Variation in Aggressiveness and Virulence Among Isolates of *Cercospora arachidicola*

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ABSTRACT

Forty-three isolates of *Cercospora arachidicola*, incitant of early leaf spot of peanut, were collected from peanut fields in Florida, Georgia, North Carolina, and Texas. Variation in aggressiveness among these isolates using the susceptible peanut cultivar Spanco was examined in greenhouse tests. Variation ($P \leq 0.05$) among the isolates was observed for the parameters of incubation period (IP), the reciprocal of the latent period (an estimate of sporulation rate), and number of lesions per leaflet (LN). Additionally, as a group, the isolates from Texas were more aggressive in terms of having a shorter latent period and greater number of lesions per leaflet than isolates from other states. When a subset of eight isolates, which differed in aggressiveness based on IP and LN, were tested on Spanco and three early leaf spot-resistant breeding lines (TX957910-5, TX957910-27, TX957910-68), the breeding lines were resistant to all isolates and, except for lesion diameter, no host genotype-by-isolate interaction was detected ($P > 0.05$). These observations suggest that success of efforts to identify resistance to early leaf spot can be affected by the aggressiveness of the pathogen isolate. Additionally, the resistance of the breeding lines tested is likely to be effective against a wide array of isolates of *C. arachidicola*.

Key Words: Early leaf spot, host resistance.

Major factors affecting the utility of resistance for management of plant disease are the stability and durability of resistance. Stability is the effectiveness of resistance across different environments and durability is the length of time that the resistance remains effective (Fehr, 1993; Shew, 1995). Resistance that lacks stability or durability is of little value because it may be effective only in a limited area and for a limited time. Stability and durability of resistance are directly related to variability in the pathogen (Dyck and Kerber, 1985; Otsuka *et al.*, 1963; Waliyar *et al.*, 1994; Liu *et al.*, 1996). When populations of the pathogen have limited variability with respect to virulence or aggressiveness, then resistance will be more stable and durable than if the pathogen is highly variable for these traits (Dyck and Kerber, 1985; McDonald and McDermott, 1993; Liu *et al.*, 1996).

Variation among isolates of *Cercospora arachidicola* Hori, incitant of early leaf spot of peanut, has been reported with respect to morphology and germination of conidia (Subba Rao *et al.*, 1992), tolerance to fungicides (Littrell, 1974), and production of the phytotoxin cercosporin (Melouk and Schuh, 1987; Fore *et al.*, 1988). No data, however, are available to characterize the variability of *C. arachidicola* with respect to aggressiveness or virulence, where aggressiveness is defined as the relative amount of disease produced on a susceptible host and where virulence is defined as the ability to incite disease on host genotypes with one or more resistance genes (Maytac and Bailey, 1988). The objectives of this research were to measure variation in aggressiveness among isolates of *C. arachidicola* collected from different peanut production regions and determine if isolates that differ in aggressiveness on a common host also differ with respect to virulence on host genotypes with different levels of resistance.

Materials and Methods

Isolates of *C. arachidicola* were collected from peanut fields in Lee County, TX; Bertie County, NC; Tift County, GA; and Gadsden County, FL. A single field was sampled in each county. Each isolate was collected as a single infected leaflet along linear transects 3 m apart in each field during July and August of 1995 and mailed to College

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Station, TX. Individual lesions were excised from infected leaflets, surface sterilized with 0.5% NaOCl for 15 sec and incubated on peanut oatmeal agar (POA), pH 4.5, at room temperature (Smith and Littrell, 1980). Cultures were successfully established for 13 isolates from Texas, seven from Georgia, seven from Florida, and 11 from North Carolina. Isolates were maintained separately on POA and were preserved as air-dried cultures on desiccated POA at room temperature. Subcultures from the original culture of each isolate were made for each experiment by streaking conidia from the original culture onto POA.

