number of microsclerotia were detected per gram of root for NC 18016 than for Florigiant or NC 8C at the root rot rating of one. For the root rot ratings of two and three, microsclerotia per gram of root were greater for NC 18016 than for Florigiant, NC 8C, and NC 18229. At the Martin County location, the number of microsclerotia per gram of root assigned a rating of two was greater for the two breeding lines than for Florigiant. When roots assigned a rating of five were compared, fewer microsclerotia were detected for NC 8C and NC 18229 than for NC 18016. At the Bladen County location in 1982, no differences occurred among the cultivars and breeding lines except at a root rot rating of five when fewer microsclerotia were detected per gram of root for NC 8C than for Florigiant or the two breeding lines.

TABLE 2. Mean root rot rating and Cylindrocladium black rot (CBR) incidence for peanut cultivars Florigiant and NC 8C, and two peanut breeding lines evaluated in North Carolina field plots with low, medium, and high inoculum density

Cultivars and lines	Inoculum density[a]					
	Root rot rating[b]			Incidence[c]		
	low	medium	high	low	medium	high
Florigiant	2.22	3.01	4.03	0.37	0.53	0.79
NC 8C	1.41	1.62	2.82	0.20	0.21	0.61
NC 18229	1.08	1.75	2.14	0.12	0.21	0.36
NC 18016	1.03	1.08	1.78	0.10	0.10	0.19

[a] Inoculum density based on microsclerotia (ms) populations per gram of soil: low (ms <1.0), medium ($3.0 >$ ms > 1.0), and high (ms > 3.0).
[b] Root rot ratings on a 0 to 5 scale (19).
[c] Incidence of CBR ~1 wk before digging measured as the number of dead and wilted plants per plot divided by stand counts.

TABLE 3. Chi-square analyses in which the hypothesis of homogeneity failed to be rejected for comparisons of distributions of number of plants per root rot rating for peanut cultivars Florigiant and NC 8C, and two peanut breeding lines grown in North Carolina field plots with low, medium, or high inoculum density[a]

Comparisons	Chi-square	P[b]
Florigiant (low) : NC 8C (medium)	9.78	0.08
Florigiant (low) : NC 18229 (medium)	10.58	0.06
Florigiant (low) : NC 18229 (high)	10.38	0.07
Florigiant (medium) : NC 8C (high)	6.14	0.29
NC 8C (medium) : NC 18229 (medium)	1.75	0.88
NC 8C (medium) : NC 18229 (high)	8.38	0.14
NC 8C (high) : NC 18229 (high)	10.87	0.05
NC 18229 (low) : NC 18016 (low)	8.40	0.14
NC 18229 (medium) : NC 18229 (high)	6.92	0.23

[a] Inoculum density based on microsclerotia (ms) populations per gram of soil: low (ms > 1.0), medium ($3.0 >$ ms > 1.0), and high (ms > 3.0).
[b] Probability of exceeding the chi-square value when the hypothesis of homogeneity is true.

DISCUSSION

Based on CBR severity, the overall evaluation of results from greenhouse, microplot, and field testing procedures indicate that NC 18016 is slightly more resistant to CBR than NC 18229; NC 8C is intermediate in resistance between Florigiant and the breeding lines. However, this ranking was not discernible from all methods of evaluating resistance.

In greenhouse and microplot evaluations, substantial differences in CBR resistance among Florigiant, NC 8C, and the breeding lines were apparent, but the subtle difference between NC 18016 and NC 18229 was not detected. In two of the three greenhouse trials, regressions of root rot rating on $\log_{10}$ inoculum density were not significantly different among NC 18016, NC 18229, and NC 3033. Analyses of root and shoot weights gave similar results as would be expected considering the relatively high correlations among these plant growth variables and root rot rating. In microplot evaluations, differences in CBR aboveground symptoms were most apparent at the late September rating date, but no difference was detected between NC 18016 and NC 18229.

