Peanut Science (1983) 10:66-69

A Comparison of Methods of Evaluating Resistance to *Cylindrocladium crotalariae* in Peanut Field Tests[1]

C. C. Green, M. K. Beute and J. C. Wynne*[2]

ABSTRACT

Three methods presently used to evaluate disease resistance in peanut (*Arachis hypogaea* L.) to Cylindrocladium black rot (CBR) were compared to identify the most efficient method to screen peanut lines in field tests. Forty randomly selected F_2 families in the F_5 generation from a cross of the CBR-resistant source NC 3033 with NC 6 and the two parents were evaluated for percent dead and diseased plants, root rot index and microsclerotia/g of root. Significant differences were detected between the parents ($p = 0.05$) and among segregates (familes) ($p = 0.01$) for percent dead and diseased plants only. Error components exceeded the mean square values for differences among segregates for the root rot index and microsclerotia/g of root resulting in no significant differences among entries for these traits and low to no correlations among traits. Percent dead and diseased plants was concluded to be the best of the three methods for screening peanut lines in the field for CBR resistance.

Key Words: *Arachis hypogaea* L., Cylindrocladium black rot, percent dead and diseased plants, root rot index, microsclerotia/g of root.

Cylindrocladium black rot (CBR) of peanut (*Arachis hypogaea* L.) caused by *Cylindrocladium crotalariae* (Loos) Bell and Sobers, is a relatively new disease in the southeastern United States. CBR was first reported in Georgia in 1965 (1) and in North Carolina in 1970 (5). Since 1970 the disease has been reported in virtually all peanut-producing counties of North Carolina (5, 14, 15). *Cylindrocladium crotalariae* causes a serious peg, pod and root necrosis of peanut. Symptoms of the disease, which can appear in the field as early as July, include chlorosis and wilting of the main axis followed by complete wilting of the remaining foliage and death of the plant. Lateral roots are either blackened or completely severed from the taproot. Pod development is greatly reduced and existing pods may be severely rotted (5, 8, 14). Microsclerotia are the primary source of inoculum as well as the primary survival structures in the field (16).

Cultural practices, chemicals and resistant peanut lines have been evaluated in an effort to develop a CBR control program. Cultural practices such as sanitation and rotation and chemical treatments have not been highly effective nor consistent in controlling the disease (14, 15). NC 3033 was identified as a resistant germplasm source in North Carolina field tests conducted in 1973-74, but it is low yielding and has small fruit (2, 19). A resistant cultivar has been developed from a cross of a CBR-resistant line and a high yielding, agronomically acceptable CBR-susceptible cultivar (18).

It is necessary in the development of a resistant line to have an accurate but rapid method of rating disease severity in order to screen large numbers of lines. Three methods presently used for evaluating CBR severity were compared in this study to determine the most effective method for distinguishing resistant lines in field tests. Correlations among these three disease measurements were also estimated to examine whether the three parameters measure the same resistance trait or different traits. The three variables compared were root rot index on a scale of 0-5, number of microsclerotia/g of root, and percent of aboveground diseased and dead plants.

Materials and Methods

Forty randomly selected advanced generation families produced from a cross of NC 3033 and NC 6 were evaluated for CBR resistance in field tests conducted in 1980. NC 3033 is a CBR-resistant line which has small fruit and is low yielding. NC 6 is a high yielding, large-fruited cultivar presently grown in North Carolina (3). Hybrids produced from this cross were increased to produce F_2 seed. Equal numbers of seed from individual F_2 plants were bulked and individual families were advanced to the F_5 generation. Forty F_2 families in the F_5 generation were selected at random and planted in a field naturally infested with *C. crotalariae*. Four replications of two-row plots (10 seed/row) were planted in a randomized complete block design. NC 3033 and NC 6 were included as checks.

Stand counts were recorded 3 weeks after planting. Three weeks prior to harvest counts were made of aboveground diseased and dead plants. Percentages of dead and diseased plants were calculated from stand counts. At harvest, two samples per plot consisting of three roots each were collected and stored at 13 C.

