Different Ratios of General:Specific Virulence Variance Among Isolates of *Cylindrocladium crotalariae* from Different Peanut Genotypes

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ABSTRACT

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One hundred and forty-five isolates of *Cylindrocladium crotalariae* were obtained from seedlings of six peanut (*Arachis hypogaea*) genotypes grown in peanut-field soil. Resistant peanut cultivar NC 3033 and susceptible cultivar Florigiant were used as host differentials to compare isolate variability for virulence. There was a trend for general virulence for Cylindrocladium black rot to differ among groups of isolates from different genotypes. The overall root rot mean was highest for isolates from resistant NC 3033 and lowest for isolates from susceptible NC 6. Isolates obtained from resistant genotypes NC 3033 and NC 18231 had high variance for virulence specific to host differentials. Isolates from NC 3033 and NC 18231 had low and intermediate ratios of general:specific virulence variance, and these ratios were the two lowest among the six sources of isolates compared.

Following the initial observation of Cylindrocladium black rot caused in peanuts (*Arachis hypogaea* L.) by *Cylindrocladium crotalariae* (Loos) Bell and Sobers (1), efforts have been made to identify sources of resistance (4,9,21) and to transfer resistance into large seeded Virginia-type peanuts (6,20). Although no pathogen races have been detected (16), there has been concern for stability and proper deployment of resistance (3,4,7). Resistance is quantitatively inherited (5,8) and this type of resistance is thought to be more stable than qualitatively inherited resistance (19).

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Some isolates of *C. crotalariae* that Hadley et al (7) obtained from resistant NC 3033 prior to harvest had a high level of specific virulence for NC 3033. They did not determine whether this adaptation to resistant plants was due to selection of rare strains of the pathogen or resulted from some type of genetic recombination during the 22 wk following planting.

The virulence of isolates of *C. crotalariae* on a set of differential peanut genotypes can be described with a model used by Hadley et al (7). Their model, $D_{hp} = \mu + K_h + G_p + S_{hp}$, describes variation in root rot severity (D_{hp}) as a function of the population mean (μ), the cultivar deviation from that mean (K_h), a general virulence component (G_p), and a specific virulence component (S_{hp}). "General virulence" was defined as virulence of *C. crotalariae* effective against all peanut genotypes. "Specific virulence" was

defined as virulence effective against one cultivar, or a group of cultivars that have in common the same genes for resistance. From the additive model, any given isolate of *C. crotalariae* would probably have some of both types of virulence. Ideally, several differentials would be used when estimating variance components, but with the logistics of this host pathogen system it is mandatory to keep experiments simple. Only two differential genotypes were used in experiments by Hadley et al (7) and in the present study.

This study was initiated to determine whether seedlings of resistant or susceptible cultivars could select for isolates of *C. crotalariae* with or without high specific virulence to a resistant differential, and to provide a basis for choosing isolates for other studies of variability under field conditions (2,3).

MATERIALS AND METHODS

Seedlings of six peanut cultivars grown in peanut-field soil were used as sources of isolates of *C. crotalariae*. Soil was collected from peanut fields with a history of Cylindrocladium black rot in Martin and Bladen counties, NC, in December 1978 and placed in cylinders (inside diameter, 3.5 cm; length, 15.5 cm) of black plastic pipe with plastic mesh glued on one end. Three-day-old seedlings of four resistant peanut cultivars (NC 3033, NC 18016 [NC 9088 × NC 3033], NC 18230 [NC 9088 × NC 3033], NC 18231 [NC 3033 × NC 2]) and two susceptible cultivars (Florigiant and NC 6) were transplanted, one plant per cylinder. The cylinders were placed in a tray with 1–2 cm of water for subirrigation and the plants were grown in the greenhouse at ~25 C. After 4 wk, roots were washed free of soil, lesions were surface sterilized in 0.5% NaClO for 30 sec and plated on a semiselective medium for *C. crotalariae* (14). After transfer on this medium to ensure pure cultures, 145 isolates were increased and microsclerotia were extracted and standardized (15) at 35 microsclerotia per gram (dry mass basis) in soil (Norfolk loamy sand from North Carolina State University Central Crops Research Station, Clayton, NC) initially free of *C. crotalariae*.

