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Spore Production and Latent Period as Mechanisms of Resistance to *Cercospora Arachidicola* in Four Peanut Genotypes[1,3]

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ABSTRACT

Arachis batizocoi, *A. monticola* and two genotypes of *A. hypogaea* ('Florigiant' and PI 109839) chosen to represent differing levels of resistance to early leafspot (*Cercospora arachidicola*) were evaluated for their effects on production of conidia per lesion, conidia per unit area of lesion and latent period necessary for sporulation. The largest lesions and the most conidia per lesion and unit lesion area were produced on cultivar 'Florigiant'. PI 109839 had smaller lesions than Florigiant. Fewer conidia per lesion and per unit lesion area were produced on PI 109839 than Florigiant. *C. arachidicola* sporulated abundantly on lesions from both Florigiant and PI 109839 15 days after inoculation. Size of lesions and conidia per lesion did not differ between *A. monticola* and PI 109839 but conidia per unit lesion area were fewer on *A. monticola*. The smallest lesions and the fewest conidia per lesion and per unit lesion area were produced on *A. batizocoi*. *C. arachidicola* did not begin sporulating on *A. monticola* and *A. batizocoi* until 18 days after inoculation. Sporulation of *C. arachidicola* was observed on defoliated leaves of *A. monticola* and *A. batazocoi* 21 days after inoculation.

Key Words; Mechanism of resistance, *Arachis hypogaea*, Groundnut, Epidemiology

Early leafspot caused by *Cercospora arachidicola* Hori is a destructive disease of the cultivated peanut (*Arachis hypogaea* L.). Leafspot epidemics occur during favorable environmental conditions. A high incidence of lesions results in extensive defoliation of leaflets.

Conidia of *C. arachidicola* are transmitted by splashing rain (4, 13), wind currents (12, 13,17) and insect vectors (17). Successive generations of conidia are produced on each lesion within a growing season. The rate of epidemic increase is usually estimated by r, the apparent infection rate, which is based upon numerous estimates of disease severity obtained throughout the season (16).

The rate of epidemic of many diseases may be reduced by mechanisms of resistance present within the host crop (9). Such mechanisms may be effective by i) reducing the infection frequency (number of lesions that develop), ii) reducing the spore production (spores per lesion, or spores per unit area of lesion, etc.) and/or iii) increasing the latent period (length of time between inoculation and sporulation).

Two of these resistance components which are effective against early leafspot have been discovered in cultivated and wild peanut species. Numberous investigators have reported a reduced infection frequency, ususally estimated by lesion number per leaf or percent leaf area infected, among both wild and cultivated peanut types (3, 6, 7, 8, 10, 14). Abdou *et al* (1) observed that *C. arachidicola* failed to sporulate on numerous wild species. This reduced sporulation was probably due to the minute lesion size. Mechanisms that increase the latent period of *C. arachidicola*, however, have not yet been noted in peanuts.

The objectives of this investigation were to i) measure the amount of sporulation of *C. arachidicola* on four peanut genotypes; and ii) to determine differences in the latent period of this pathogen on these genotypes.

Materials and Methods

Four peanut lines of diverse genetic background were selected: (1) Florigiant, a widely grown cultivated variety chosen primarily to serve as a susceptible check; (2) PI 109839, a plant introduction from Venezuela described as cercospora resistant by other investigators 914); (3) *A. monticola* Krap. *Et.* Rig., a tetrapoloid wild species highly susceptible to infection (1, 5); and (4) *A. batizocoi* Krap. *et.* Greg., a susceptible diploid species (5) previously observed to exhibit reduced sporulation of *C. arachidicola* in a field study in North Carolina.

Trial 1

In the fall of 1978 seed of each of the genotypes were treated with a mixture of 90% Captan-Maneb and 10% Ethrel (Amchem 72-A152 dust containing 15% Ethephon). Seedlings, germinated in moist vermiculite, were transferred to 10-cm diameter plastic pots filled with a 2:2:1 mixture of soil, sand and peat moss. After plants were 3 weeks old, they were placed outside the greenhouse each day for approximately 10 hours. Plants were exposed to this weathering period to produce symptoms similar to those on field-grown plants (6). This treatment was continued for a period of 3 weeks.