The root samples were weighed and their disease severity was rated on a scale of 0 (no visible disease) to 5 (completely decayed) (13). Root tissues were ground in water in a Waring blender for 2.5 min (low speed for 1.5 min, high speed for 1 min). Microsclerotia were extracted by washing samples with running water through a series of nested sieves of 400, 38 and 8-μm openings, respectively (16). Extracted samples were surface-sterilized for 1 min in 0.28% sodium hypochloride and then rinsed. The samples were suspended in water and the subsamples dispensed such that microsclerotia were in a sufficient concentration to detect at the lower root rot indices (indices 1 and 2) but were not too concentrated as to prevent colony counts at the higher sample indices (indices 4 and 5). This was accomplished by suspending samples with a root rot index of 0-4 in a standard volume of 200 mL water or 400 mL water for samples with a root rot index of 5. Subsamples of

[1]Paper No. 8822 of the North Carolina Agricultural Research Service, Raleigh, NC 27650.

[2]Graduate Research Assistant of Crop Science, Professor of Plant Pathology and Associate Professor of Crop Science, respectively, North Carolina State University, Raleigh, NC 27650.

5 or 10 mL of the suspensions (5 mL for a root rot index of 3, 4 or 5, 10 mL for a root rot index of 0, 1 or 2) were added to 100 mL of a specialized isolation medium at 45 C (11), swirled and dispensed into 10 petri dishes for each subsample. The petri dishes were incubated 5 to 6 days at room temperature under continuous cool-white light (Econo-Light, Westinghouse Electric, Pittsburg, PA 15212). The total number of *C. crotalariae* colonies for 10 petri dishes were recorded for each subsample. Microsclerotia counts for each subsample were multiplied by the appropriate dilution factor and converted to total microsclerotia/g of root weight for each sample.

The root rot index and microsclerotia count for the two samples per plot were averaged and recorded as one value per plot. An arcsin transformation was used for percent dead and diseased plants to stabilize the error variance and reduce the coefficient of variation. An analysis of variance was computed for each of the three traits as follows:

Source of variation	df	For trait i EMS	For trait i,j EMCP$_{ij}$
Entries	41		
Parents vs segregates	1		
NC 6 vs NC 3033	1		
Among segregates	39	$\sigma^2_{e_i} + 4\sigma^2_{s_i}$	$\sigma_{e_{ij}} + 4\sigma_{s_{ij}}$
Error	123	$\sigma^2_{e_i}$	$\sigma^2_{e_{ij}}$

where: $\sigma^2_{s_i}$ = the genetic variance of the i^{th} trait.

$\sigma_{s_{ij}}$ = the covariance component between the i^{th} and j^{th} traits.

$\sigma^2_{e_i}, \sigma^2_{e_{ij}}$ = the error variance and covariance, respectively.

Means for the 40 families plus the two parents were ranked for each of the three traits measured. Spearman rank correlations and Pearson product-moment correlations among family means for all pairs of traits were computed (17). The Pearson product-moment correlations among family means were considered an approximation of genotype correlations.

Results

The parent means were significantly different ($p = 0.05$) from the segregates for percent dead and diseased plants. Significant variation ($p = 0.01$) among the 40 F_5 families for percent dead and diseased plants was observed. Error mean squares exceeded the mean squares among segregates for the traits root rot index and microsclerotia/g of root thus there were no significant differences among entries for these traits (Table 1).

There were no significant correlations (Pearson product-moment) between family means for percent dead and diseased plants and root rot index or microsclerotia/g of root. There was a low but significant positive correlation between root rot index and microsclerotia/g of root (Table 2). There were no significant rank correlations for F_5 entries ranked according to means for percent dead and diseased plants and root rot index or microsclerotia/g of root. There was a low but significant positive rank correlation between root rot index and microsclerotia/g of root (Table 3). The resistant parent NC 3033 had the lowest mean percent dead and diseased plants. The susceptible parent NC 6 ranked 16 (1 = lowest, 42 = highest) for mean percent dead and diseased plants with 26 entries exceeding it in susceptibility. NC 3033 had a high mean root rot index and mean microsclerotia/g of root, but NC 6 ranked among the best 10 entries for these traits (Table 4).