Field evaluations revealed the more subtle difference in CBR resistance between the breeding lines, even though problems

TABLE 4. Correlation coefficients for Cylindrocladium black rot incidence ~1 wk before digging and mean root rot per plot for peanut cultivars Florigiant and NC 8C, and two peanut breeding lines evaluated in field trials at three locations in North Carolina

Cultivars and lines	Location and year		
	Martin County	Bladen County	
	1981	1981	1982
Florigiant	0.826[a]	0.524	0.947
NC 8C	0.851	0.621	0.929
NC 18229	0.871	N.S.	0.886
NC 18016	0.745	0.746	0.901

[a] Correlations significant at $P < 0.05$.

TABLE 5. *Cylindrocladium crotalariae* microslerotia per gram of root for roots of peanut cultivars Florigiant and NC 8C, and two peanut breeding lines collected from three field trials in North Carolina and rated for root rot on a 0 to 5 scale

Location and year	Cultivars and lines	Microsclerotia per gram of root with root rot rating[a]					
		0	1	2	3	4	5
Martin County, 1981	Florigiant	11	39	58	116	1,881	3,336
	NC 8C	4	190	153	607	1,720	853
	NC 18229	8	179	295	849	1,207	2,781
	NC 18016	57	13	237	794	2,559	6,850
	FLSD ($P = 0.05$)	N.S.	N.S.	152	N.S.	N.S.	3,716
Bladen County, 1981	Florigiant	0	3	12	295	1,803	5,245
	NC 8C	6	2	6	549	7,150	7,624
	NC 18229	4	45	160	1,091	6,897	9,784
	NC 18016	20	77	1,182	2,682	9,770	6,516
	FLSD ($P = 0.05$)	N.S.	54.4	398	1,494	N.S.	N.S.
Bladen County, 1982	Florigiant	19	600	401	893	1,829	4,761
	NC 8C	49	209	402	898	2,965	1,581
	NC 18229	4	149	1,150	1,910	2,476	5,841
	NC 18016	18	416	729	1,179	2,731	3,985
	FLSD ($P = 0.05$)	N.S.	N.S.	N.S.	N.S.	N.S.	2,621

[a] Root rot rating scale: 0 = no visible disease symptoms and 5 = completely decayed root (19).

associated with the spatial pattern of microsclerotia in naturally infested field soils were apparent. In plots with low inoculum density, CBR incidence ~1 wk before digging and mean root rot rating were high for Florigiant, intermediate for NC 8C, and low and similar for the two breeding lines. In plots with medium and high inoculum densities, CBR incidence and mean root rot rating were high for Florigiant, low for NC 18016, and intermediate and similar for NC 8C and NC 18229. Consequently, interpretations of field evaluations suggest that NC 18229 is not as resistant as NC 18016, but NC 18229 is more resistant than NC 8C.

Problems with field evaluations of CBR resistance can occur because the spatial pattern of microsclerotia in field soils is related to the inoculum density-dependent nature of CBR resistance. This can best be illustrated when comparisons of frequency distributions of plants per root rot rating are examined (Table 3, Fig. 2). When comparisons are made across inoculum density classes, the ranking of the two cultivars and breeding lines may change. For example, if comparisons are made among Florigiant and NC 8C at low inoculum density and NC 18016 and NC 18229 at high inoculum density, results may be interpreted as follows: NC 8C, most resistant; NC 18229 and Florigiant, similar and most susceptible; and NC 18016, slightly more resistant than Florigiant and NC 18229. Likewise, other comparisons across inoculum density classes could result in similar erroneous interpretations of the actual resistance of a genotype. Additionally, the clustering of inoculum within individual plots probably caused many of the frequency distributions to be bimodal at 0 to 1 and 4 to 5. This is probably due to the clustering of inoculum within individual plots. Within a plot, plants were probably severely diseased regardless of genotype when grown in a "cluster" of inoculum for which microsclerotial populations are high. These "hot spots" of CBR are commonly observed in fields infested with *C. crotalariae* and were responsible for the mode at 4 to 5 in the frequency distributions. Plants grown near the "hot spots" were probably subjected to intermediate inoculum densities if gradients of microsclerotia occur. Florigiant plants grown at these intermediate inoculum densities were probably severely diseased, while plants of resistant lines were probably healthy or less diseased than Florigiant. Plants grown more than 1 m from "hot spots" were probably escapes (ie, not subjected to the pathogen) and were responsible for the mode at 0 to 1 in the frequency distributions.