For comparison of virulence among 145 isolates on host differentials Florigiant and NC 3033, infested soil was packed in cylinders and one seedling was transplanted per cylinder as described above. The experimental design was a split plot design with five replications (290 plants per replication) and in which main plots were isolates and subplots were cultivars. Twelve isolates were increased after 15 mo in agar culture at 25 C (15) and retested.

Root rot was evaluated at 4 wk on a 0 (no visible lesions) to 5 (completely rotted) scale (16). Variances for general ($\hat{\sigma}^2$ due to isolates) and specific ($\hat{\sigma}^2$ due to isolate × differential interaction) virulence and their ratio (7) were calculated from expected mean squares for the mixed model in a split plot design (17). Main plots for isolates were random and subplots for differentials were fixed effects. Severity of root rot caused by each isolate on Florigiant was regressed against severity of root rot caused by the same isolate on NC 3033 for the group of isolates obtained from each of the six peanut cultivars and for 12 isolates retested after 15 mo in culture.

RESULTS

A trend was detected for differences ($P<0.12$) in mean root rot (averaged for resistant NC 3033 and susceptible Florigiant differentials) among the six cultivar sources of isolates (Table 1). Isolates from resistant NC 3033 and susceptible NC 6 had the highest and lowest mean virulence, respectively.

Data were analyzed a second time without regard for cultivar origin of isolates in the model, ie, 145 isolates were compared on two host differentials. Isolate, differential, and isolate × differential effects were all significant ($P<0.05$). Thirdly, data were then analyzed separately for each cultivar used as a source of isolates (Table 1). Variance due to specific virulence was highest for isolates from NC 3033 and NC 18231 and lowest for isolates from Florigiant and NC 18016. The isolate × differential interaction was highly significant ($P<0.01$) for NC 18231 and the ratio of general:specific virulence variance was intermediate (an arbitrary designation). The isolate × differential interaction trended towards significance for NC 6 ($P = 0.08$) and NC 3033 ($P = 0.15$) with intermediate and low general:specific virulence variance ratios, respectively. The interaction term was not significant for Florigiant, NC 18016, and NC 18230 which had high general:specific virulence variance ratios (Table 1).

The magnitude of difference among the ratios of general:specific virulence variance ranged from twofold (NC 3033 versus NC 18231) to 23-fold (NC 3033 versus NC 18016) (Table 1).

Regression analyses of root rot on Florigiant with root rot on NC 3033 are illustrated (Fig. 1). Slopes and intercepts were similar among cultivars used as sources of isolates. Twelve isolates labeled on Fig. 1 were chosen for further study of variability under field conditions based upon proximity to the upper or lower 95% confidence limits (2,3).

Analysis of variance (Table 2) and regression analysis (Fig. 2B) were conducted for the 12 selected isolates that were retested after 15 mo in culture. Mean virulence of the selected isolates was similar after 1.5 and 15 mo in culture. At 1.5 mo, general virulence variance was less than specific virulence variance (low ratio), but after 15 mo in culture, these same isolates had comparatively more variance for general than for specific virulence variance (intermediate ratio). The regression model for isolates on the two differentials was not significant at 1.5 mo but was significant ($P<0.001$) at 15 mo.