Table 1. Mean squares and cross products for percent dead and diseased plants, root rot index, and microsclerotia/g of root.

Source of variation	Trait: % Dead & diseased[a]	Root rot index[b]	Microsclerotia per g of root
	% Dead and diseased		
Entries	1947.25	3.13	4281.50
Parents vs segregates	5769.97*	11.79	-95419.40
NC 3033 vs NC 6	2584.75	-35.95	-136109.00
Among segregates	1832.89**	3.91	10437.40
Error	921.35		
	Root rot index		
Entries		1.08	558.51
Parents vs segregates		0.02	-195.04
NC 3033 vs NC 6		0.02	-195.04
Among segregates		1.12	543.61
Error		1.19	
	Microsclerotia/g of root		
Entries			1126301.00
Parents vs segregates			1577972.00
NC 3033 vs NC 6			7167274.00
Among segregates			959823.00
Error			1255213.00

[a]Percent dead and diseased transformed to arcsin ($\sqrt{Y/100}$).

[b]Root rot index on a scale of 0 (no disease) to 5 (completely decayed).

*,**Denote significance at the .05 and .01 levels, respectively.

Table 2. Pearson product-moment correlations between all pairs of three traits for CBR resistance.

	Root rot index	Microsclerotia/g of root
% Dead and diseased[a]	0.068	0.091
Root rot index[b]		0.507**

[a]Percent dead and diseased plants transformed to arcsin ($\sqrt{\%}$).

[b]Root rot index on a scale of 0 (no disease) to 5 (completely decayed).

**Denotes significance at the .01 probability level.

Table 3. Rank correlations between all pairs of three traits for CBR resistance.

	Root rot index	Microsclerotia/g of root
% Dead and diseased[a]	-0.008	0.175
Root rot index[b]		0.515**

[a]Percent dead and diseased plants transformed to arcsin ($\sqrt{\%}$).

[b]Root rot index on a scale of 0 (no disease) to 5 (completely decayed).

**Denotes significance at the .01 probability level.

Discussion

The variability in the data and the large error com-

Table 4. Ranking from lowest to highest followed by entry number and entry mean for percent dead and diseased plants, root rot index, and microsclerotia/g of root.

Rank	% Dead & diseased[a]		Root rot index[b]		Microsclerotia/g of root	
	Entry no.	Mean	Entry no.	Mean	Entry no.	Mean
1	NC 3033	15.41	20	1.375	20	195
2	2	21.49	32	1.625	21	207
3	32	23.45	2	1.750	13	215
4	20	27.12	3	1.875	4	279
5	25	31.01	29	1.875	NC 6	342
6	21	32.32	36	1.875	27	355
7	10	32.35	4	2.000	9	380
8	40	33.65	34	2.000	34	412
9	16	33.71	5	2.125	39	415
10	22	41.45	NC 6	2.250	23	432
11	39	42.81	27	2.250	36	483
12	1	43.04	38	2.250	38	487
13	28	44.26	40	2.250	2	493
14	7	48.44	7	2.375	40	510
15	37	48.61	15	2.375	7	522
16	NC 6	51.36	13	2.500	5	540
17	27	51.80	21	2.500	28	593
18	24	53.91	28	2.500	1	634
19	14	58.55	33	2.500	22	668
20	8	59.54	9	2.625	10	678
21	17	59.91	16	2.625	32	727
22	34	65.41	22	2.625	35	756
23	30	66.93	23	2.625	19	771
24	31	68.78	26	2.625	16	774
25	4	68.84	30	2.625	17	840
26	13	70.43	31	2.625	11	914
27	38	73.07	NC 3033	2.750	6	969
28	18	73.53	11	2.750	3	1024
29	11	73.92	17	2.750	24	1042
30	19	73.95	19	2.750	33	1043
31	12	75.18	25	2.750	8	1049
32	35	77.77	39	2.750	18	1080
33	5	78.26	1	2.875	31	1144
34	6	77.79	10	2.875	26	1210
35	33	83.43	35	2.875	37	1298
36	15	83.87	12	3.125	25	1341
37	23	85.04	6	3.125	29	1530
38	29	85.58	8	3.250	30	1537
39	36	86.49	18	3.250	12	1574
40	9	89.59	24	3.375	15	1715
41	3	93.39	37	3.500	NC 3033	2235
42	26	95.41	14	3.875	14	2515
LSD (p = .05)		43.38		1.560		1601

[a]Percent dead and diseased plants transformed to arcsin ($\sqrt{\%}$).

