Variability of *Cylindrocladium crotalariae* Response to Resistant Host Plant Selection Pressure in Peanut

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ABSTRACT

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.

Seventy-nine isolates of *Cylindrocladium crotalariae* originally obtained from plants of the resistant peanut cultivar NC 3033 and 11 isolates obtained from susceptible plant species including peanut were tested by inoculating replicates of the susceptible cultivar Florigiant and resistant cultivar NC 3033. The mean virulences of isolates from the susceptible hosts did not differ significantly from those of isolates from the resistant host. When disease data were fitted to a pathogen virulence model, however, differences were noted among isolates from the resistant host. As isolates became adapted to NC 3033, they tended to become less adapted to Florigiant. Following only one cropping cycle of the resistant host, virulence specific for NC 3033 increased in a previously nonselected pathogen population. The results indicate that a potential exists for race development in *C. crotalariae* even though corresponding resistance in the host appeared to be quantitatively inherited.

Van der Plank (10) proposed the concepts of vertical and horizontal resistance, which provided a basic genetic description of host plant resistance to pathogens. In host-pathogen interactions, vertical resistance effects a differential interaction between host plant cultivars and pathogenic races. In contrast, no differential interactions between host and pathogen occur when only horizontal resistance is present.

Van der Plank (11) presented two methods for determining differential interactions. The first method involves a direct statistical analysis of variance to test host × pathogen interaction variance. A significant F-test indicates a differential interaction and, consequently, vertical host resistance. The second method involves ranking pathogen isolates for virulence on host cultivars. Statistically significant changes in the ranking order of isolates on different cultivars demonstrates a differential interaction and vertical resistance.

Recently, deficiencies have been noted in the concepts of vertical and horizontal resistance. According to Van der Plank (10) vertical resistance affects epidemics by reducing initial inoculum and horizontal resistance reduces the rate of disease increase. Consequently, reduced rate of disease increase customarily has been associated with horizontal resistance. By definition, there should be no differential interactions with horizontal resistance. Caten (1), working with potato cultivars presumed to be horizontally resistant to races of *Phytophthora infestans,* demonstrated that intra-racial variation in the pathogen resulted in differential interaction on host cultivars. Race 4 isolates of *P. infestans* that were isolated from cultivars with resistance that reduces the rate of disease increase, showed significant adaptations toward the original host cultivar when inoculated onto potato tubers.

Similar results for differential interactions between barley cultivars and *Puccinia hordeii* were reported by Clifford and Clothier (2) and Parlevliet (4). Clifford and Clothier (2) also found indications that increased adaptation or compatibility on a particular horizontally resistant host cultivar may result in decreased compatibility on other cultivars.

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Rowe and Beute (9) reported that physiologic races of *C. crotalariae* did not exist at detectable levels in 1973. Six peanut cultivars were utilized as host differentials for assaying variability in virulence of isolates obtained from widely differing geographic areas of the United States. At that time, however, peanut genotypes resistant to Cylindrocladium black rot (CBR) had not yet been discovered, thus adequate host differentials did not exist. The purpose of this study was to re-evaluate the variability in virulence of *C. crotalariae* isolates by using CBR-resistant and susceptible peanut genotypes as host differentials to determine the effect of resistant host plant selection pressure on the degree of differential interactions between hosts and pathogen isolates. Knowledge of potential race development in the fungus may be directed toward future *C. crotalariae*-resistant peanut germplasm evaluation.

MATERIALS AND METHODS

Source of isolates. In 1976, seeds of peanut cultivar NC 3033 were planted in 1–2 hectare (ha) blocks at each of five locations in North Carolina. All blocks were located in fields with previous histories of CBR and diverse geographic locations and soil types.

At each of the five locations, 20 randomly chosen NC 3033 plants with typical CBR symptoms were removed from the field and root biopsy tissue samples were incubated on a selective medium (7) for recovery of the fungus. Of the 100 diseased plants that were sampled, 79 yielded cultures of *C. crotalariae.* These 79 resistant host isolates, together with 11 isolates originally isolated from susceptible hosts which included peanut (*Arachis hypogaea* L.), soybean (Glycine max [L.] Merr.), blueberry (*Vaccinium corymbosum* L.), *Acacia koa,* and sicklepod (*Cassia obtusifolia* L.), were tested to determine their virulence on susceptible and resistant peanut cultivars.

Isolate evaluation. The isolates were screened for virulence characteristics in a greenhouse in which soil temperatures were maintained at approximately 25 C and soil moisture at field capacity for maximum disease development (5). The isolates were tested on five replications each of the susceptible cultivar Florigiant and resistant cultivar NC 3033 with two plants per replication. A peanut field soil known to be free of *C. crotalariae* was infested artificially to a density of 35 microsclerotia (ms) per gram of soil

according to the method described by Phipps et al (8). This particular inoculum density was chosen to avoid overwhelming the resistance of NC 3033 (6) and to allow identification of any isolates with increased virulence which may have been selected by the resistant host.