The single spore isolate of *C. arachidicola* used in this investigation was obtained from Lewiston, N. C. Conidia, prepared in the manner described by Smith (12), were suspended (2.7×10^3/ml) in an emulsion of Tween 80 (3 drops/100 ml H_2O). Conidia were sprayed on the foliage of plants with a DeVilbiss atomizer. Four plants of each genotype were sprayed with the conidial suspension, plants were covered with clear plastic bags (to prevent conidia from being washed off the leaves), pots were transferred beneath a greenhouse bench and misted with water (12-second spray every 6 minutes) to insure that relative humidity was high enough to promote conidial germination and infection. The bags were removed after 1 week and plants were transferred to greenhouse benches. Tiny necrotic lesions were visible on some plants 8 days after inoculation.

Fifteen days after inoculation plants of each genotype were randomly separated into two groups. The three largest lesions were removed from leaflets within each group with a 9-mm

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diameter cork borer, and the six lesions were placed on moist filter paper in a petri dish. Lesions were transferred to a vial containing 1 ml of Tween 80 emulsion after 2 days. Following manual agitiation, the concentration of conidia in each vial was estimated with a hemacytometer. Eight estimations per vial were determined. Numbers were averaged for data comparison. Lesions were removed from plants a total of three times at 4-day intervals for each test.

Trial 2

The second study was initiated in the spring of 1979. Plants were kept in the greenhouse where temperatures ranged between 22-23 C. Six plants of each genotype were inoculated with conidia of *C. arachidicola*. Inoculum, prepared as previously described, consisted of approximately 1 x 10^4 conidia/ml. Leaves of 5-wk old plants were inoculated with cotton swabs dipped into the conidial suspension. Plants, covered with plastic bags and placed under a greenhouse bench, were misted for 11 days instead of one week. Temperature under the bench ranged between 16-34 C. Bags were removed and plants transferred to high humidity chambers (temperature 22-37 C) to promote disease development.

Each genotype was randomly separated into two groups of three plants 15 days after inoculation. Methods used to induce and measure sporulation were the same as those used in trial 1.

Using data obtained from this trial, the mean number of conidia per lesion, mean number of conidia per mm² of lesion, and mean lesion size estimates were obtained for each of the sample dates for each genotype. An analysis of variance was performed for each measurement with the variation sources being replications, dates, genotypes, interactions, and error. As is often true with disease count data, the error variance of the smaller counts was less than that of the larger counts. Consequently an additional analysis of variance for both conidia per lesion and conidia per mm² of lesion was tabulated using a square root transformation of the data. Lesion size was fairly homogeneous across genotypes so no variance-stabilizing transformation was used on these data. Missing data values (*A. batizocoi*, date 15) were estimated (15).

Results and Discussion

Trial 1 (Fall 1978)

Mean numbers of conidia per lesion for each entry averaged over dates, ranged from 0 to 9,133 (Table 1). For each date that sporulation was studied, *C. arachidicola* produced more conidia per lesion on Florigiant than on the other three genotypes. Conidia were not produced on lesions from *A. batizocoi* in this test.

Sporulation of *C. arachidicola* on lesions from Florigiant decreased on each sampling date. There are two likely explanations for this decrease. Plants were watered with a hose and the forceful impact of water droplets on the leaves could have washed away some of the conidia that had formed. Secondly, though the three largest lesions on each plant were removed on each date, it is possible that the largest remaining lesions on the second or third date were actually smaller than those removed the preceding time(s). No record was made of the size of the lesions that were removed.

Table 1. Mean number of conidia per lesion based upon the largest three lesions on each of two plants for each of two replications (trail 1).

Genotype	Date 15	19	23	Mean
PI 109839	1600	1083	1333	1339
A. monticola	717	2383	2300	1800
A. batizocoi	0	0	0	0
Florigiant	16083	7933	3383	9133
LSD (.05)				2467
(.01)				3500

Trial 2 (Spring 1979)

More conidia per lesions and per mm² were produced by *C. arachididola* on Florigiant than on the other three genotypes studied (Table 2). There were usually more conidia produced on PI 109839 on a per lesion and per mm² basis than on *A. monticola* and *A. batizocoi*, though the differences were not always statistically significant. The numbers of conidia recovered from lesions on *A. monticola* and *A. batizocoi* were very similar for the first two sample dates. On date 21, however, more conidia were obtained from lesions on *A. monticola*. The fact that some conidia had developed on lesions on *A. batizocoi* is contrary to what was found in the preliminary sporulation study.

