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Evaluating Peanuts for Resistance to Cylindrocladium Black Rot[1]

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ABSTRACT

Cylindrocladium black rot (CBR), a devastating disease of peanut (*Arachis hypogaea* L.), is caused by *Cylindrocladium crotalariae* (Loos) Bell and Sobers [perfect state, *Calonectria crotalariae* (Loos) Bell and Sobers]. No effective chemical control is known. Resistant cultivars would be the best way to control the disease. Two screening methods, employing sterile and nonsterile media, were developed and used for large-scale systematic screening of peanut genotypes for resistance. Results were reproducible with either method. The nonsterile method was more economical, required less handling, and permitted immediate transplanting of selections. Using both methods, we evaluated 929 different peanut lines from the world germplasm pool. Some 130 lines (14%) exhibited sufficient resistance to be reevaluated. After repeated screening by single-plant selection, six derived inbreds with greater resistance than the standard resistant Spancross and NC 3033 peanuts were named CBR-R1 to CBR-R6 and released as germplasm lines for breeding use. They include representatives from three of the four botanical varieties of *A. hypogaea*.

Key Words: *Arachis hypogaea, Calonectria crotalariae, Cylindrocladium crotalariae,* disease-resistance, groundnut, plant-diseases, peanut breeding.

Cylindrocladium black rot (CBR) was first identified in 1965 as a disease of peanut (*Arachis hypogaea* L.) in Georgia. The pathogen, *Cylindrocladium crotalariae* (Loos) Bell and Sobers [perfect stage, *Calonectria crotalariae* (Loos) Bell and Sobers], has been isolated from hypocotyls, roots and fruits (pegs, pods and seed) of diseased peanuts (3). The disease was found in Japan (17, 19) and South Carolina (9) by 1968, in Virginia and North Carolina in 1970 (9), in Alabama in 1972 (23), in Florida (A. J. Norden, pers. comm.) and India in 1976 (27). CBR is a major threat to production in all these areas, but is most serious in Virginia and North Carolina (4).

Although CBR continues to spread slowly in Georgia (2), where it is limited to some extent by higher soil temperatures (1), the loss potential is enhanced by monoculture of the susceptible Florunner peanut cultivar.

The biology, infection process and host range of the causal fungus were reviewed recently (4). The disease can be very devastating. Whole fields may be lost; in other fields the yield, grade and quality of peanuts are seriously impaired. The influence of inoculum density (14, 20), soil moisture (21), temperature (1, 21), plant age (1), nematodes (8), cropping sequences (15, 22, 24), and other factors upon the incidence, spread and severity of CBR are well known.

No consistently effective or economical chemical control has been found (14, 26).

All commercial cultivars presently grown in the USA (7,10,30) and in Japan (17) are susceptible to CBR. Early studies suggested that Spanish-(subsp. *fastigiata* var. *vulgaris*) type peanuts were more tolerant than the Virginia- or Runner-(subsp. *hypogaea* var. *hypogaea*) types (2,10,26,30). Significant differences in yields, however, have been reported among Virginia/Runner cultivars grown on land infested with *C. crotalariae* (18). A wide range of virulence occurred among fungal isolates (11), but the disease severity pattern indicated an absence of physiological races (25).

Early screening tests indicated less susceptibility in Spanish-type cultivars (2,26,30), and Spancross has consistently appeared among the more resistant cultivars in subsequent tests (7,10).

Two small-seeded Virginia-type breeding lines have been developed, released, and registered (5, 6, 30) as sources of resistance. Each of these has Spanish-type cultivars in its pedigree: NC 3033 was selected from crosses where Spanish 18-38 and Spanish 2B were ancestors (5,30); VGP 1 traces to a cross where Schwartz 21 was a parent (6). Because resistance in NC 3033 is thought to be primarily the response of additive effects of genes, early generation selection should be restricted to environments optimum for disease development (11,12).

Three advanced lines, NC 17941, NC 18016, and NC 18229, developed in North Carolina, exhibit considerable promise and are undergoing extensive further testing for agronomic potential, quality, and disease resistance (29).

Few programs of selection for resistance can rely solely on natural infection. Characterization of the degree of resistance in a wide range of *A. hypogaea* genotypes is essential to the peanut breeding programs. The aims of this research were (a) to identify additional peanut germplasm with resistance to the CBR pathogen, and (b) to develop methods for screening large numbers of seedlings for CBR resistance.