Variation in inoculum density among and within plots is probably of greater importance in peanut breeding nurseries than in this study. In this study, we were able to evaluate 62 field plots of each cultivar and breeding line and consequently, we were able to distinguish differences among genotypes in levels of resistance despite variation in microsclerotial populations and patterns. However, a peanut breeder attempting to evaluate many entries must often rely on three to six replications per location. In previous field evaluations of CBR resistance, significant genotype × location interactions have occurred (3,5,6,24). These interactions have been interpreted to indicate differences in strains or isolates of *C. crotalariae*, environments, or inoculum density. Differences in isolates of *C. crotalariae* have been detected (2,10,19) but have been subtle and probably are not responsible for genotype × location interactions. Our results suggest that genotype × location interactions and large error variances obtained in analyses of some field trials are largely due to the clustered pattern of microsclerotia in naturally infested fields.

Because of the variation in field trials resulting from the spatial distribution of inoculum, field evaluations can be more effective if microsclerotial populations are estimated and considered in comparisons of treatments and if fields with more uniform spatial patterns of inoculum are used. To achieve more uniform microsclerotial patterns, CBR breeding nurseries will probably need to be established, monitored for populations of *C. crotalariae* and properly maintained. These nurseries may also allow for greater sensitivity and reproducibility of field trials of chemicals, rotations or other control practices since problems associated with spatial patterns of microsclerotia will be present in all field evaluations.

Problems associated with greenhouse and microplot evaluations were also evident in our study. Results for Florigiant in the first trial of our greenhouse evaluations were different from those of the second and third trials and from a previous evaluation of Florigiant (16). This was probably due to the age of the cultures used in the first trial and suggests that cultures of *C. crotalariae* freshly isolated from soil or host tissue should be used in greenhouse and laboratory studies. In our microplot evaluations, the range of inoculum densities obtained by mixing soil from previously established microplots was from 1.7 to 9.3 microsclerotia per gram of soil. Aboveground CBR symptoms and root rot ratings were significantly different among inoculum densities only for the low inoculum level. This result and results of field plot evaluations suggest that 0 to 4 microsclerotia per gram of soil may be a more useful range of inoculum density for evaluations of resistance in microplot trials so that detection of the more subtle differences among moderately resistant genotypes can be made.

Green et al (8) have suggested that early generation screening and selection in the greenhouse would be useful in a CBR breeding program if greenhouse and field results are correlated. Our results suggest that greenhouse and field results are similar, but subtle differences among resistant lines would not be detected in greenhouse evaluations. Therefore, advanced generation lines should be evaluated in infested fields or in improved microplot trials.

Our evaluations of microsclerotia production were highly variable as were those of others (7,21). Results of field and microplot evaluations of microsclerotial production suggest that more microsclerotia were produced in moderately diseased roots of NC 18016 than in moderately diseased roots of the other lines. Therefore, based on microsclerotial production, NC 18016 appears to be less desirable for CBR resistance than the other lines tested. However, since root rot is less at a given inoculum density for NC 18016 than for the other lines, the number of microsclerotia produced in roots of NC 18016 plants grown at a given inoculum density may be similar to or less than the number of microsclerotia produced in roots of the other lines. While reduced production of microsclerotia is a trait that may be very useful in a CBR management program, it would probably be extremely difficult to detect because of the complex assay procedure and variation in results, and therefore could not be practically selected for in existing peanut breeding programs.