The artificially infested soil was placed in 10-cm plastic pots and two seeds of either NC 3033 or Florigiant were planted in each pot. The experimental design was split plots in randomized complete blocks. Fungal isolates represented whole plots, and the host cultivars, the subplots. Plants were grown for eight wk and then the roots were washed and rated for disease development. A subjective root disease index was used which ranged from 0 (healthy and no lesions) to 5 (root system completely decayed and plant dead). The root disease indices for the two plants in each pot were averaged (9).

Pathogen virulence model. To detect rank changes in virulence of isolates following resistant host plant selection pressure, the data, which consisted of replicate root disesae indices, were fitted to a mathematical model adapted from the host-pathogen interaction studies of Caten (1). The model, $D_{hp} = \mu + K_h + G_p + S_{hp}$, specifies that disease (D_{hp}) equals the additive effects of the mean (μ) or general susceptibility of peanut, the varietal deviation from that mean (K_h), a general virulence component (G_p) of the pathogen that is effective against all peanut genotypes, and a specific virulence component (S_{hp}) of the pathogen that is specifically effective against peanut genotype h. Variances were calculated for the general and specific virulence components from isolates originating from the resistant and susceptible hosts. The mathematical relationships of components in the pathogen virulence model (and also Caten's [1] model) correspond to those in the linear additive model for the analysis of variance of factorial experiments. The general and specific virulence components can be directly obtained as the mean squares of isolates and of the interaction between hosts and isolates, respectively.

TABLE 1. Variance comparisons of the general and specific virulence components of *Cylindrocladium crotalariae* isolates that originated from resistant and susceptible host populations

Host Population	Number of Isolates	Virulence component	Variance	Virulence variance ratio (general:specific)
Resistant	79	General	2.62 **	2.06:1
		Specific	1.27 **	
Susceptible	11	General	1.84 **	7.87:1
		Specific	0.23 NS	

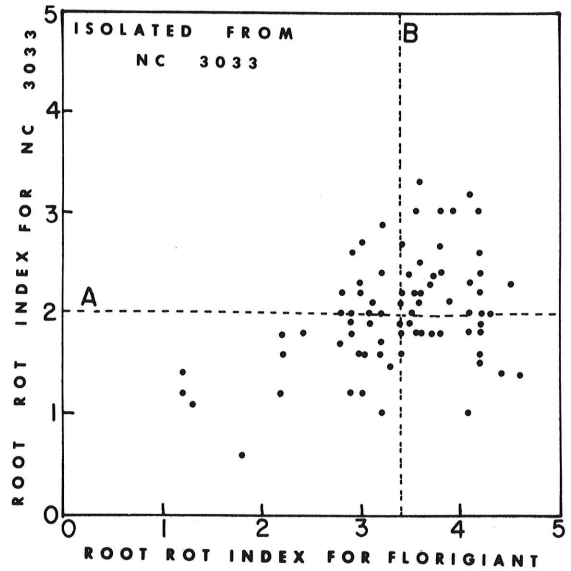

Fig. 1. Average virulence of *Cylindrocladium crotalariae* isolates originating from Cylindrocladium black rot-resistant NC 3033 peanut. Lines A and B are average virulence of isolates on NC 3033 and Florigiant, respectively. Among isolates with greater than average virulence on Florigiant, virulence on NC 3033 is not correlated ($P = .05$) with virulence on Florigiant. Among isolates with less than average virulence on Florigiant, virulence on NC 3033 is correlated with virulence on Florigiant.

RESULTS

Taken as a group, the 79 isolates from resistant hosts showed a wide range of virulence on both Florigiant and NC 3033 (Fig. 1). The same was true of the 11 isolates from susceptible hosts (Fig. 2). The means and standard errors for virulence of isolates from the resistant and susceptible hosts were 2.84 ± .22 and 2.71 ± .22,

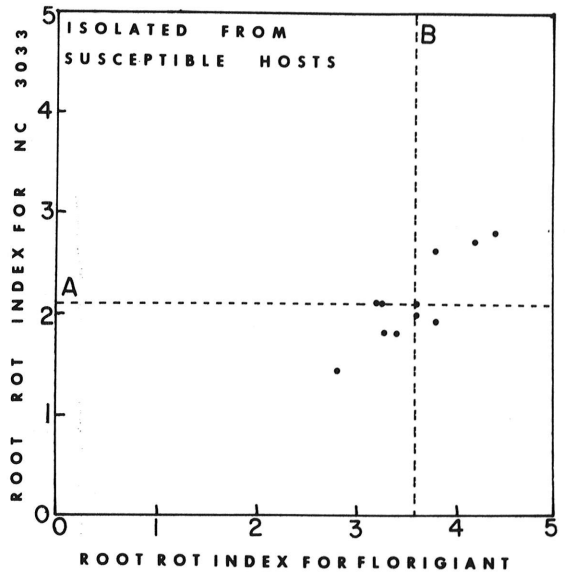

Fig. 2. Average virulence of *Cylindrocladium crotalariae* isolates originating from CBR-susceptible hosts. Lines A and B are average virulence of isolates on peanut cultivars NC 3033 and Florigiant, respectively.

