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Resistance to Late Leafspot Peanut of Progenies Selected for Resistance to Early Leafspot[1]

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ABSTRACT

Fifty-six F_7 peanut (*Arachis hypogaea* L.) lines previously selected for resistance to early leafspot (*Cercospora arachidicola* Hori) were evaluated in the field and greenhouse for resistance to late leafspot [*Cercosporidium personatum* (Berk. & Curt.) Deighton]. After growing in field plots for 12 weeks, differences for numbers of lesions per 15 leaves were found among the lines and between these lines and NC 3033, the susceptible control. Eleven lines with the fewest numbers of lesions and their parents were screened in the greenhouse for components of resistance. These lines had lesions that were significantly smaller, produced lesions with longer latent periods, and produced fewer conidia than NC 3033. Latent periods ranged from 23 to 26 days for the selections compared to 20 days for NC 3033. GP-NC 343 and NC 5 were the most resistant parents with latent periods of 24 days each. A rank correlation of greenhouse and field data revealed that the rank of an entry in the greenhouse for latent period, lesion area and amount of sporulation was correlated with the rank of the entry in the field. Thus, these variables could be used as measurements of resistance to predict the performance of a line in the field for this population. Lines with resistance to late leafspot can be selected from a population of lines with this parentage which have been selected for resistance to early leafspot.

Key Words: *Arachis hypogaea*, components of resistance, groundnut, latent period, sporulation, epidemiology.

Leafspots are considered the most important fungal diseases of peanut, *Arachis hypogaea* L., and occur wherever peanuts are grown (4,13). The geographical distribution of the causal agents, *Cercospora arachidicola* Hori and *Cersosporidium personatum* (Berk. and Curt.) Deighton, is similar; but the incidence of either disease can differ greatly depending on location and environment (14). In North Carolina, for example, early leafspot (*C. arachidicola*) has been the more destructive of the two diseases in the past few years, usually becoming visible early in July and increasing in incidence and severity throughout the growing season; whereas late leafspot (*C. personatum*) does not appear until late August and is considered less important (17). Although these two leafspot diseases are successfully controlled by fungicides in the U. S., the cost is increasing and the use of one of these chemicals has led to benomyl-resistant strains of leafspot (6,7). Furthermore, in many less developed countries fungicides may not be available or are economically unfeasible for use by subsistence farmers. Thus the development of leafspot-resistant cultivars has received increased attention as an alternative form of control (1,2,3,10,15,16).

Hassan and Beute (3) reported that NC 3033, NC 5, and Ac 3139 were useful parents in breeding for resistance to *C. arachidicola*. Kornegay *et al.* (5) crossed these three lines plus Florigiant, NC 2, and GP-NC 343 in a complete diallel to determine the inheritance of resistance to both early and late leafspots. Several lines including NC 3033, NC 5, and GP-NC 343 produced progenies in the F_1 and F_2 generation with partial re-

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sistance to both pathogens. Evaluation of the progenies produced from these crosses has continued at North Carolina and, in 1982, F_7 generation breeding lines were selected for resistance to early leafspot by a regular pedigree breeding method. The objectives of this investigation were to (a) field screen these selected lines for their resistance to late leafspot to determine if *C. personatum*-resistant lines could be selected and (b) evaluate the most resistant late leafspot lines in the greenhouse to determine the most important components of resistance.

Materials and Methods

Field Screening

Seventy peanut lines were screened in the field during the 1983 growing season for resistance to late leafspot at the Central Crops Research Station in Clayton, NC. Entries included 56 breeding lines selected in 1982 for resistance to early leafspot from a population of lines generated in 1977 by a diallel cross of NC 3033, NC 5, NC 2, GP-NC 343, Ac 3139, and Florigiant. Other entries in this test included all of the parents of the diallel as well as NC Ac 17090, NC Ac 17132, NC Ac 17133 (RF), NC Ac 17135, and PI 259747 which had been reported as resistant to late leafspot in India (16). These lines served as the resistant checks, whereas NC 3033 served as the susceptible check.