DISCUSSION

Hadley et al (7) showed that some isolates of *C. crotalariae* obtained from resistant NC 3033 peanut plants 22 wk after planting had high virulence for NC 3033. In this study, 4-wk-old peanut seedlings were used as sources of isolates. Groups of isolates with different virulence characteristics were obtained from seedlings of different genotypes. Mean overall virulence tended ($P<0.12$) to differ and was highest for isolates from resistant NC 3033 and lowest from susceptible NC 6 (Table 1). Hadley et al (7) found no

TABLE 1. Mean virulence over two differential peanut cultivars (susceptible Florigiant and resistant NC 3033) and variances due to general and specific virulence of isolates of *Cylindrocladium crotalariae* obtained from seedlings of six peanut cultivars

Isolate source	Reaction[a]	Mean root rot[b]	Number of isolates	CV (*a*)[c]	CV (*b*)[c]	Variance[d]		Variance ratio (G:S)
						General (G) virulence	Specific (S) virulence	
NC 3033	R	3.01	31	28	34	0.195	0.130	1.5:1
NC 18016	R	2.92	28	28	32	0.305	0.009	34.2:1
NC 18230	R	2.88	25	28	36	0.606	0.025	24.2:1
NC 18231	R	2.94	25	25	32	0.438	0.120	3.7:1
Florigiant	S	2.95	18	32	35	0.238	0.009	25.6:1
NC 6	S	2.75	18	28	34	0.258	0.053	4.9:1

[a] R = resistant, S = susceptible.

[b] 0 for no lesions, 5 for completely rotted roots; standard error = 0.23 from overall analysis; the mean is over the two differential cultivars (five plants/isolate/cultivar), susceptible Florigiant and resistant NC 3033.

[c] CV(*a*) = coefficient of variability for main plots and CV(*b*) = coefficient of variability for subplots from six separate analyses according to source of isolates.

[d] General virulence variance is $\hat{\sigma}^2$ due to isolates, specific virulence variance is $\hat{\sigma}^2$ due to isolate × differential interaction (from split plot expected mean squares, isolate was main plot and differential was subplot).

difference in mean virulence between two groups of isolates.

C. crotalariae can frequently be isolated from asymptomatic peanut roots (18). Perhaps such isolates did not have sufficient virulence to initiate a visible lesion. This would suggest that lesions on roots of a susceptible genotype could be induced by variants of *C. crotalariae* over a broad range of virulence (low to high), and that the fewer lesions on a resistant genotype should represent only the more virulent variants of the fungus population. Therefore, isolates obtained from lesions on resistant plants should have different characteristics than isolates from a susceptible cultivar on which lesions might represent a random sample of microsclerotia.

The significant isolate × differential interaction from analysis of variance for 145 isolates led us to look at characteristics of isolates according to their source. For each group of isolates we calculated the ratio between variances of general virulence and specific virulence (Table 1). A low ratio indicated that a relatively large amount of the variability within an isolate group was due to specific virulence. Conversely, a high ratio indicated that specific virulence contributed little to total variance in that group of isolates. In turn, the ratios were compared and the group of isolates from NC 3033 had a 2- to 23-fold greater proportion of their variance as specific virulence variance than did isolates from other cultivars. Specific virulence variance was also a relatively large component of total variance for NC 18231. With the specific virulence variance of NC 6 being less than half that of isolates from NC 3033 or NC 18231, and with the tendency for isolates from NC 6 to have low overall virulence, NC 6 apparently would not select strains of *C. crotalariae* effective against resistant NC 3033.

In agreement with Hadley et al (7), NC 3033 and now NC 18231 appear to be capable of selecting some strains of *C. crotalariae* that