[b]Root rot index on a scale of 0 (no disease) to 5 (completely decayed).

ponents obtained in the analysis of variance for microsclerotia/g of root and the root rot index may have resulted from several factors. Field evaluation of lines is initially complicated by the lack of uniformity in inoculum distribution in the field as well as by lack of uniformity in infection time of the roots. Sampling roots at harvest time results in a collection of all stages of infection as well as exclusion of roots lost to disease early in the season. Another problem is that highly susceptible lines may have such badly decayed roots that much of the root system remains in the soil when the plant is dug. Therefore high numbers of microsclerotia associated with the more susceptible plants probably remain in the soil. It is also possible that highly susceptible lines are hypersensitive and roots are killed rapidly upon infection. The dead peanut root is then colonized by secondary organisms and *C. crotalariae,* being a poor saprophyte, is unable to produce microsclerotia. A resistant line remains healthy and maintains a large root system. Microsclerotia may infect these roots but lesions develop slowly and the roots resist decay (6). High numbers of microsclerotia would then be recovered from these roots because of the relatively larger root area recovered when root samples are collected. This could be more accurately defined as tolerance.

Although microsclerotia were recovered in large numbers from the resistant parent NC 3033, the microsclerotia population in a field planted to a resistant cultivar may not increase over time. Phipps (10) reported no significant increase in the microsclerotia populations in microplots planted to NC 3033, whereas microsclerotia populations did increase significantly in soil from microplots planted to the susceptible cultivar Florigiant. Krigsvold (9) reported less germination of microsclerotia in the rhizospheres of resistant peanut plants than for susceptible plants.

Low to no correlations between disease measurements may indicate that more than one resistance mechanism exists as suggested by Coffelt (4). The existence of several mechanisms of resistance would further complicate the use of microsclerotia counts from roots as well as the root rot index to screen or evaluate lines in the field. The root rot index could be misleading because tolerant plants would have "determinant" root lesions (6); therefore, they would receive a midrange root rot rating of approximately 2.5 on the scale of 0-5. It would then be difficult on the basis of the root rot index alone to distinguish between tolerant plants receiving a moderate rating for root rot and susceptible plants that have moderate ratings only because the lesions had not progressed at the time the samples were taken.

In summary, the lack of uniformity in initial infection time in the field, the rapid death and decay of roots of highly susceptible lines, and the possible existence of several resistance mechanisms, particularly tolerance, may explain why no differences were observed between families or between the parents of these families for the root rot index and microsclerotia/g of root. These factors can also account for low to no correlations found among traits. Therefore, counts of microsclerotia recovered from peanut roots and the root rot index are confounded by several factors and, thus, are not useful methods to screen lines in field tests. Because the resistant parent NC 3033 had the lowest percent dead and diseased plants and because there were statistical differences between the entries for this trait, percent dead and diseased plants is the best of these three methods to evaluate lines in the field.

A field used for evaluation of lines for disease resistance should ideally have an even distribution of the pathogen, although *C. crotalariae* has been found to have a clustered distribution in the field (7). Thus it may be necessary to have a large number of replications or to repeat the experiment over years and locations to account for the natural variation in the pathogen distribution. A susceptible line could be eliminated whenever any replication has an extremely high number of dead and diseased plants.