Materials and Methods

Two methods of screening peanut germplasm for resistance to CBR were developed. The first method utilizes a sterile environment and is particularly adapted to the laboratory. *C. crotalariae* was grown at 26 C

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in flasks containing 40 ml of malt extract broth consisting of 20 g malt extract, 3 g sodium nitrate, 1.5 g monobasic potassium phosphate, and 1.5 g dibasic potassium phosphate in demineralized water to make 1 liter. After 14 days growth 5 cultures were drained of excess medium and homogenized for 30 sec in 800 ml of sterile water Test tubes 25x150 mm, containing 50 mm of vermiculite were plugged with cotton and autoclaved for 1 hr at 15 psi. Peanut seed were fumigated with 2.2% cyano-(methylmercuri) guanidine (Panogen-15) under a negative pressure of 700 mm Hg for 48 hr at 36 C. Eight ml of inoculum in suspension were placed in each tube. Twenty seed of each variety or germplasm line were planted individually in the tubes. After 9 days incubation in the dark at 26 C the plants were removed and damage to tap roots and hypocotyls was compared with the resistant control Spancross. Plants exhibiting greater resistance were transplanted to 18 cm pots for seed increase and repeated test.

The second method consisted of growing seedlings in environmental chambers in a nonsterile environment. Seeds were planted in 32x67 cm styrofoam flats with 200 compartments, each containing nonsterile vermiculite and 10 ml of inoculum. Flats were grown in simulated sunlight in chambers set for a 12 hr day at 26-28 C. After 9 days the plants were "pulled" and compared with Spancross and/or NC 3033.

Entries with susceptibility ≥ Spancross (a moderately resistant cultivar) were not tested further. Those with uniform resistance > Spancross, or with a range less-to-greater than Spancross were reevaluated. These lines were reinoculated and incubated 14 days to provide additional advantage for the fungus. Germplasm lines with uniform resistance ≤ NC 3033 (5) were eliminated. Those lines with a range of resistance less-to-greater than NC 3033 were retained as individual plant selecitons, along with entries exhibiting greater resistance.

Plants were selected where no visible disease symptoms were apparent. Selections were immediately transplanted into the greenhouse. At maturity the seeds were sent either to the USDA winter nursery in Puerto Rico or to the agronomy farm near Tifton for increase, and their progeny was retested.

The plant material consisted of 929 different peanut lines from the world collection. The accessions sampled the variation from 43 countries other than the U.S., including representatives from all of the principal peanut producing countries on all six arable continents and from several Oceanic islands. The material ranged from standard cultivars and established genetic stocks through advanced breeding lines to early generation segregants. The germplasm included Spanish and Valencia types in subspecies *fastigiata*, Virginia and Runner types in subspecies *hypogaea*, and a few *Arachis* wild species.

Results and Discussion

Two methods - one sterile and the other nonsterile - were developed for rapid evaluation of peanut germplasm for resistance to Cylindrocladium black rot. The nonsterile method required less manipulation, was more economical, and permitted immediate transplant of individual plant selections with minimal injury. An estimated 6,200 breeding lines/yr (125,000 seed) could be evaluated easily in a typical plant pathological laboratory.

The 929 accessions that have been evaluated represent a "random" sample of the peanut germplasm available. Some 130 lines (14%) gave resistant plants that were retained for generation advance and second cycle screening of their progeny (Table 1). When second cycle plants exhibited resistance no greater than Spancross, the lines were discarded. However, these 130 accessions represent a reservoir of germplasm where additional resistant lines could be sought.

Among 49 lines we found 371 plants with no visible signs of infection. Twelve separate strains, or 1% of the total, had resistance equal or greater than the NC 3033 standard.

Table 1. Peanut germplasm evaluated for resistance reaction to *Cylindrocladium crotalariae*: Accessions from which resistant plants were isolated for generation advance and reevaluation as inbred lines.†

a) Cultivars (7 of 44): GK-3, Jenkins Jumbo, Pearl, Pearl Black, NC 3033, Spancross, New Mexico Valencia A.

b) Aspergillus flavus-tolerant germplasm (1 of 23): A2-74-34.

c) Univ. Georgia-USDA Experimental lines (10 of 88): C200-52-3, GC 32, GC 258-1-1, GA 123, Georgia Jumbo, MC 266CE, Tifton-8, Tifton 1108, GA 722105, VA 7329043.

d) University of Florida breeding lines (first cycle 26 of 124; second cycle 5 of 26): F393-7-1-b4-B, F440A-18-8-B, UF 562B-4-3-1, UF 534A-5-1-2-4-1, UF 520-1-6-1-2-1-2-1-B.

e) Arachis spp. (2 of 4): White-flower selection A-4 from A. monticola, Arachis sp. Acc. A-170.

f) A. hypogaea plant introductions (84 of 646):

119880	139917	145044	196756	226253	244603	268521
268562	268572	268573§	268689	270773	270806	331281
331296	331301	331326§	336931§	336932	337379	337405
338555	338560	339945	339953	339958	339970	339974§
343371	362137§	362141	362142§	362144	363061	385939
385940	390597	393516	393517	393518	393519	393520
393525	393643	399568	403715	403723(flesh)		403737
403739	403740	403741	403744	403748	403752	403757
403758	403762	403768	403769	403770	403771	403772
403773	403774	403775	403782	403785	403786	403788
403790	403792	403793	403794	403795	403796	403797
403799	403802	403827	403829	403831	403832	403836
407670						

† The list will be made available upon request to the author.