The 70 lines evaluated for resistance to late leafspot were planted in a randomized complete block design with 15 replications. Each plot consisted of a single five-seed row 1.2-m long, with 1 m between rows and 1 m between plots. No infector rows were used and recommended cultural practices were followed during the growing season. Because *C. arachidicola* is the predominant pathogen early in the season in North Carolina, the field was sprayed once in late June to control leafspot. The original plan was to continue a spraying program until late July to reduce infection by *C. arachidicola*, but due to dry conditions during June and July of 1983 further spraying for *C. arachidicola* was unnecessary.

On August 1, 1983 the field was irrigated for 3 hours with an overhead sprinkler system which dispersed approximately 2 cm of water per hour to raise the humidity in the leaf canopy in preparation for inoculation with *C. personatum*. Conidia were collected with a cyclone spore collector (ERI Machine Shop, Iowa State University, Ames) from infected leaves of NC 3033 maintained in the greenhouse and suspended in water (2000/mL). The following day each plot was inoculated using a Chapin compressed air sprayer (Chapin Manufacturing Works, Batavia, NY). Approximately 20 mL of conidial suspension was sprayed on the foliage of each plot. For the next 2 weeks, the field was irrigated for 1 hour every other day to increase the humidity and provide favorable leaf wetness conditions for infection. Since the spore concentration was low for the inoculum applied by spraying, two weeks later dried infected leaves from NC 3033 were finely ground in a Waring blender and applied to each plot at a rate of approximately 10-15 g/plot. No attempt was made to estimate the conidial concentration. Because of the limited amount of inoculum, only seven of the 15 replications were treated with the leaves.

On September 30, 1983 a total of 15 leaf samples were taken from each plot. The samples were taken at random from the middle one-third of the plant canopy to reduce the probability of sampling very young or very old leaves which are less suseptible to leafspot (12). The total number of *C. personatum* lesions was recorded for the 15 leaves sampled for each plot; however, data were taken only on the seven replications that received both inoculations because of the absence of *C.* personatum in the other replications. The error variance for means of lesion counts was less for smaller than for larger counts, and analyses of the residuals revealed that the data were not normally distributed. Therefore, a logarithmic transformation was used to stabilize the variance and achieve normality. An analysis of variance of the transformed data was used with the sources of variation being entry, replications and error. Mean number of lesions for each entry was used to rank the lines form highest to lowest.

Greenhouse Screening

The 11 F_7 lines with the lowest mean lesion counts in the field were selected for evaluation of resistance to late leafspot in the greenhouse using a detached leaf technique (8). NC 3033, NC 2, NC 5, GP-NC 343 and Florigiant were also included. Four seeds of each entry were planted in 22-cm plastic pots containing a 2:2:1 mixture of sand, peat and soil and arranged on a greenhouse bench in a randomized complete block design with four replications. The plants were grown for 10 weeks receiving normal care and fertilization but were not sprayed with pesticides to avoid residue on the leaves. After 10 weeks fully expanded leaves were detached from each plant at the fourth node from the terminal bud and placed in 45 x 33.5-cm plastic trays with the pulvinus buried in moist sand. The detached leaves were misted with water for 10 seconds every 3 minutes for 3 days to allow the leaves to regain lost turgor. After 3 days the trays were removed from the mist bed and the leaves were allowed to dry in preparation for inoculation. Conidia of *C. personatum* were collected as previously described and suspended in water with a few drops of Tween 80. The spore concentration was adjusted to 34,000 spores/mL. Detached leaves were then inoculated with a fine mist of spore suspension using a Paasche-H air brush (Paasche Airbrush Co., Chicago, IL). Immediately after inoculation, the trays were returned to the mist bed and sprayed with warm water (30C for 30 seconds every 3 minutes during the day). To prevent direct spray from hitting the leaves, trays were placed in a wooden frame covered with plastic on the top and two sides and cheesecloth on the two ends, which was kept moist at all times. Temperature recorded in the chambers ranged from 25 to 32 C and the humidity ranged from 80 to 95%.