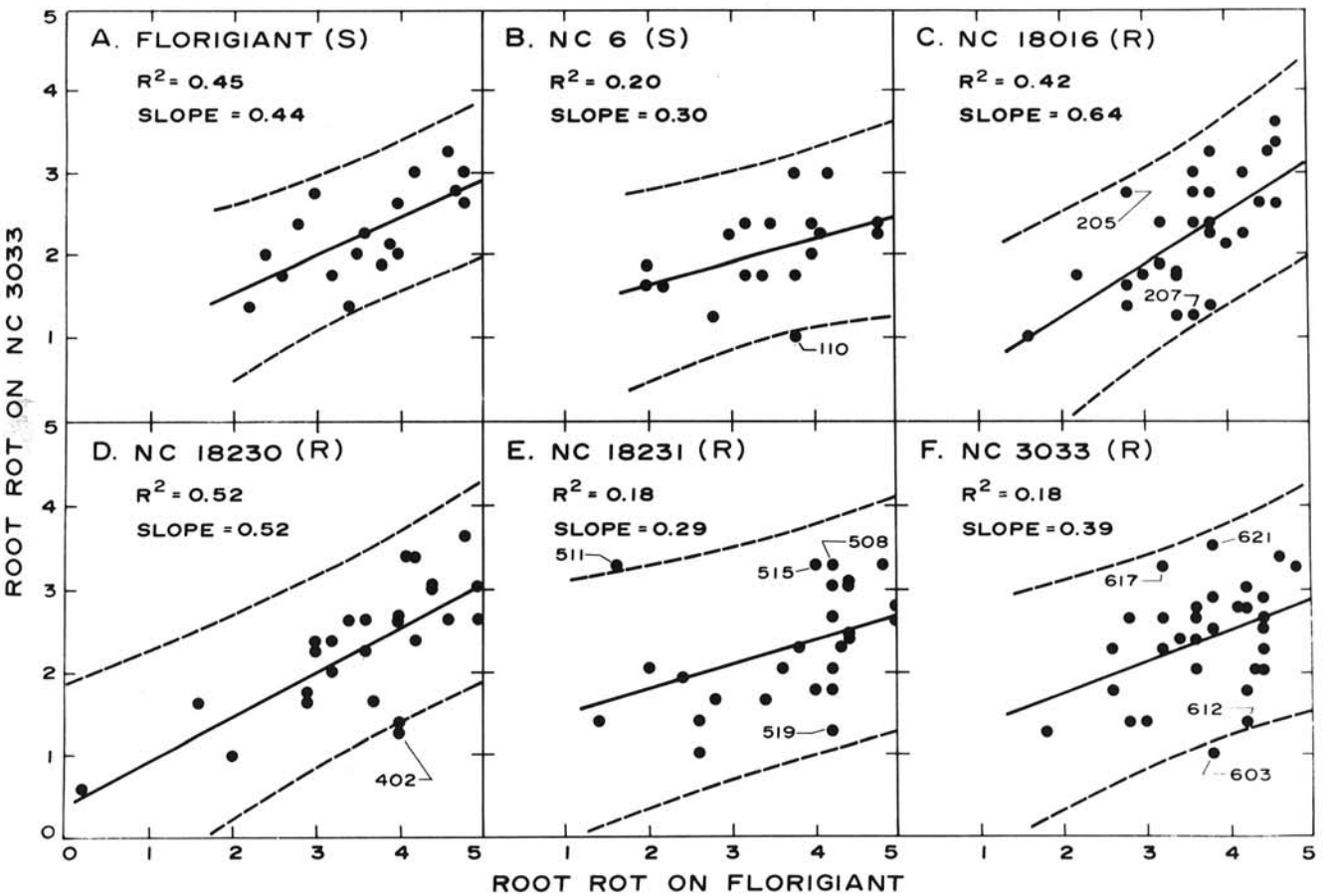

Fig. 1. Regressions for peanut root rot caused by *Cylindrocladium crotalariae* on host differentials Florigiant and NC 3033 according to peanut cultivars **(A to F)** used as a source of isolates from peanut-field soil. Solid line is the regression line, dotted lines are upper and lower 95% confidence limits. Regression models are significant ($P<0.04$) except NC 6 ($P<0.06$). Isolates designated with numbers were chosen for other studies in the field.