The susceptible cultivar Spanco was used as the host for the aggressiveness study. Seeds were planted in a steam-pasteurized soil mix (6 parts coarse sand:1 part peat moss) in 12.7-cm dia. pots. *Bradyrhizobium* inoculum was applied to seeds prior to planting and 3 g of slow release fertilizer (Osmocote, Scotts Sierra Horticultural Products, Marysville, OH) with 14% N, 14% P, and 14% K added to each pot.

Inocula were collected from 20- to 25-d-old cultures by flooding each petri dish with 10 mL sterile distilled water containing three drops Tween 20 per 100 mL. Conidia were removed by gently brushing all colonies on each plate with a small brush. Resulting conidial suspensions for each isolate were adjusted to 2.5×10^4 conidia/mL using a hemacytometer.

The foliage of each 5-wk-old plant was inoculated by spraying with 10 mL of conidial suspension using an artist's air brush. Three plants were inoculated per isolate. Inoculated plants were then placed on greenhouse benches in a randomized complete block design and covered with clear polyethylene to maintain 98 to 100% relative humidity at 22 to 28 C. The polyethylene cover was removed after 96 hr.

Disease parameters of incubation period (IP), latent period (LP), lesion diameter (LD), and lesion number (LN) were recorded every other day beginning with the appearance of the first lesion. Incubation period was defined as the time between inoculation and first lesion development, and latent period was the time from inoculation to sporulation. Isolates that did not sporulate during the designated test period were assigned a LP value of the length of the test period (35 d) plus 1. Additionally, the reciprocal of LP was calculated as an estimate of the rate of sporulation with respect to time(Kenerley and Jeger, 1990). Lesion diameter was the mean of the three largest lesions per plant at 16 d after inoculation. Infection rate was measured as the number of lesions on the most heavily infected leaflet for each individual plant.

To determine variation in virulence among selected isolates of *C. arachidicola*, seeds of Spanco and the resistant peanut breeding lines TX957910-5, TX957910-27, and TX957910-68 (Tuggle, 1998) were planted in the steam-pasteurized soil medium (6:1 sand to peat moss, v/v) in 12.7-cm diam. pots. Eight isolates, which differed with respect to IP and LN in the aggressiveness test, and two representatives from each geographic region were selected for use in this test. The inoculum concentration was 5×10^4 conidia/mL for each isolate. Inoculation and plant growth conditions were as described previously.

Disease parameters of IP, LP, LD, and LN were measured as described above. Additionally, days to defoliation were measured as the number of days between inoculation and abscission of first infected leaflet. Sporulation rate was estimated from the reciprocal of the LP. The test was a randomized complete block design with four single plant replications for each treatment.

Data were analyzed using the SAS (SAS Institute Inc., Cary, NC) general linear model procedure to evaluate treatment effects. Fisher's LSD was used for separation of treatment means. Correlations between the components of disease were determined using the SAS Pearson correlation procedure.

Results

Variation ($P \leq 0.05$) among isolates was observed with respect to IP, 1/LP, and LN in the test of aggressiveness on the susceptible cultivar Spanco (Table 1). The shortest mean IP observed was 9 d for isolates no. 104 and 105 from Texas, whereas the longest mean IP was 14.7 d for isolate no. 315 from Florida (Table 2). Negative correlations were observed between IP and 1/LP ($r = -0.380$, $P \leq 0.01$), and between IP and LN ($r = -0.562$, $P \leq 0.01$). No effect of isolate ($P > 0.05$) on LP or LD was observed (Table 1). The number of lesions per leaflet (LN) exhibited greater variation than did any of the other components of disease (Table 2). Eighty-nine percent of the isolates were within one standard deviation of the overall test mean of 7.9 ± 13.4, but four isolates had numbers of lesions that were greater than three times the overall mean (Table 2).