The following data were taken on the lesions which began to appear on the detached leaves 10-12 days following inoculation: (a) the total number of lesions per leaf (final count taken at day 20 to prevent the counting of secondary lesions), (b) lesion size in mm^2 (determined at day 30 by taking an average of five lesions/leaf), (c) leaf area in cm^2 [determined with a Li-Cor Model LI3000 leaf area machine (Li Cor, Ltd., Lincoln, NB)], (d) total necrotic area (determined by taking lesion number x lesion size/leaf area x 100), (e) the percent sporulating lesions (determined by taking counts of sporulating lesions every other day), (f) latent period (determined as the number of days from inoculation until 50% of the lesions were sporulating on a leaf) and (g) sporulation index (rated on a scale of 1-5 with 1 indicating no lesions sporulating and 5 indicating heavy sporulation).

Using the data obtained, an analysis of variance was performed for each variable and a Waller Duncan multiple range mean separation test was performed. Plots made from the mean percent sporulating lesions against days after inoculation for each entry gave typical sigmoid curves. These curves were linearized to facilitate comparison of the treatments and interpolation of the values. A comparison of the equality of the regression lines was made with the General Linear Approach of Neter and Wasserman (9).

From the predicted values for the slopes and intercepts for each entry, a predicted latent period was computed and compared with the latent periods calculated from the data. Rank correlations were computed between the field and greenhouse data in order to determine the relationship between the two tests.

Results and Discussion

Field Analysis

Evaluation of the data for number of lesions/15 leaves indicated differences ($p=0.01$) among the 70 lines screened. As both highly resistant and susceptible cultivars were screened in the field test, analysis excluding checks revealed differences ($p=0.01$) among the 56 F_7 lines indicating that there was variability in this population for resistance to late leafspot (Table 1). Differences ($p=0.01$) were also found among the parents of the F_7 lines with NC 3033 having the highest number of lesions and NC 5 having the fewest (Table 1).

The 11 F_7 lines with the lowest mean number of lesions/15 leaves were selected from the 56 lines screened in the field and compared with NC 3033, the susceptible control. Differences ($p=0.01$) were found indicating that these selected lines were partially resistant to late leafspot infection in the field. Further evaluation in the greenhouse compared the level and components of re-

Table 1. Means of F_7 generation peanut breeding lines and checks for numbers of late leafspot lesions per 15 leaves grown in field plots at Clayton, NC during 1983.

Entry	Cross/check	No. lesions/15 leaves: Transformed log (10)	No. lesions/15 leaves: Not transformed	Entry	Cross/check	No. lesions/15 leaves: Transformed log (10)	No. lesions/15 leaves: Not transformed
1.	NC 2 x NC 5	1.12	20.71	36.	GP-NC 343 x NC 5	1.01	15.29
2.	"	1.84	96.43	37.	"	0.94	9.57
3.	"	1.80	76.14	38.	"	1.04	22.86
4.	"	1.03	13.00	39.	"	0.91	11.42
5.	"	1.18	17.29	40.	"	1.15	14.86
6.	"	1.19	14.43	41.	"	1.26	20.14
7.	GP-NC 343 x NC Ac 3139	1.17	17.57	42.	"	1.24	21.43
8.	"	1.17	18.71	43.	"	0.88	8.71
9.	NC 5 x GP-NC 343	0.91	9.71	44.	"	1.09	19.71
10.	"	0.85	6.86	45.	"	1.16	17.14
11.	"	1.08	19.29	46.	"	0.92	11.00
12.	"	1.21	21.57	47.	"	0.91	9.57
13.	"	0.92	8.14	48.	"	1.09	14.57
14.	"	1.34	27.29	49.	"	1.09	14.71
15.	"	1.09	17.00	50.	"	1.01	14.71
16.	"	0.95	8.57	51.	"	1.24	20.86
17.	"	0.95	9.14	52.	"	0.88	10.00
18.	"	1.07	16.00	53.	"	0.91	11.14
19.	UF 82206	0.69	5.85	54.	NC 3033 x GP-NC 343	0.74	6.00
20.	Florigiant x GP-NC 343	1.11	25.29	55.	"	0.90	10.57
21.	"	0.86	7.57	56.	"	0.86	10.28
22.	"	1.14	16.29	57.	"	0.81	6.29
23.	"	1.00	15.14	58.	Florigiant	1.05	13.00
24.	GP-NC 343 x NC 2	1.10	15.29	59.	NC 7	1.00	10.71
25.	"	1.09	27.00	60.	NC 5	0.76	6.29
26.	"	1.20	23.71	61.	NC 6	1.30	30.00
27.	"	1.09	17.00	62.	GP-NC 343	0.90	8.86
28.	"	0.98	14.14	63.	NC 3033	1.80	70.86
29.	"	0.84	8.00	64.	NC 17090	0.77	6.00
30.	"	0.93	8.86	65.	NC 17133(RF)	0.15	0.57
31.	"	0.92	11.00	66.	NC 17132	0.39	3.71
32.	"	1.17	18.86	67.	NC 17135	0.38	1.57
33.	"	1.30	35.00	68.	FESR-11-P11-B2	1.12	18.71
34.	"	1.19	17.43	69.	FESR-5-P2-B1	0.90	10.71
35.	NC Ac 3139 x GP-NC 343	0.99	13.14	70.	PI 259747	0.23	1.14