TABLE 2. Mean virulence over two differential peanut cultivars (susceptible Florigiant and resistant NC 3033), mean virulence by differential, and variances due to general and specific virulence of 12 isolates of *Cylindrocladium crotalariae* after 1.5 and 15 mo in culture

Months in culture	Mean root rot[a]		CV(*a*)[b]	CV(*b*)[b]	Variance[c]		Variance ratio (G:S)
					General (G) virulence	Specific (S) virulence	
1.5	Overall	2.88	28	36	0.147	0.458	0.32:1
	Florigiant	3.60					
	NC 3033	2.16					
15	Overall	2.85	21	26	0.876	0.134	6.56:1
	Florigiant	3.76					
	NC 3033	1.94					

[a] 0 for no lesions, 5 for completely rotted roots. For 1.5 mo, there were five plants per isolate per cultivar and for 15 mo there were 12 plants per isolate per cultivar.

[b] CV(*a*) = coefficient of variability for main plots and CV(*b*) = coefficient of variability for subplots.

[c] General virulence variance is $\hat{\sigma}^2$ due to isolates, specific virulence variance is $\hat{\sigma}^2$ due to isolate × differential interaction (from split plot expected mean squares).

are adapted to our resistant differential. Resistant NC 18016 and NC 18230 were not as capable of selecting isolates highly variable in specific virulence from soil as were NC 3033 and NC 18231 (Table 1).

The significant regression analyses illustrate that variance for general virulence was always greater than variance for specific virulence (Fig. 1). However, the low coefficients of determination (R^2) indicate that the genetics of virulence in *C. crotalariae* to *A. hypogaea* is complex. That is, one cannot consistently predict virulence of an isolate on a differential based on that isolate's virulence on another differential. Width of the confidence band around the regression line is an illustration of the magnitude of specific virulence variance. Slopes less than 1.0 are consequences of susceptibility of Florigiant (root rot severity scale on the *x*-axis) and resistance of NC 3033 (root rot severity scale on the *y*-axis).

Testing 12 isolates after 15 mo in culture revealed that mean virulence over both differentials did not decline (Table 2).

