Effects of Host Resistance on Germination of *Cercospora arachidicola* on Peanut Leaf Surfaces[1]

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ABSTRACT

Conidial germination by a North Carolina field-isolate of *Cercospora arachidicola* Hori was studied on leaf surfaces of two highly resistant and two susceptible peanut (*Arachis hypogaea* L.) genotypes under 5 day/night temperature regimes. Conidia germinated at all temperature regimes, but a greater percentage germinated at the coolest temperature regimes of 26 C day/ 20 C night and constant 24 C than in warmer temperature regimes. Percentage germination differed significantly with respect to time and genotype in each temperature regime. Fewer conidia germinated on resistant compared to susceptible genotypes, with lowest germination on the resistant genotype 91 PA 150 (a virginia type derived from a cross of *A. hypogaea* and *A. cardenasii* Krapov. and W. C. Gregory). Conidia germinated more quickly and more conidia germinated overall on the susceptible NC 7 than on the other genotypes tested.

Key Words: *Arachis* spp., groundnut, early leaf spot.

Early leaf spot caused by *Cercospora arachidicola* Hori severely damages peanut (*Arachis hypogaea* L.) grown in the United States and in other peanut-cropping regions of the world. Yield losses due to this pathogen alone vary from 10 to 50%, depending on the climate, disease management inputs, and peanut genotype.

Improved levels of resistance to *C. arachidicola* in locally adapted cultivars would substantially increase peanut yields in developing countries. Elsewhere, planting of partially resistant cultivars would reduce fungicide use, decrease environmental impacts, and increase profits of peanut production. However, if pathogenic races of *C. arachidicola* are present, disease resistance could

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lose effectiveness over time or locations. Significant isolate by genotype interactions were found for several components of resistance when genotypes were inoculated with isolates of *C. arachidicola* collected from various locations around the world (10). Conidia from these isolates also differed significantly in germination percentages when incubated under uniform conditions.

The frequency of conidial germination undoubtedly influences development of epidemics caused by *C. arachidicola*. Genetic variability in the pathogen and host and the conduciveness of pre- and post-infection environments also influence the rate of disease increase (13). The effects of temperature on conidial germination previously have been reported for *C. arachidicola* (2, 4, 5, 10), but most experiments either examined germination in water or under a range of constant temperatures. Alderman and Beute (2) studied germination on intact leaf surfaces of susceptible genotypes and on glass slides coated with chloroform extracts of wax and other materials from leaf surfaces. Germination and germ tube elongation by *C. arachidicola* were a function of relative humidity and incubation temperature, with maximum germination after 24 or 48 hr incubation at 16 to 25 C. Under conditions favorable for germ tube growth, 95% of germinated conidia penetrated stomata of a susceptible genotype after 2 to 12 d incubation at constant 24 C. Earlier, Abdou *et al.* (1) compared post-germination behavior of *C. arachidicola* on the leaf surfaces of wild species and cultivated peanut genotypes. Although they did not report differential effects of genotypes on conidial germination, the behavior of subsequent germ tube elongation differed on highly resistant and susceptible genotypes. Germ tubes apparently were not attracted toward stomata on highly resistant genotypes but were attracted toward stomata on susceptible genotypes. They also reported that conidia and their germ tubes quickly lost their stainability and became transparent on resistant genotypes.

We previously showed that temperature regimes significantly influenced expression of resistance to early leaf spot in 14 peanut genotypes inoculated with a field isolate of *C. arachidicola* (14). The objective of the current study was to determine if peanut genotype influences germination by conidia of *C. arachidicola* under day/night temperature regimes representing the range of environments where peanuts are grown. Leaves of two highly resistant and two susceptible peanut genotypes (14) were used in the study.

