Variability in Virulence of Cylindrocladium crotalariae Isolates on Peanut

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ABSTRACT

A wide range of virulence was found in isolates of *Cylindrocladium crotalariae* collected from Virginia, North Carolina, South Carolina, Alabama and Hawaii. This variability was not related to linear growth rate in culture or to geographic origin of the isolates. The disease severity pattern on a series of six peanut varieties was essentially the same for all isolates tested, indicating the absence of detectable physiologic races in the fungus.

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Cylindrocladium black rot (CBR) [caused by *Cylindrocladium crotalariae* (Loos) Bell & Sobers] is a relatively new disease of peanut (*Arachis hypogaea* L.) of increasing importance in the southeastern United States (1, 2, 3, 7). Intensive efforts to develop disease control strategies for CBR, including breeding for resistance, are in progress (1, 7).

Knowledge of the existence of physiologic races in a pathogenic population is essential to breeding disease-resistant cultivars. Extreme variability in CBR severity under field conditions and the prevalence of the pathogen's sexual stage, *Calonectria crotalariae* (Loos) Bell & Sobers (2, 6), suggested the possibility of physiologic specialization in this fungus. The purposes of this study were (i) to ascertain whether races of *C. crotalariae* now exist that should be included in the screening of CBR-resistant peanut selections, and (ii) to investigate variability in virulence among isolates of the fungus, and (iii) to determine if any relationship exists between variability and geographic distribution of the pathogen.

MATERIALS AND METHODS.—*Isolate collection.*—CBR-infected peanut plants were collected from every major peanut-producing county throughout North Carolina during the summers of 1972 and 1973. The fungus was isolated from infected tap roots on acidified potato-dextrose agar (APDA) and monoconidial cultures were established. In a few cases, *C. crotalariae* was isolated from the following legumes: soybean [*Glycine max* (L.) Merr.], partridge pea (*Cassia fasciculata* Michx.), and sickle pod (*C. obtusifolia* L.). Peanut and soybean isolates from Virginia, South Carolina, and Alabama; a blueberry (*Vaccinium corymbosum* L.) isolate from North Carolina (4); and papaya (*Carica papaya* L.) and *Acacia koa* isolates from Hawaii (5) were obtained from K. H. Garren, J. D. Arnett, R. G. Linderman, R. D. Milholland, and M. Aragaki, respectively. Cultures were maintained on APDA at approximately 25 C. Linear growth rates of selected isolates over the range 15-30 C were compared after 3 days.

Peanut cultivars.—Because CBR-resistant peanut cultivars have not yet been developed, this attempt to identify potential races of *Cylindrocladium crotalariae*

TABLE 1. Variability in virulence among North Carolina monoconidial isolates of *Cylindrocladium crotalariae* used to inoculate the peanut cultivar Florigiant

Original isolate	Disease index for indicated monoconidial isolates[a]				Average disease severity[b]
	1	2	3	LSD ($P = 0.05$)	
I	1.6	1.6	2.2	0.6	1.8
C	2.9	2.3	2.5	NS	2.6
H	2.4	2.8	2.8	NS	2.7
L	2.5	3.7	2.7	0.7	3.0
D	2.8	3.4	3.2	NS	3.1
A	4.9	4.1	2.2	1.0	3.7
B	3.6	3.2	4.4	0.7	3.7
G	3.6	3.1	4.5	0.7	3.7
E	4.9	3.5	3.7	0.7	4.0
N	4.3	4.4	4.0	NS	4.2
F	4.5	4.8	4.0	0.6	4.4
K	4.4	4.0	4.9	0.7	4.4
J	4.6	4.8	4.0	0.4	4.5
M	5.0	4.1	4.5	0.4	4.5

[a]Disease indices based on root infection evaluated from 0 (no visible damage) to 5 (completely decayed). Each figure represents the average of 20 plants.

[b]Mean index for the three monoconidial isolates. LSD ($P = 0.05$) = 0.5.