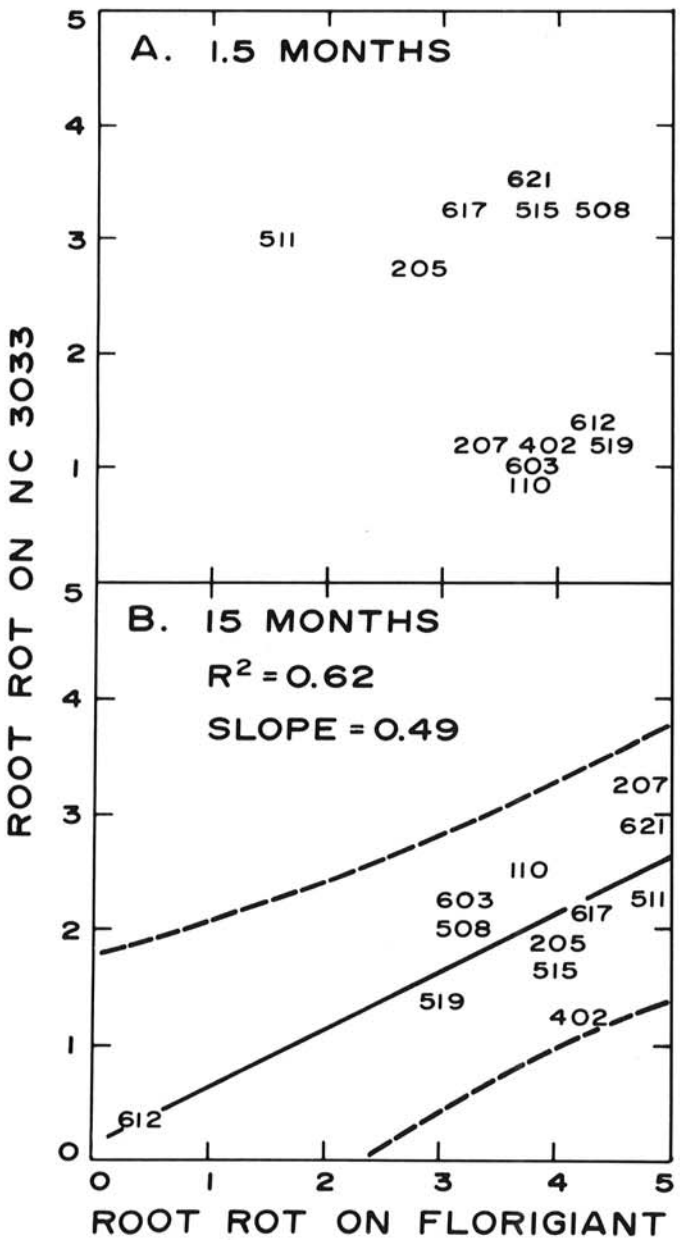

Fig. 2. Response of 12 selected isolates of *Cylindrocladium crotalariae* on peanut differential cultivars Florigiant (susceptible) and NC 3033 (resistant). **A,** At 1.5 mo after isolation from peanut seedling roots there was no relationship between the response on these two cultivars. **B,** Regression with isolates retested after 15 mo in culture. The solid line is the regression line and dotted lines are upper and lower 95% confidence limits. The regression model was significant ($P<0.001$).

However, variance for specific virulence decreased and variance for general virulence increased with time in culture. The increase in the general:specific virulence variance ratio indicates that specific virulence is not a stable trait when isolates are kept as agar cultures at 25 C. This is illustrated by the change from no relationship between root rot severity on differentials for 12 selected isolates (Fig. 2A) to a significant relationship (Fig. 2B). The low level of specific virulence previously reported for isolates pooled from several susceptible hosts (7) may be related to how long those isolates were in culture. Hadley et al (7) did not use fresh cultures for isolates from susceptible hosts in their experiment. Perhaps isolates should be maintained as dormant microsclerotia in field soil to avoid changes in virulence during storage in agar cultures.

This study indicates that some peanut cultivars can select strains of *C. crotalariae* with high specific virulence variance within 4 wk after exposure. There may be several possible mechanisms whereby isolates with specific virulence to a resistant cultivar could be selected. Germination would not occur if exudates from resistant cultivars do not overwhelm fungistasis for some microsclerotia, especially under suboptimal conditions for disease development (12). Or microsclerotia may germinate and mycelium may contact host tissue, but infections may still be unsuccessful because of root defense mechanisms (10). Successful infections may proceed at different rates, with the most rapidly enlarging lesions on the root system (initiated by microsclerotia with greater virulence) forming the most microsclerotia for subsequent seasons.

Mycelium of *C. crotalariae* is reported to be predominately binucleate (11) which probably serves as a reservoir of variability for this pathogen. On quantitative assay plates (14), colonies from different microsclerotia become intermingled with no indication of aversion in most cases (M. C. Black, *unpublished*). Hyphal anastomosis between different isolates has been observed under laboratory conditions (J. E. Hollowell and M. C. Black, *unpublished*). Perhaps unique strains arise when anastomosis of hypha from coalescing lesions on resistant cultivar roots is followed by parasexual recombination of genes for virulence.

A gradual erosion of the effectiveness of quantitatively inherited resistance to *C. crotalariae* may occur if resistance is mismanaged. All known resistance to this pathogen is inoculum density dependent (13). Therefore, even highly resistant peanuts should not be planted in fields with high inoculum densities. To do so would drastically increase the probability of selecting variants of *C. crotalariae* virulent to resistant cultivars. Data from this study are in agreement with those of field studies, which indicates that monoculture of resistant peanuts in infested fields should be avoided. This would minimize the probability of developing specifically virulent strains, even if inoculum density does not increase to high levels in the first one or two seasons (3).

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