Materials and Methods

Resistant and susceptible peanut genotypes from breeding programs at North Carolina State University (NCSU) and the International Crops Research Institute of the Semi-Arid Tropics (ICRISAT) were used. The susceptible genotype from NCSU was the virginia-type cultivar NC 7; the resistant virginia-type germplasm 91 PA 150 {=NC 5 x [PI 270806 x (PI 261942 x PI 262141-107A)]} was derived from a cross of *A. hypogaea* and *A. cardenasii* Krapov. and W. C. Gregory (PI 262141; GKP 10017) (9). The susceptible ICRISAT genotype (ICG 10900) is a valencia type and the resistant genotype (ICG 7878) is a spanish type.

Genotypes were planted in 15-cm diameter pots and grown in a greenhouse at NCSU, Raleigh. Pots contained a 2:1 mixture (v:v) of pasteurized sandy loam soil and greenhouse potting mix (Metromix 220, Grace Sierra Company, Milpitas, CA). A commercial *Bradyrhizobium* inoculant (cowpea group, Keel Peanut Company, Greenville, NC) was added to the soil mixture.

The second or third fully expanded leaves were excised from the main stems of 8- to 12-wk-old plants. Leaf petioles were placed in 75-mL beakers containing steamed sand and water and then placed in a mist chamber for 24 hr prior to initiation of experiments.

All experiments were conducted in growth chambers at the Southeastern Plant Environment Laboratory (Phytotron) at NCSU. Closed plexiglass boxes (30.5 x 30.5 x 16 cm) were used to create and maintain high humidity during the experiments (7, 14). Growth chambers were calibrated to maintain the following day/night temperatures within the boxes: 24/24, 26/20, 32/26, 38/26, and 38/32 C. A 12-hr photoperiod was obtained with fluorescent lights; intensity was approximately 350 to 400 μmol m^{-2} sec^{-1}. Leaves first were placed in boxes in growth chambers set at moderate temperatures (24/24 and 26/20 C day/night) and selected beakers were moved to progressively higher temperatures each 24 hr to allow acclimation to high temperature treatments.

All tests used conidia of *C. arachidicola* obtained from lesions that previously were collected at the Peanut Belt Research Station near Lewiston, NC and stored dry at 4 C. Conidia were suspended in a solution containing 1 drop of Tween 80 per 100 mL of deionized water and spore concentration was adjusted to 5×10^4 conidia per mL by counts with a hemacytometer. The conidial suspension was sprayed uniformly on leaflets by an artist's air brush at about 50 kpa air pressure. After inoculation, leaves were allowed to dry and beakers were arranged in plexiglass boxes in four randomized complete blocks of the four genotypes in each experiment. One experiment was conducted in each temperature regime.

Leaves were examined at 24-hr intervals up to 120 hr to determine the number of conidia germinated. The examination of leaves involved removing each leaflet, pressing clear tape (Scotch Magic Tape; 3M, St. Paul, MN) on the adaxial surface, and then adding cotton blue dye before placing the tape imprint on a slide. A compound microscope at 500X magnification was used to examine conidial germination on one slide for each leaflet. Conidia were counted as germinated if one or more germ tube was clearly visible (>.05-1.0 mm). Approximately 100 to 200 conidia per slide were counted and the percentage germination was calculated.

Data for each temperature regime were subjected to a split-plot analysis of variance with genotype as the whole plot and incubation time as the subplot. Temperature main or interaction effects were not evaluated statistically because temperature treatments were not replicated.

Conidial germination in water was monitored in each experiment. A conidial suspension (25×10^4 conidia mL^{-1}) was prepared in distilled water and 1.5 mL were distributed into 0.5-mL plastic capsules (Better Equipment for Electron Microscopy, Inc., Bronx, NY). Five capsules were placed in each of the Phytotron growth chambers as described for the leaf surface studies. After the initial 24-hr incubation, one capsule from each chamber was removed

every 24 hr for 4 additional days and the percentage germination was determined by microscopic examination of the entire sample at 500X magnification.