LSD (0.05): Transformed data = 0.31

sistance of the 11 F_7 lines.

Greenhouse Analysis

In the greenhouse, differences occurred among the 16 lines screened for lesion area, latent period, and amount of sporulation (Table 2). However, no differences were detected for total number of lesions/leaf or total necrotic area. No differences were found among the F_7 selections for lesion area, latent period, or amount of sporulation; however, differences were found when these lines were compared with NC 3033. Differences ($p = 0.01$) were also found among the parents of the selections.

Table 2. Significance of variables affecting resistance to late leafspot from analysis of variance of greenhouse data.

Source of variation	df	Total lesion no.	Lesion size	Total necrotic area	Latent period	Sporulation index
Among entries	15	ns	**	ns	**	**
Among selections	10	ns	ns	ns	ns	ns
Among parents	3	ns	*	ns	**	**
NC 3033 vs Selections	1	ns	**	ns	**	**
Parents vs Selections	1	ns	**	ns	**	**

*,** Significant at 0.05 and 0.01 probability levels, respectively.

The latent periods (Table 3) ranged from 20 days for NC 3033 to 26 days for NC 3033 x GP-NC 343 (line 55), the best selection. GP-NC 343, the best parent, had a latent period of 25 days. NC 3033 and Florigiant had the greatest sporulation, while GP-NC 343 x NC 2 (line 29), NC 3033 x GP-NC 343 (line 57), and NC 3033 x GP-NC 343 (55) had the least sporulation (2.25, 2.50, and 2.50, respectively). Lesion size (Table 3) ranged from 5.53 mm^2 for GP-NC 343 to 11.05 mm^2 for NC 2. The best line, GP-NC 343 x NC 2 (line 29), produced lesions of 5.62 mm^2.

The rank of an entry in the greenhouse for lesion area, latent period, and amount of sporulation was correlated with the rank of the entry in the field for number of lesions/15 leaves (Table 4). Thus, these variables could be used as measurements of resistance for predicting the performance of a line in the field for this population.

The nonsignificant differences for total lesion number/leaf in the greenhouse and the lack of correlation between the greenhouse and field data for this variable suggest that total lesion numbers/leaf in the greenhouse should not be used as the sole measurement of resistance. Since only one primary infection period

Table 3. Means of F_7 peanut selections and parents for three components of resistance to late leafspot.