One method for screening the CBR resistance would be to use percent dead and diseased plants in field tests and microplot studies of microsclerotia populations. Field evaluation using percent dead and diseased plants would allow for the elimination of poor genotypes for yield as well as susceptibility, thus reducing the size of the test population for further, more extensive evaluation. A small number of promising genotypes can be further evaluated in microplots to determine the long-

term effects of each genotype on the microsclerotia populations in the soil. Microplots would be uniformly inoculated and changes in microsclerotia populaitons in the soil as well as the viability of the microsclerotia produced could be examined over a period of years.

Literature Cited

1. Bell, D. K., and E. K. Sobers. 1966. A peg, pod and root necrosis of peanuts caused by a species of *Calonectria*. Phytopathology 56:1361-1364.
2. Beute, M. K., J. C. Wynne, and D. A. Emery. 1976. Registration of NC 3033 peanut germplasm. Crop Sci. 16:887.
3. Campbell, W. V., J. C. Wynne, D. A. Emery, and R. W. Mozingo. 1977. Registration of NC 6 peanuts. Crop Sci. 17:346.
4. Coffelt, T. A. 1980. Reaction of spanish-type peanut genotypes to Cylindrocladium black rot. Peanut Sci. 7:91-94.
5. Garren, K. H., M. K. Beute, and D. M. Porter. 1972. The Cylindrocladium black rot of peanut in Virginia and North Carolina. J. Am. Peanut Res. Educ. Asso. 4:66-71.
6. Harris, N. E., and M. K. Beute. 1982. Histological responses of peanut germplasm resistant and susceptible to *Cylindrocladium crotalariae* in relationship to inoculum density. Phytopathology 72:1250-1256.
7. Hau, F. C., C. Lee Campbell, and M. K. Beute. 1982. Inoculum distribution and sampling methods for *Cylindrocladium crotalariae* in a peanut field. Plant Dis. Reptr. 66:568-571.
8. Johnston, S. A., and M. K. Beute. 1975. Histopathology of Cylindrocladium black rot of peanut. Phytopathology 65:649-653.
9. Krigsvold, D. T., G. J. Griffin, and M. G. Hale. 1982. Microsclerotial germination of *Cylindrocladium crotalariae* in the rhizospheres of susceptible and resistant peanut plants. Phytopathology 72:859-864.
10. Phipps, P. M., and M. K. Beute. 1979. Population dynamics of *Cylindrocladium crotalariae* microsclerotia in naturally infested peanut soil. Phytopathology 69:240-243.
11. Phipps, P. M., M. K. Beute, and K. R. Barker. 1976. An elutriation method for quantitative isolation of *Cylindrocladium crotalariae* microsclerotia from peanut field soil. Phytopathology 66:1255-1259.
12. Rowe, R. C., and M. K. Beute. 1973. Susceptibility of peanut rotational crops (tobacco, cotton and corn) to *Cylindrocladium crotalariae*. Plant Dis. Reptr. 57:1035-1039.
13. Rowe, R. C., and M. K. Beute. 1975. Variability in virulence of *Cylindrocladium crotalariae* isolates on peanut. Phytopathology 65:422-425.
14. Rowe, R. C., M. K. Beute, and J. C. Wells. 1973. Cylindrocladium black rot of peanuts in North Carolina — 1972. Plant Dis. Reptr. 57:387-389.
15. Rowe, R. C., M. K. Beute, J. C. Wells, and J. C. Wynne. 1974. Incidence and control of Cylindrocladium black rot of peanuts in North Carolina during 1973. Plant Dis. Reptr. 58:348-352.
16. Rowe, R. C., S. A. Johnston, and M. K. Beute. 1974. Formation and dispersal of *Cylindrocladium crotalariae* microsclerotia in infected peanut roots. Phytopathology 64:1294-1297.
17. Snedecor, G. W., and W. G. Cochran. 1967. Correlation. *In* Statistical Methods. Iowa State Univ. Press, Ames. pp. 172-195.
18. Wynne, J. C., and M. K. Beute. 1983. Registration of NC 8C peanut (reg. no. 27). Crop Sci. 23:185.
19. Wynne, J. C., R. C. Rowe, and M. K. Beute. 1975. Resistance of peanut genotypes to *Cylindrocladium crotalariae*. Peanut Sci. 2:54-56.

Accepted August 3, 1983