Lesions that developed on Florigiant were gen-

Table 2. Means of conidia per lesion, conidia per mm² of lesion and lesion size based upon the largest two lesions on each of three plants for each of two replications (trial 2).

Date[a]	Genotype	Conidia per lesion	Conidia per mm² of lesion	Lesion size (mm²)
15	PI 109839	541.67	198.39	2.71
	A. monticola	0.00	0.00	3.00
	A. batizocoi	0.00	0.00	2.29
	Florigiant	1020.83	284.19	3.50
18	PI 109839	1375.00	295.63	4.63
	A. monticola	125.00	28.85	4.17
	A. batizocoi	125.00	42.86	2.54
	Florigiant	2104.17	329.44	6.25
21	PI 109839	2020.83	268.24	7.63
	A. monticola	1354.17	344.93	3.83
	A. batizocoi	20.83	7.14	3.42
	Florigiant	6125.00	596.35	10.25
LSD (.05)		1601.34	177.96	2.68
(.01)		2277.67	253.12	3.81
LSD (.05)[b]		1961.23	217.96	3.28
(.01)		2789.57	310.01	4.66

[a] Dates represent number of days after inoculation.

[b] LSD used when comparing mean of A. batizocoi on date 15 with any other mean (due to missing observation).

erally larger than those of the other genotypes. Lesions on PI 109839 were larger than those on *A. monticola* which, in turn, were larger than those on *A. batizocoi*.

The fact that both the greatest conidial production and the largest lesions occurred on Florigiant is in agreement with the levels of susceptibility normally associated with this cultivar. It is surprising, however, that more conidia per lesion and per mm^2 of lesion were found on PI 109839 than on *A. monticola*, because PI 109839 has been reported as having a high level of resistance. Although fewer conidia were shown to have been produced on *A. monticola* than on PI 109839, the converse may have been true if sporulation had been calculated on a unit of leaf area basis. There was a proliferation of lesions on the leaves of *A. monticola*, and consequently there were probably more conidia produced on the total leaf than were produced on PI 109839. The abundant lesions on *A. monticola* leaves often coalesced. Since only isolated lesions were measured and removed, the estimate of lesion size on this entry was not based upon the largest lesions present.

Sporulation was evident on lesions from PI 109839 and Florigiant on the first sampling date (date 15). Therefore, it may be concluded that the latent period; i. e., the length of time between inoculation and sporulation, was shorter on these genotypes than on the wild species. On the third sampling date (date 21) the amount of sporulation on lesions from *A. monticola* was much higher than that on lesions from *A. batizocoi;* therefore, it may also be concluded that *A. batizocoi* possesses some mechanism which reduces sporulation. Lesions on *A. batizocoi* were small with slight halos, and they were generally sunken below the leaf surface.

In general, as the lesions increased in number and size, the leaflets became chlorotic and abscised. Because the lesions removed for study were only taken from intact leaves, no inferences may be drawn as to the level of sporulation on the leaves after they have abscised. Some leaves which had large lesions on date 21 were incubated in petri dishes for 2 days. It was found that sporulation was abundant on these older, abscised leaves. This phenomenon could explain why sporulation was not observed on *A. batizocoi* prior to this present experiment. Defoliation probably occurred before the pathogen had sufficient time to sporulate.

The mean squares from the analysis of variance of conidia per lesion, conidia per mm^2 of lesion, and lesion size, as well as the mean squares of the transformed data, were calculated. The variation due to dates and genotypes was significant ($P=.01$) for all of the parameters. There was also a significant ($P=.05$) date by genotype interaction. These results indicate that when the amount of sporulation on different genotypes is to be estimated and compared, the number of days after inoculation is a vital consideration. A linear relationship was also shown between both conidial production and lesion size, with time. In general, more conidia were recovered on each successive sampling date.

In conclusion, our results indicated suppressed sporulation on *A. batizocoi*. Furthermore, *C. arachidicola* exhibited longer latent periods on *A. batizocoi* and *A. monticola* than the cultivated lines, Florigiant and PI 109839. These characteristics may be useful if incorporated into commercial cultivars because they could reduce the rate of increase (r) of the cercoseora epidemic. However, since sporulation was evident on *A. batizocoi* leaves following abscision, the actual value of the suppressed sporulation on this genotype is unknown.

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