Entry	Avg lesion size (mm^2) (24 DAI*)	Latent period**	Sporulation index†
GP-NC 343	5.53d‡	24.5a-d	2.75def
NC 2	11.05a	22.0def	4.50a
NC 5	7.66bcd	23.5bcd	4.13c
Florigiant	10.28ab	20.5ef	5.00a
NC 3033	9.83abc	20.0f	5.00a
NC 3033 x GP-NC 343 (55)	6.03d	26.5a	2.50ef
NC 3033 x GP-NC 343 (56)	7.22bcd	25.0abc	3.37cd
NC 3033 x GP-NC 343 (57)	5.17d	24.5a-d	2.50ef
GP-NC 343 x NC 5 (52)	5.92d	23.5cde	3.16de
GP-NC 343 x NC 5 (46)	7.82a-d	24.5bcd	3.50cd
GP-NC 343 x NC 5 (39)	6.54d	24.0bcd	3.37cd
NC 5 x GP-NC 343 (10)	5.84d	24.0a-d	3.33cd
NC 5 x Florigiant (31)	8.03a-d	23.0cde	3.33cd
NC 3033 x GP-NC 343 (54R)	6.69cd	26.0ab	2.88def
NC 3033 x GP-NC 343 (54T)	7.14bcd	23.5bcd	3.00de
GP-NC 343 x NC 2 (29)	5.62d	24.5a-d	2.25f

*Days after inoculation.

**Determined as the number of days from inoculation in which 50% of the lesions were sporulating.

†Rated on a scale of 1-5 where 1 indicated no lesions sporulating and 5 indicated heavy sporulation.

‡Means in each column followed by the same letter do not differ (P = 0.05) according to the Waller-Duncan multiple range test.

was used to determine the total lesion count in the detached leaf test, estimates do not account for secondary cycles that probably occurred in the field and, thus, differences in genotypes would be due to a cumulative effect of all resistance factors such as decreased infection frequency, increased latent period, etc. operating together. Infection frequency is greatly influenced by the environment and the artificial, highly optimum environment of the detached leaf test could influence the number of successful infections.

According to Parlevliet (11), partial resistance tends to be coincident with a longer latent period, reduced sporulation, lower infection frequency, and a reduced lesion size and these components generally reinforce one another (11,18). Therefore, correlations were used to determine the relationship among the different components of resistance in the greenhouse (Table 4). All components measured in the greenhouse were correlated with the exception of lesion number with spore production and total necrotic area. The lines selected for resistance to *C. personatum* from the field evaluations produced smaller lesions with longer latent periods and reduced sporulation indicating that these components of resistance are operating sequentially in *C. personatum* epidemics and could explain why the original lines were identified as resistant.

The results of the regression analyses of mean percent sporulating lesions against days after inoculation confirmed that the regression lines among the 16 lines in the greenhouse study were not equal. Tests performed for heterogeneity of slopes and intercepts were both significant at 0.05 and 0.01, respectively (Fig. 1-3). From the regression analyses, the predicted values for the slopes and intercepts were used to compute a latent period for each entry (Table 5). The resistant selections had longer latent periods than the susceptible control. The predicted values for the latent periods were compared with the latent periods computed from the data using a chi-square goodness of fit test. No differences between the observed and expected values were found indicating that the values obtained from the regression analyses can be used to predict the latent period for these lines. The regression method can be used without having to take data on percent sporulating lesions on a daily basis. The time saved would allow screening of larger populations in the greenhouse.

Table 4. Rank correlation entry means for measurements of resistance to late leafspot from greenhouse and field tests.

	Lesion size	Total necrotic area	Total lesion no.	Latent period	Spore production	# Lesions/ 15 leaves (field)
Lesion size	--	.83**	.57*	.78**	.72**	.59*
Total necrotic area		--	.52	.59*	.52*	.46
Total lesion no.			--	.69**	.34	.38
Latent period				--	.65**	.73**
Spore production					--	.52*

*,** Significant at 0.05 and 0.01 probability levels, respectively.