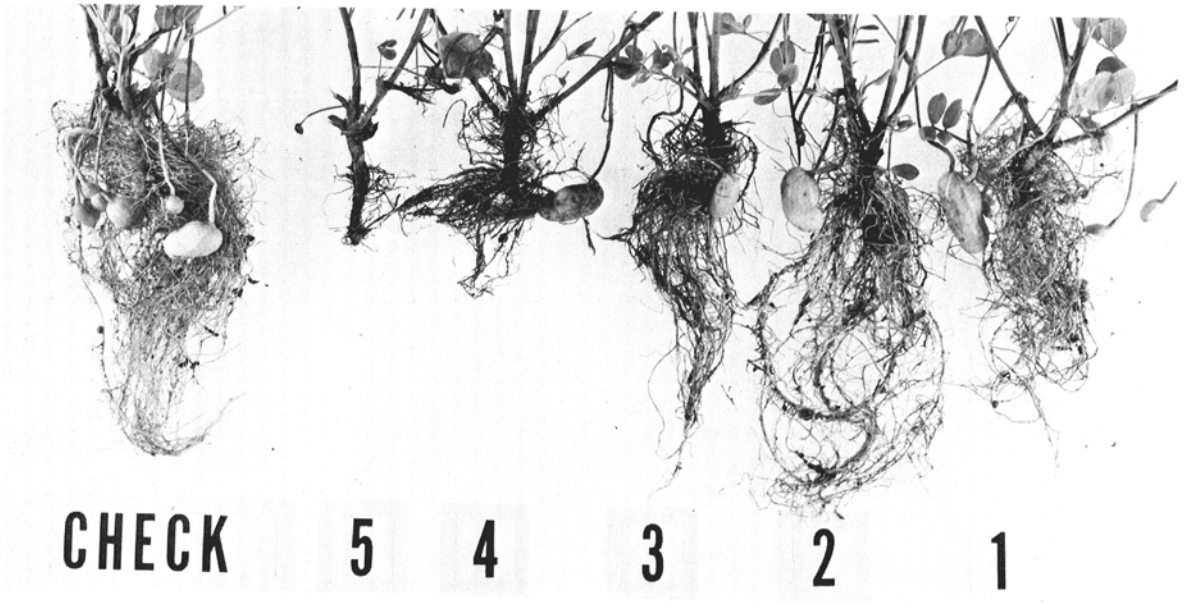

Fig. 1. Root symptom rating scale for Cylindrocladium black rot of peanuts.

TABLE 2. Variability in virulence among three monoconidial daughter isolates taken from monoconidial isolates of *Cylindrocladium crotalariae* and used to inoculate the peanut cultivar Florigiant

Monoconidial parent isolate	Disease index for indicated monoconidial daughter isolates[a]				Average disease severity[b]
	1	2	3	LSD (P=0.05)	
C-2	0.8	0.8	1.5	0.7	1.0
N-1	2.0	1.1	1.5	0.7	1.5
G-2	2.1	3.4	4.3	1.2	3.3
H-2	3.5	3.2	3.5	NS	3.4
J-1	3.7	3.1	3.3	NS	3.4
K-3	3.6	4.0	3.6	NS	3.7
I-1	4.4	2.3	4.6	1.1	3.8
F-2 (ATCC 26110)	4.4	3.3	4.6	0.9	4.3

[a]Disease indices based on root infection evaluated from 0 (no visible damage) to 5 (completely decayed). Each figure represents the average of 10 plants.

[b]Mean disease index for monoconidial daughter isolates. LSD (P = 0.05) = 0.6.

was based on the response of six cultivars with varying degrees of susceptibility. These were selected from a previous screening of 23 cultivars (7) representing genetic diversity in the three botanical types of cultivated peanuts. The cultivars used (with corresponding botanical types listed in order of increasing susceptibility) were: Spantex (Spanish), Florunner (Virginia runner), P. I. 337396 (Valencia), N.C. 5 (Virginia bunch), Florigiant (Virginia bunch), and New Mexico Valencia (Valencia). The criteria used to recognize pathogenic races were differential host reactions to isolates of the fungus.

Inoculum preparation, inoculation procedure, and evaluation of host response.—Inoculum suspensions of each *Cylindrocladium crotalariae* isolate were prepared from four cultures (grown at approximately 25 C in 2% malt extract in 250-ml flasks for approximately 8 weeks) which were comminuted in a Waring Blendor for 15 seconds in 250 ml of sterile distilled water. Four-week-old peanut plants grown in the greenhouse in methyl bromide-fumigated soil in 10-cm-diameter pots were inoculated by placing 3 ml of fungal suspension 2-3 cm below the soil surface at the base of each plant. The soil was then saturated with water. Disease severity was evaluated 10-14 weeks later by washing root systems free of soil and evaluating them for CBR damage on a scale of 0 (no visible damage) to 5 (completely decayed) (Fig. 1).