§ Denotes accession from which resistant genotype was derived by consecutive cycles of screening inbred lines.

A majority of the lines screened exhibited heterogeneity with respect to CBR resistance, varying from highly susceptible plants to those, in some cases, that are highly resistant. Rescreening the progeny from seed increases of the initial selections has produced only small changes in the numbers of plants reselected. This phenomenon may be attributable to the allopolyploid genetic system of *A. hypogaea*.

Six CBR-resistant lines were developed by repeated single plant selection and reevaluation. Their levels of resistance were compared against the resistant Spancross and NC 3033 checks and against 28 cultivars. The derived lines had resistance equal or greater than NC 3033 or Spancross but lack some of the attributes necessary for commercial production. After initial seed multiplication in nurseries at Tifton, Ga., and Isabella, Puerto Rico, the six peanuts were named and released in March 1981 (13) as germplasm lines CBR-R1 to CBR-R6 (Table 2).

A potentially more serious problem exists by the presence of other species of *Cylindrocladium* in the Southeast and elsewhere where peanuts are grown. Eight species in addition to *Calonectria crotalariae* are pathogenic to peanuts (28). One of these, *C. floridanum* (incorrectly identi-

Table 2. Peanut germplasm lines resistant to Cylindrocladium black rot disease.

Released Germplasm Line	Selection: derived from PI	Sel. No.[1]	Seed size[2]	Testa color	Plant type[3]	Source & Designation
CBR-R1	268573	2	.33	brown	Sp	Morocco Rabat No. 27
CBR-R2	331326	1	.40	white	Va,R	Argentina -- (HL 161)[4]
CBR-R3	336931	2	.30	light brown	Sp	Argentina Correntino (HL 635)[4] Blanco Comun
CBR-R4	339974	1	.34	red	Val	Argentina Hemsy No. 11
CBR-R5	362137	5	.33	brown	Sp	India K 3
CBR-R6	362142	2	.30	brown	Sp	Sudan U4-4-24

1/ Number of resistant selections retained after 3 or 4 consecutive cycles of screening. These inbreds were pooled to form the released germplasm line (cf. ref. 13).

2/ Size of seed in g.

3/ Type: Sp = Spanish; Va = Virginia; Val = Valencia; R = Runner.

4/ Collection number, R. O. Hammons and W. R. Langford, 1968.

fied as *C. scoparium*) is the cause of CBR on peanuts in Japan (19). The extremely wide host range of those two species, and their similarity to that of *C. crotalariae* (3), presents a potentially dangerous situation.

These other species are not temperature sensitive and pathogenicity tests show all are pathogenic to at least two peanut cultivars (28). *C. floridanum* and *C. scoparium* have also been associated with peach decline and have been isolated from an array of ornamental and forest trees in Georgia. The microsclerotia produced by all species of *Cylindrocladium* can persist in the soil for years (16).

Seeds of the 6 resistant germplasm lines are maintained by the Agronomy Department, University of Georgia, Coastal Plain Station, and are available upon request (13).

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Literature Cited

1. Bell, D. K. 1967. Effects of soil temperature and plant age on the severity of Cylindrocladium rot of peanut. Plant Dis. Rep. 51:986-988.

2. Bell, D. K., B. J. Locke, and S. S. Thompson. 1973. The status of Cylindrocladium black rot of peanut in Georgia since its discovery in 1965. Plant Dis. Rep. 57;90-94.

3. 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.

4. Beute, M. K. 1980. Cylindrocladium black rot (CBR) disease of peanut. ICRISAT (Internat'l. Crops Res. Inst. for the Semi-Arid Tropics). Proceedings of the International Workshop of Groundnuts, 13-17 Oct. 1980. (India). Pp. 171-176.

5. Beute, M. K., J. C. Wynne, and D. A. Emery. 1976. Registration of NC 3033 peanut germplasm (Reg. No. GP 9). Crop Sci. 16:887.

6. Coffelt, T. A. 1980. Registration of VGP 1 peanut germplasm (Reg. No. GP-11). Crop Sci. 20:419.

7. Coffelt, T. A. 1980. Reaction of Spanish-type peanut genotypes to Cylindroclaidum black rot. Peanut Sci. 7(2):91-94.

8. Diomande, M., and M. K. Beute. 1979. Influences of *Meloidogyne hapla* and *Macroposthonia ornata* on the effectiveness of microsclerotial inocula of *Cylindrocladium crotalariae* in causing black rot (CBR) on peanuts. (Abst). Phytopathology 69:526.