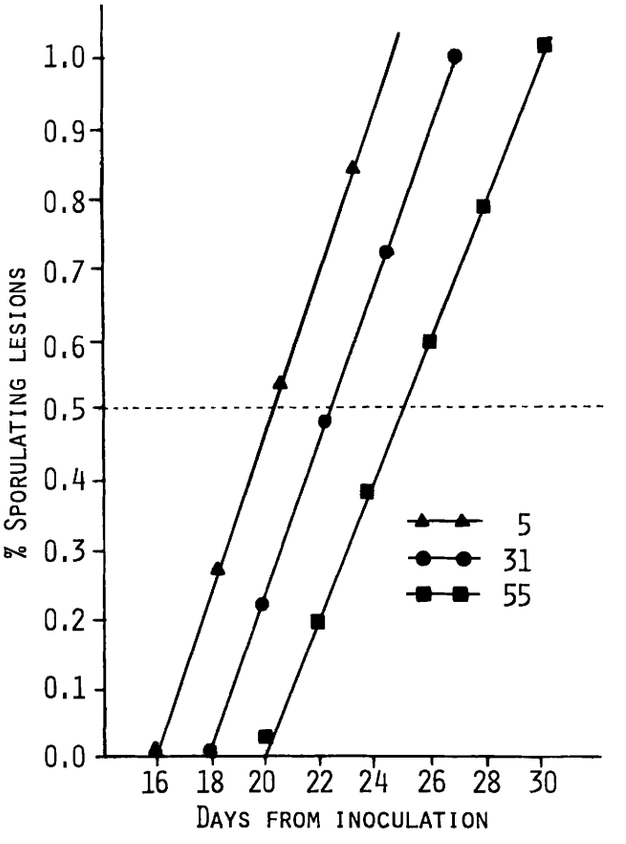

Fig. 1. Regression of percent sporulating lesions on days from inoculation for NC 3033, the susceptible control (line 5); NC 3033 x GP-NC 343, the best selection (line 55); and NC 5 x Florigiant, the worst selection (line 31).

In conclusion, the results from this investigation indicate that lines with resistance to late leafspot can be selected from this population of lines selected for resis-

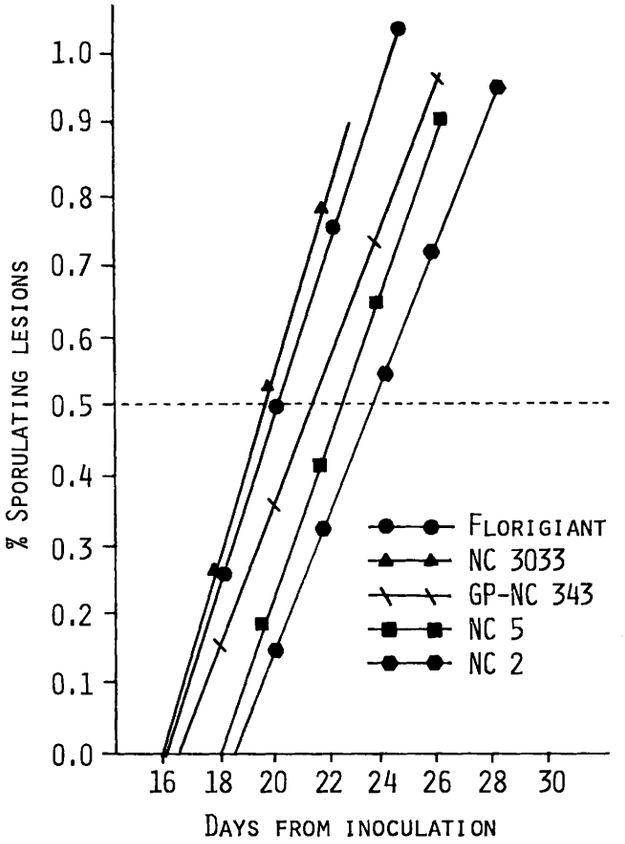

Fig. 2. Regression of percent sporulating lesions on days from inoculation for the parents of the selected crosses.