Results and Discussion

Hours of incubation significantly (P< 0.0001) influenced germination on the peanut leaf surfaces in all experiments, but the effects of incubation time depended on genotype in the 24/24 and 26/20 C regimes (Table 1). The greatest rates of increase in germination over the first 72 hr of incubation were found in the two coolest temperature regimes (Fig. 1), resulting in high levels of germination on all genotypes. The maximum germination (84%) was observed on NC 7 in the 24/24 C regime. Differences in germination on the two ICRISAT lines generally were significant only in the 24/24 C experiment after extended incubation (Table 1; Fig. 1). The highest percentage germination (78%) was observed on ICG 10900 after 96 hr incubation at 26/20 C (Fig. 1).

Leaf genotype significantly influenced germination in the 24/24, 26/20, 32/26, and 38/32 C temperature regimes (Table 1). Percentage germination was significantly greater on NC 7 than on other genotypes at all incubation temperatures except 38/26 C. Percentage germination was lowest on the resistant genotype 91 PA 150; the difference between this and all other genotypes was significant in the 26/20 and 32/26 C day/night temperature regimes.

The effects of genotype on conidial germination may have important implications for the epidemiology of leaf spot caused by *C. arachidicola*. Partial resistance to Cercospora leaf spot involves multiple biological components (6, 12), but the consistently high resistance of 91 PA 150 may be derived in part from a reduction in conidial germination on leaf surfaces. Under conditions optimal for germination (24/24 and 26/20 C), about 25% fewer conidia germinated on leaves of 91 PA 150 than on susceptible NC 7 (Table 1; Fig. 1). If fewer germ tubes are present on leaf surfaces (assuming equal conidial density on all genotypes), and these germ tubes are less capable of penetrating stomata (1), the cumulative effect would be a reduction in the number of infections induced per unit of inoculum. Lower inoculum efficiency of *C. arachidicola* has been reported on 91 PA 150 compared to NC 7 (14). This and other components of resistance would be complementary in providing high performance by 91 PA 150 in field and greenhouse tests (14).

Studies on components of resistance to *C. arachidicola* indicate that inoculum efficiency does not consistently differentiate genotypes of *A. hypogaea* (6). Similarly, in the current experiment, germination on the susceptible valencia type (ICG 10900) and the resistant spanish type (ICG 7878) differed only slightly. In contrast, inoculum efficiency appears to contribute to differences in resistance observed when wild species of *Arachis* and genotypes of *A. hypogaea* are compared (3). Genotype 91 PA 150 probably inherited unique genetic factors that suppress inoculum efficiency because it was derived from an interspecific cross between *A. cardenasii* and *A. hypogaea* (9).

Table 1. Analysis of variance of genotype and incubation time effects on percentage germination by conidia of *Cercospora arachidicola* in five temperature experiments.

	Temperature (C)[a]				
	24/24	26/20	32/26	38/26	38/32
			P > F		
Source					
Replicate	0.34	0.11	0.01	0.12	0.01
Genotype	<0.01	<0.01	<0.01	0.10	0.03
Time	<0.01	<0.01	<0.01	<0.01	<0.01
Genotype x time	<0.01	<0.01	0.24	0.22	0.42
			Germination (%)[b]		
Genotype[c]					
NC 7	65.4 a	65.2 a	29.5 a	11.2 a	6.5 a
91 PA 150	49.6 c	46.6 c	19.5 c	7.3 a	4.0 b
ICG 10900	57.1 b	60.0 b	25.8 b	9.9 a	4.7 b
ICG 7878	49.4 c	57.9 b	25.6 b	11.4 a	4.4 b

[a] Day/night temperatures are indicated for 12-hr photoperiods.

[b] Data presented are germination percentages averaged across four replicates and five incubation times. Means within columns followed by the same letter were not significantly different according to Waller-Duncan procedure with k=100.

[c] NC 7 and ICG 10900 are susceptible to early leaf spot and 91 PA 150 and ICG 7878 are resistant.