9. 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. Assoc. 4:67-71.

10. Garren, K. H., and T. A. Coffelt. 1976. Reaction to Cylindrocladium black rot in Virgina-type peanut cultivars. Plant Dis. Rep. 60:175-178.

11. Hadley, B. A., M. K. Beute and K. J. Leonard. 1979. Variability of *Cylindrocladium crotalariae* response to resistant host plant selection pressure in peanut. Phytopathology 69:1112-1114.

12. Hadley, B.A., M. K. Beute, and J. C. Wynne. 1979. Heritability of Cylindrocladium black rot resistance in peanut. Peanut Sci. 6:51-54.

13. Hammons, Ray O. 1981. Registration of six peanut germplasm lines (Reg. Nos. GP 12 to GP 17). Crop Sci. 21: (in press).

14. Hanounik, S. B., W. R. Pirie, and W. W. Osborne. 1977. Influence of soil chemical treatment and host genotype on the inoculum density disease relationships of Cylindrocladium black rot of peanut. Plant Dis. Rep. 61:431-435.

15. Krigsvold, D. T., K. H. Garren, and G. J. Griffin. 1977. Importance of peanut field cultivation and soybean cropping in the spread of *Cylindrocldium crotalariae* within and among peanut fields. Plant Dis. Rep. 61:495-499.

16. Menge, J. A., and D. W. French. 1976. Effect of plant residue amendments and chemical treatments upon the inoculum potential of *Cylindrocladium floridinum* in soil. Phytopathology 66:1085-1089.

17. Misonou, T. 1973. New black root rot disease in soybeans and peanuts by *Calonectria crotalariae*. Shokubutsu Bocki 27:77-82. (Japanese). Tech. Trans. 75-53102. Saad Publ. Trans. Div. 31 p. (English). 1976.

18. Morton, C. S., and L. W. Baxter, Jr. 1979. Response of selected peanut cultivars to Cylindrocladium black rot in South Carolina. (Abst.) Phytopathology 69:530.

19. Ogoshi, A. 1970. A root rot disease of peanuts (*Arachis hypogaea* L) caused by *Cylindrocladium scoparium* Morgan. Bull. Nat. Inst. Agric. Sci., Tokoyo, Ser. C. 24: 153-163 (Japanese, English summary).

20. Phipps, P. M., and M. K. Beute. 1977. Sensitivity of susceptible and resistant peanut cultivars to inoculum densities of *Cylindrocladium crotalariae* microslerotia in soil. Plant Dis. Reptr. 61:300-303.

21. Phipps, P. M., and M. K. Beute. 1977. Influence of soil temperature and moisture on the severity of Cylindrocladium black rot in peanut. Phytopathology 67:1104-1107.

22. Phipps, P. M., and M. K. Beute. 1979. Population dynamics of *Cylindrocladium crotalariae* microsclerotia in naturally-infested soil. Phytopathology 69:240-243.

23. Rodriquez-Kabana, R., and P. A. Backman. 1975. Control of Cylindrocladium black rot of peanuts in Alabama. Highlights of Agric. Res. (Auburn Univ.) 22:16.

24. Rowe, R. C., and M. K. Beute. 1973. Susceptibility of peanut rotational crops (tobacco, cotton and corn) to *Cylindrocladium crotalariae*. Plant Dis. Rep. 57:1035-1039.

25. Rowe, R. C., and M. F. Beute. 1975. Variability in virulence of *Cylindrocladium crotalariae* isolates on peanut. Phytopathology 65:422-425.

26. Rowe, R. C., M. K. Beute, J. C. Wells, and J. C. Wynne. 1973. Incidence and control of *Cylindrocladium crotalariae* black rot of peanuts in North Carolina during 1973. Plant Dis. Rep. 58:348-352.

27. Sharma, N. D., S. C. Vyas, and A. C. Jain. 1978. A groundnut disease new to India. Sci. Cult. 44:119-120.

28. Sobers, E. K., and D. K. Bell. 1975. Pathogenicity of 9 species of *Cylindrocladium* to a variety of Valencia peanuts. (Abstr.) Proc. Am. Phytopathol. Soc. 2:141.

29. Wynne, J. C., and M. K. Beute. 1980. Black root rot resistance found. Virginia-Carolina Peanut News 26:16.

30. Wynne, J. C., R. C. Rowe, and M. K. Beute. 1975. Resistance of peanut genotypes to *Cylindrocladium crotalariae*. Peanut Sci. 2:54-56.

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