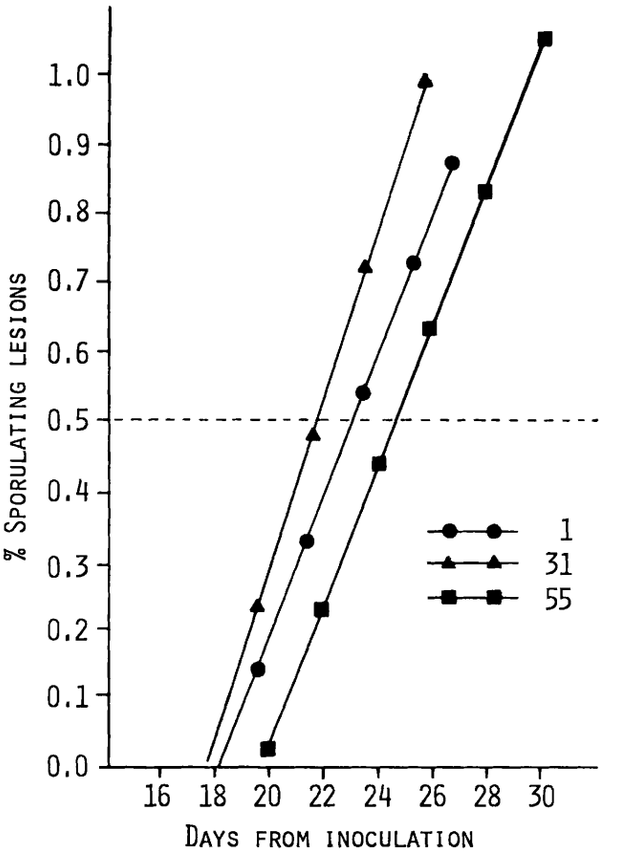

Fig. 3. Regression of percent sporulating lesions on days from inoculation for GP-NC 343, the best parent (line 1); NC 3033 x GP-NC 343, the best selection (line 55); and NC 5 x Florigiant, the worst selection (line 31).

Table 5. Predicted and observed latent periods for late leafspot from greenhouse data for 11 selections and parents.

Entry	Intercept	Slope	Predicted latent period*	Latent period from data**
GP-NC 343	-1.73	.095	24	24
NC 2	-1.53	.095	22	22
NC 5	-1.93	.110	23	24
Florigiant	-2.05	.130	20	20
NC 3033	-2.05	.130	20	20
NC 3033 x GP-NC 343 (55)	-1.92	.097	25	26
NC 3033 x GP-NC 343 (56)	-2.21	.110	24	25
NC 3033 x GP-NC 343 (57)	-1.60	.088	24	24
GP-NC 343 x NC 5 (52)	-1.62	.088	24	23
GP-NC 343 x NC 5 (46)	-2.24	.122	24	24
GP-NC 343 x NC 5 (39)	-1.59	.092	23	24
NC 5 x GP-NC 343 (10)	-2.87	.147	23	24
NC 5 x Florigiant (31)	-2.15	.119	22	23
NC 3033 x GP-NC 343 (54R)	-1.37	.078	24	26
NC 3033 x GP-NC 343 (54T)	-1.50	.090	22	24
GP-NC 343 x NC 2 (29)	-2.02	.107	24	24

*Calculated as .50 sporulating lesions = intercept + slope (day) and solving for day.

**Computed from actual greenhouse data.

tance to early leafspot. In North Carolina where *C. arachidicola* is the predominant leafspot pathogen, but where *C. personatum* also occurs, the release of a cultivar with resistance only to early leafspot could result in a shift in the incidence and severity of late leafspot. In this population of early leafspot-resistant lines, sufficient variability remains for selection of late leafspot-resistant lines.

The lines selected from the field for resistance to late leafspot produced smaller lesions with reduced sporulation with latent periods of up to 5 days longer than the susceptible entry. Since *C. personatum* does not initiate infection in North Carolina until late August, a longer latent period would be significant in reducing the number of reproductive cycles of the pathogen and, therefore, decreasing the severity of disease. With further evaluation and selection, agronomically acceptable lines with partial resistance to both early and late leafspot can be developed and, when used in conjunction with other control measures, will result in a substantial reduction in the cost of production.

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