LITERATURE CITED

1. Beute, M. K., Wynne, J. C., and Emery, D. A. 1976. Registration of NC 3033 peanut germplasm. Crop Sci. 16:887.
2. Black, M. C., and Beute, M. K. 1981. Response of twelve *Cylindrocladium crotalariae* isolates to selection pressure from susceptible and resistant peanut cultivars. (Abstr.) Phytopathology 71:861.
3. Coffelt, T. A. 1980. Reaction of spanish-type peanut genotypes to Cylindrocladium black rot. Peanut Sci. 7:91-94.
4. Coffelt, T. A. 1981. Registration of VGP1 germplasm (Reg. No. GP-11). Crop Sci. 20:419.
5. Coffelt, T. A., and Garren, K. H. 1982. Screening for resistance to Cylindrocladium black rot in peanuts. Peanut Sci. 9:1-5.
6. Garren, K. H., and Coffelt, T. A. 1976. Reaction to Cylindrocladium black rot in virginia-type peanut cultivars. Plant Dis. Rep. 60:175-178.
7. Green, C. C., Beute, M. K., and Wynne, J. C. 1983. A comparison of disease parameters used for evaluation of resistance to *Cylindrocladium crotalariae* in peanut field tests. Peanut Sci. 10:(In press).
8. Green, C. C., Wynne, J. C., and Beute, M. K. 1983. Genetic variability and heritability in early generation crosses of large-seeded virginia-type peanuts with lines resistant to Cylindrocladium black rot. Peanut Sci. 10:47-51.
9. Green, C. C., Wynne, J. C., Beute, M. K., and Campbell, W. V. 1983. Quantitative genetics for yield, CBR, and insect resistance. Peanut Sci. 10:(In press).
10. Hadley, B. A., Beute, M. K., and Leonard, K. J. 1979. Variability of *Cylindrocladium crotalariae* response to resistant host plant selection pressure in peanut. Phytopathology 69:1112-1114.
11. Hadley, B. A., Beute, M. K., and Wynne, J. C. 1979. Heritability of Cylindrocladium black rot resistance in peanut. Peanut Sci. 6:51-54.
12. Hammons, R. O. 1980. Registration of six peanut germplasm lines

(Reg. Nos. GP12 to GP17). Crop Sci. 21:992-993.
13. Hammons, R. O., Bell, D. K., and Sobers, E. K. 1981. Evaluating peanuts for resistance to Cylindrocladium black rot. Peanut Sci. 8:117-120.
14. Harris, N. E., and Beute, M. K. 1982. Histological responses of peanut germplasm resistant and susceptible to *Cylindrocladium crotalariae* in relationship to inoculum density. Phytopathology 72:1250-1256.
15. Hau, F. C., Campbell, C. L., and Beute, M. K. 1982. Inoculum distributions and sampling methods for *Cylindrocladium crotalariae* in a peanut field. Plant Dis. 66:568-571.
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. 61:300-303.
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. Rhode, R. A. 1972. Expression of resistance in plants to nematodes. Annu. Rev. Phytopathol. 10:233-252.
19. Rowe, R. C., and Beute, M. K. 1975. Variability in virulence of *Cylindrocladium crotalariae* isolates on peanuts. Phytopathology 65:422-425.
20. Rowe, R. C., Beute, M. K., Wells, J. C., and Wynne, J. C. 1974. Incidence and control of Cylindrocladium black rot of peanuts in North Carolina during 1973. Plant Dis. Rep. 58:348-352.
21. Taylor, J. D., Griffin, G. J., and Garren, K. H. 1980. Influence of peanut cultivars and stage of shoot symptom development on the production of microsclerotia by *Cylindrocladium crotalariae*. (Abstr.) Proc. Am. Peanut Res. and Ed. Assoc. 12:40.
22. Taylor, J. D., Griffin, G. J., and Garren, K. H. 1981. Inoculum pattern, inoculum density-disease relationships and population fluctuations of *Cylindrocladium crotalariae* microsclerotia in peanut field soil. Phytopathology 71:1297-1302.
23. Wynne, J. C., and Beute, M. K. 1983. Registration of NC 8C peanut (Reg. No. 27). Crop Sci. 23:(183-184).
24. Wynne, J. C., Rowe, R. C., and Beute, M. K. 1975. Resistance of peanut genotypes to *Cylindrocladium crotalariae*. Peanut Sci. 2:54-56.