Variation ($P \leq 0.05$) due to origin of the isolates was observed for LP, 1/LP, and LN (Table 1). As a group, the isolates from Texas had the shortest LP (17.9 d), the greatest 1/LP (0.59), and greatest LN (12.4 lesions/leaflet). Isolates from Florida, collectively, had the longest mean LP (29.8 d)and the smallest 1/LP (0.49), whereas isolates from North Carolina had the fewest number of lesions per leaflet (3.18).

In the comparison of effects of resistant and susceptible peanut genotypes on variation in components of

Table 1. F-values for incubation period, latent period, the reciprocal of latent period, lesion diameter, and lesion number from greenhouse comparisons of aggressiveness of isolates of *Cercospora arachidicola* collected from Florida, Georgia, North Carolina, and Texas.

Source	df	F-value
Incubation period		
Origin	3	2.23
Isolate	34	2.07**
Latent period		
Origin	3	3.41*
Isolate	34	1.43
1/Latent period		
Origin	3	4.19**
Isolate	34	1.72*
Lesion diameter		
Origin	3	0.36
Isolate	34	0.98
Lesion number		
Origin	3	51.65**
Isolate	34	49.63**

*,**Indicate significance at $P \leq 0.05$ and 0.01, respectively.

Table 2. Variation in incubation period (IP), latent period (LP), the reciprocal of latent period (1/LP), lesion diameter (LD), and lesion number (LN) among isolates of *Cercospora arachidicola* collected from TX, GA, FL, and NC on Spanco. Incubation period and latent period are measured in days after inoculation (DAI).

Isolate	IP	LP	1/LP	LD	LN
	DAI	DAI		cm	no.
TX 101	11.7	18.3	0.055	2.3	1.3
TX 104	9.0	16.3	0.062	2.3	50.0
TX 105	9.0	15.7	0.074	2.7	50.0
TX 106	11.0	15.7	0.064	1.7	6.3
TX 107	12.3	15.7	0.064	2.7	6.7
TX 109	10.3	15.0	0.067	2.0	29.7
TX 110	13.7	23.0	0.049	1.0	2.3
TX 112	12.3	19.7	0.052	2.7	1.7
TX 113	11.7	20.3	0.050	3.0	3.0
TX 114	12.3	17.0	0.059	2.3	1.3
TX 116	12.3	19.7	0.053	2.3	3.3
TX 117	12.3	19.0	0.053	2.0	3.0
TX 119	13.7	19.7	0.052	2.7	2.3
GA 205	13.7	21.7	0.049	4.0	1.7
GA 207	11.7	17.7	0.057	2.0	2.7
GA 208	13.0	18.3	0.055	1.7	3.0
GA 215	12.3	15.7	0.064	2.0	12.3
GA 219	12.3	23.0	0.049	2.0	6.5
GA 220	12.3	19.7	0.052	2.7	5.0
FL 304	14.3	15.7	0.042	1.5	1.5
FL 308	12.3	17.0	0.059	1.7	2.3
FL 311	13.0	29.7	0.037	4.0	2.3
FL 312	9.7	18.3	0.056	2.7	43.3
FL 314	13.0	18.3	0.055	3.7	7.0
FL 315	14.7	27.0	0.039	2.5	4.0
FL 316	13.0	19.7	0.053	3.6	2.3
FL 327	10.7	19.0	0.054	2.7	3.0
NC 401	10.3	17.0	0.059	2.3	3.3
NC 402	12.3	22.0	0.045	2.0	6.3
NC 403	13.0	20.0	0.053	3.0	3.0
NC 404	13.0	26.3	0.040	3.0	1.3
NC 405	14.3	14.3	0.041	3.0	1.5
NC 406	12.3	18.3	0.055	2.0	5.3
NC 409	13.0	16.3	0.061	1.7	3.0
NC 410	13.0	25.0	0.048	2.0	4.3
NC 411	12.0	20.0	0.053	2.5	1.0
NC 412	11.7	17.7	0.057	2.3	2.3
NC 413	13.0	17.0	0.060	2.0	2.0
$LSD_{0.05}$	2.7	ns	0.017	ns	5.7

disease for eight arbitarily selected isolates, isolate no. 405 from Florida incited disease only on the susceptible Spanco and, hence, was not included in further analyses. An analysis of variance indicated a significant effect ($P \leq 0.05$) of peanut genotype across the other seven isolates of *C. arachidicola* on all measured components of disease (Table 3). Spanco had shorter IP and LP (Fig. 1), greater LD, and greater number of lesions per leaflet than did the three resistant breeding lines (Fig. 2).