RESULTS.—An extremely wide range of virulence was observed among North Carolina isolates of *C. crotalariae* on the peanut cultivar Florigiant (Table 1). Comparison of three monoconidial isolates taken from each original isolate showed uniform virulence within the sampled population in some cases (isolates C, D, H, N) and wide variability in other cases (isolates A, B, E, G, L).

Eight monoconidial isolates representing the observed range in virulence were selected for further study. A comparison of linear growth rates after 3 days in the range 15-30 C showed that all eight isolates developed maximum colony diameter (11-14 mm) at 25-28 C. The test was repeated with similar results. These isolates were then again single-spored and inoculated to cultivar Florigiant. Although (in most cases) the relative virulence of the daughter monoconidial isolates (Table 2) was similar to that of the parent monoconidial isolates (Table 1), significant variability was still apparent in many cases, with one or more of the daughter isolates less virulent than its parent isolate (F-2, G-2, N-1). In the case of isolate I-1, however, the daughter isolates appeared much more virulent than the parent isolate.

The same eight parent isolates were then tested on six peanut cultivars in an attempt to detect possible physiologic specialization. The pattern of host response of the six varieties, however, was essentially the same for all isolates tested (Table 3).

Three Hawaiian isolates of *C. crotalariae* obtained from papaya and *Acacia koa* were compared with three North Carolina peanut isolates on the six peanut varieties. Although the isolates tested came from vastly divergent geographic areas, the pattern of host response was again essentially the same.

TABLE 3. Response of six peanut varieties to infection by North Carolina monoconidial isolates of *Cylindrocladium crotalariae*

Monoconidial isolate	Disease index[a,b]						Mean disease index[c]
	Spantex	Florunner	P.I. 337396	NC 5	Florigiant	New Mexico Valencia	
C-2	0.0[a,b,c]	0.1	0.8	0.3	0.5	0.4	0.4
N-1	0.3	0.1	0.0	0.6	1.1	0.4	0.4
H-2	0.6	0.4	1.2	1.4	1.5	2.1	1.2
G-2	1.3	1.6	1.5	2.0	2.2	3.4	2.0
I-1	1.4	2.0	2.4	1.3	2.9	3.7	2.3
K-3	2.2	2.1	2.2	1.8	2.4	3.4	2.4
J-1	1.0	1.9	2.6	3.0	3.0	3.3	2.5
F-2 (ATCC 26110)	1.3	3.7	3.2	3.7	4.1	4.2	3.4
Mean cultivar susceptibility[c]	1.0	1.5	1.7	1.8	2.2	2.6	

[a]Disease indices based on root infection evaluated from 0 (no visible damage) to 5 (completely decayed). Each figure represents the average of 10 plants.

[b]LSD (P = 0.05) = 0.8.

[c]LSD (P = 0.05) = 0.3.

During 1973, 57 monoconidial isolates of the fungus were obtained representing every major peanut-producing county in North Carolina and Virginia, plus one isolate each from South Carolina and Alabama. These isolates were also tested on the same six peanut varieties. Average disease severity ratings on specific varieties ranged from 0.9 to 4.5, but in all cases, the pattern of host response was essentially the same. When the mean disease indices of each isolate on all six varieties were grouped and averaged on the basis of the isolates' geographic origin, i.e., S. E. Virginia, N. E. North Carolina, central North Carolina and S. E. North Carolina, the range of pathogenicity among isolates was the same at all sampled locations.

DISCUSSION.—All isolates tested, regardless of diverse geographic origins, elicited the same general pattern of host response on the six peanut varieties, indicating no evidence for detectable physiologic specialization in *Cylindrocladium crotalariae*. Physiologic races of this pathogen may, however, exist in nature at undetectable levels, since there are presently no resistant cultivars in use to selectively favor them within the fungal population. A wide range of virulence does appear to be inherent in the fungus. This variability is not related to linear growth rate in culture nor is it correlated with geographic distribution of the pathogen.

Although control of CBR on peanuts and other leguminous crops in the future will probably be based on the development of disease-resistant cultivars, varieties with commercially acceptable levels of CBR-tolerance have not yet been identified. Preliminary tests, however, indicate that high levels of CBR-resistance may be present in certain wild *Arachis* species. Changes in selection pressures resulting from the future use of commercial varieties which may be developed with high *C. crotalariae* resistance may then favor certain portions of the fungal population already present at undetectable levels. Moreover, because of the variability inherent in the fungus, the prevalence of its sexual stage and its wide host range, these changes could encourage the future development of physiologic races of *C. crotalariae*.

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