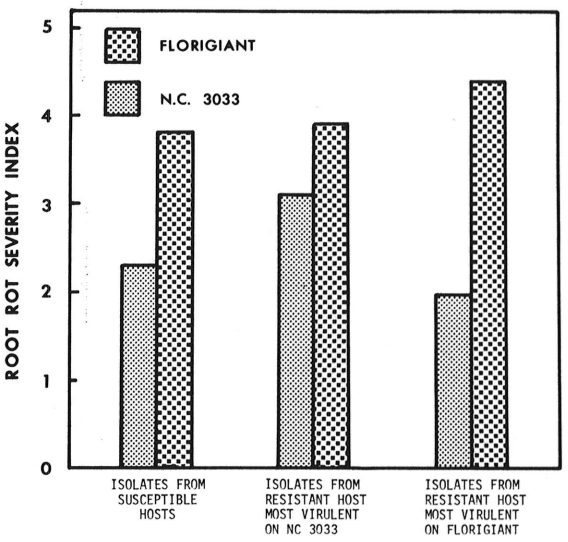

Fig. 3. Effect of *Cylindrocladium crotalariae* isolate origin and resistant host selection pressure on root rot severity of resistant and susceptible peanut cultivars.

respectively. Upon close examination, however, it was noted that among the isolates initially isolated from the resistant hosts, those which were most virulent on NC 3033 were not the same ones which were most virulent on Florigiant (Fig. 1). Graphically, these cultivar × isolate interactions can be demonstrated by comparing the heights of the bars which represent disease severity in the resistant and susceptible cultivars (Fig. 3).

Variances for the general and specific virulence components and the ratio of general virulence to specific virulence of isolates from the resistant and susceptible hosts were determined (Table 1). General virulence (virulence toward all peanut genotypes regardless of their resistance level) was slightly greater in pathogen isolates that originated from the resistant host population. More dramatic, however, was the difference in specific virulence, or virulence toward a particular host. By comparing the ratios of general virulence to specific virulence from each of the two host populations, an estimate of the relative change in the pathogen population can be made. After only one cropping cycle, the pathogen population from the resistant host showed nearly a fourfold increase in the proportion of total virulence specific to the cultivar NC 3033 compared to the pathogen population that originated from susceptible hosts.

DISCUSSION

The pathogen virulence model is adapted from Caten's studies (1) involving the growth rate of *P. infestans* on different potato cultivars. The model itself is simply a mathematical relationship in which a measured response is equal to the additive effects of the individual components and the interaction between the components. The terms general and specific virulence are proposed to give biological meaning to the mathematical expression. The interaction component or specific virulence is of interest, because it may determine the stability of resistant cultivars. As Caten (1) pointed out, the magnitude of the adaptive response (host × isolate interaction) should determine the emphasis in breeding for disease resistance.

C. crotalariae isolates that originated from susceptible hosts had about eight times more general virulence than specific virulence. This was expected, since specific virulence is a measure of the interaction between isolates of the pathogen and host cultivars that differ in their resistance to the isolates. If resistance is not encountered by a pathogen population, specific virulence should not develop in that population. Conversely, pathogen populations that encounter a resistant host population show considerably more specific virulence.

The relatively small amount of specific virulence in isolates from the susceptible hosts may be considered to be a background level. The essential genetic information for specificity is present in the population, but unnecessary in the absence of resistant hosts. However, when a resistant host is exposed to a pathogen population, strains with the genetic mechanism for specificity to that host have greater ability to cause disease and subsequently increase in the population. In only one cropping cycle of resistant host NC 3033, specific virulence increased fourfold in the previously nonselected pathogen population. This may be an indication of potential for race development in *C. crotalariae,* even though corresponding resistance in the host appears to be quantitatively inherited (3).

The importance of pathogen adaptation should be considered in the development of CBR-resistant peanut cultivars. The effectiveness of a single resistant cultivar, such as NC 3033 or its hybrid progeny, may be relatively short-lived and alternate sources of resistance must be located.

As is typical of many soil-borne pathogens, the spread of *C. crotalariae* among field sites is strongly restricted, when compared to that of foliar pathogens such as those causing rust diseases. Due to these circumstances, populations of *C. crotalariae* strains should be stabilized in their soil environment during crop rotational sequences. A pathogen population, once it has been changed by resistant host plant selection pressure, should remain intact until further selection pressures are applied. Pathogen specificity is directed toward particular resistant plant genotypes and appears to be mutually exclusive among host cultivars. For example, as isolates became more adapted to NC 3033, they tended to become less adapted to Florigiant. This may be important in extending the useful life of CBR-resistant cultivars through rotation of resistance sources. As diverse, genetically distinct, and agronomically acceptable germplasms are developed, care in their use may prevent rapid pathogen adaptation to any single germplasm.

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