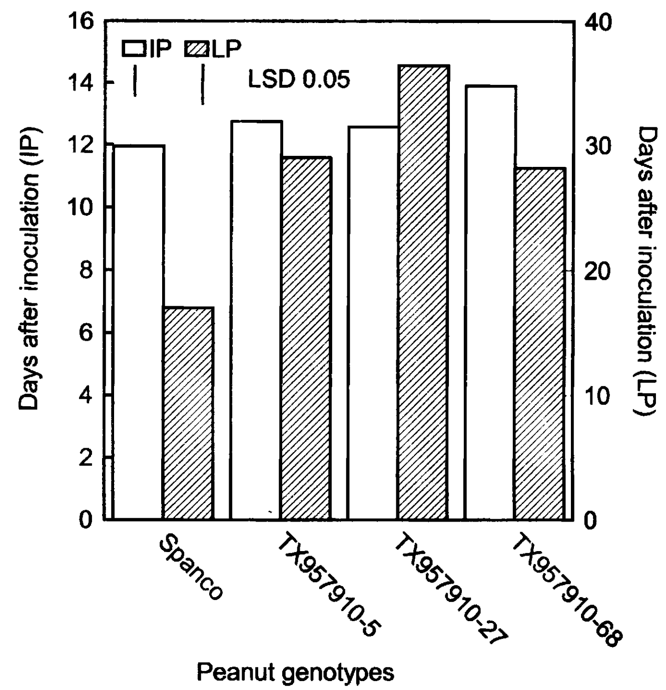

Fig. 1. Effect of peanut genotype on mean incubation period (IP) and latent period (LP) when inoculated separately with seven isolates of *Cercospora arachidicola* that differed in aggressiveness. Spanco is an early leaf spot-susceptible cultivar whereas TX957910-5, TX957910-28, and TX95790-68 are early leaf spot-resistant breeding lines.

Additionally, the estimated sporulation rate was faster on Spanco than on the resistant breeding lines (data not shown). The breeding lines could not be differentiated from each other with respect to LD or LN ($P > 0.05$), but did differ from each other with respect to IP and LP ($P \leq 0.05$) (Table 3). Breeding line TX957910-68 had the longest IP ($P \leq 0.05$), whereas TX957910-28 had the longest LP ($P \leq 0.05$) (Fig. 2).

No effect of isolate on IP, LP, or 1/LP was observed ($P > 0.05$), but differences among isolates were found for LD, LN, and days to defoliation (Table 3). All isolates of *C. arachidicola* had fewer ($P \leq 0.05$) and smaller ($P \leq 0.05$) lesions on the resistant breeding lines than on Spanco (Fig. 2) A peanut genotype by *C. arachidicola* isolate interaction ($P \leq 0.05$) was observed only for LD (Table 3) and appeared to be due to variation in the response of resistant breeding lines to different isolates of *C. arachidicola* (Fig. 2). No consistent trend was detected with respect to the effects of pathogen isolate or host genotype on days to defoliation (Fig. 2).

Discussion

Significant variation in aggressiveness across all isolates of *C. arachidicola* was observed for IP, 1/LP, and LN. Further, the amount of variation was similar within each of the geographical groups of isolates. Despite this variability in aggressiveness among isolates of *C. arachidicola*, disease resistance of the three peanut breeding lines to early leaf spot was effective against all

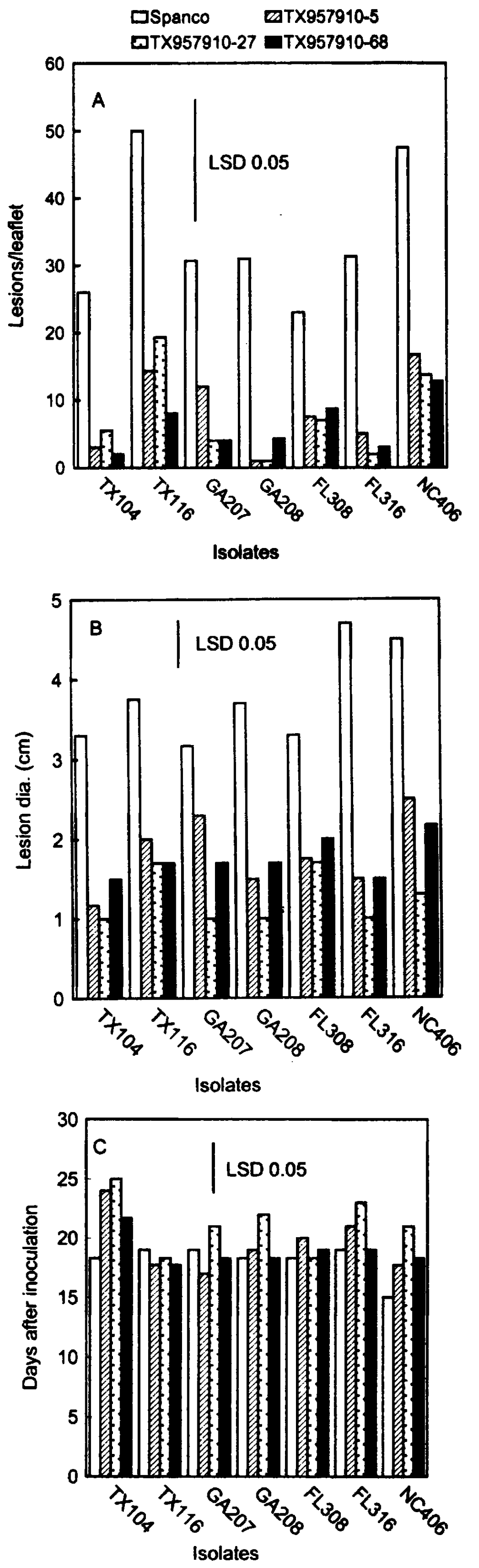

Fig. 2. Effects of differences in aggressiveness of seven isolates of *Cercospora arachidicola* on components of disease in the susceptible cultivar Spanco and the early leaf spot-resistant breeding lines TX957910-5, TX957910-28, and TX957910-68. A) lesions per leaflet; B) lesion diameter; and C) days to defoliation.

Table 3. F values from analysis of variance of effects of selected isolates of *Cercospora arachidicola* and peanut genotypes on components of disease in a greenhouse test.

		F values					
Source	df	Incubation period	Latent period	Sporulation rate	Lesion diam.	Lesion no.	Defoliation
Isolate	6	0.27	1.09	1.29	4.09**	3.85*	2.47*
Genotype	3	5.31**	32.28**	37.91**	109.92**	31.70**	3.29*
Iso × Gen	18	0.54	0.91	0.91	2.21*	0.46	0.57

*,**Indicates significance at $P \leq 0.05$ and 0.01, respectively.

isolates. With the exception of LD, no pathogen isolate-by-peanut genotype interaction was observed. Collectively, these observations indicate that the three peanut breeding lines have resistance that is likely to be stable across locations in peanut producing states. However, the durability of resistance can only be determined after the resistance has been deployed for several years.

Because of the variation in aggressiveness among isolates of *C. arachidicola*, selection of an isolate for use in greenhouse tests for resistance can affect the apparent resistance of peanut genotypes being evaluated. It is important when screening for resistance to early leaf spot, that one use isolates of sufficient aggressiveness to ensure a rigorous test.

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