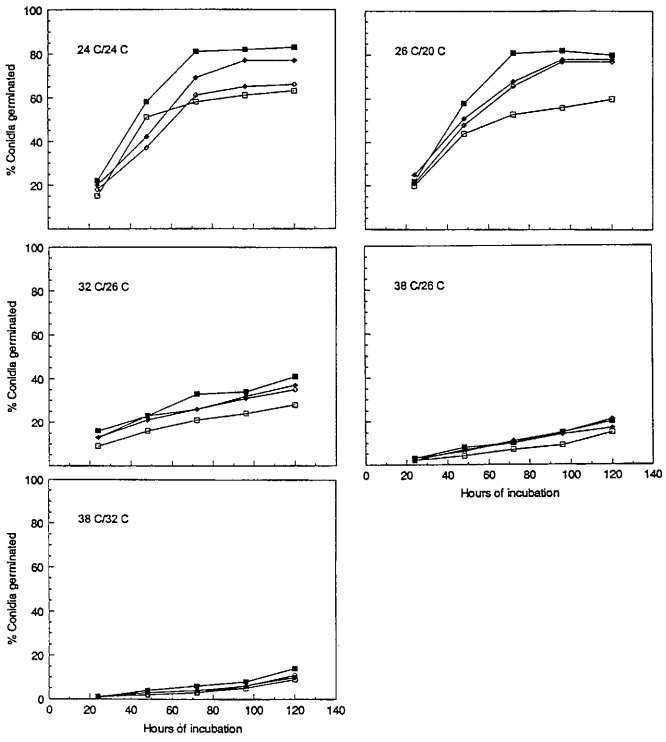

Fig. 1. Relationships between hours of incubation at various day/night temperature regimes and percentage germination by conidia of *Cercospora arachidicola* on leaves of NC 7, susceptible (■); 91 PA 150, resistant (❑); ICG 10900, susceptible (◆); and ICG 7878, resistant (◊) peanut genotypes.

Daytime temperatures in peanut-growing regions usually exceed 20 to 25 C, which consistently have been reported as most favorable for conidial germination by *C. arachidicola* (2, 4, 5, 10). Most of the reported studies used constant day/night temperatures, but we conducted experiments with cycling day/night temperatures and constant high relative humidity. Germination percentage at the most conducive temperatures, 24/24 and 26/20 C day/night, reached high levels after only 3 d of incubation, whereas the high temperature regimes of 38/26 and 38/32 C strongly inhibited germination on all genotypes (Fig. 1). Mean germination rates on leaves at each combination of temperature and incubation time were highly correlated with the corresponding mean germination in water ($r=0.95$, $n=25$; $P<0.0001$).

The 32 C day/26 C night temperature regime was of special interest because temperatures often range between 26 and 32 C in many peanut-growing regions. Germination percentage at 32/26 C was about one-half that observed at the most conducive temperatures. Apparently, the high daytime temperature slowed the germination process, but germination continued to increase when conducive conditions (temperature and wetness) persisted long enough at night.

Subba Rao *et al.* (10) recently reported that significant differences in germination (60-90%) of *C. arachidicola* conidia were observed among isolates from various agro-ecological locations. The host-status of peanut used for increasing inoculum has been reported to influence conidial morphology and germination in *Cercosporidium personatum* (Berk. and Curt.) Deighton (8), but Subba Rao *etal.* (11) used a single peanut cultivar for inoculum production. It is apparent that the differences they reported were the result of genetic characteristics of the pathogen. Geographic differences in genotype performance thus appear to be influenced by a complex of different factors, including host and pathogen genetics, local climatic conditions, and their interactions.

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Literature Cited

1. Abdou, Y.A.M., W.C. Gregory, and W.E. Cooper. 1974. Sources and nature of resistance to *Cercospora arachidicola* Hori and *Cercosporidium personatum* (Berk. & Curt.) Deighton in *Arachis* species. Peanut Sci. 1:6-11.
2. Alderman, S.C., and M.K. Beute. 1986. Influence of temperature and moisture on germination and germ tube growth of *Cercospora arachidicola* on peanut. Phytopathology 76:715-719.
3. Foster, D.J., H.T Stalker, J.C. Wynne, and M.K. Beute. 1981. Resistance of *Arachis hypogaea* L. and wild relatives to *Cercospora arachidicola* Hori. Oléagineux 36:139-143.
4. Gobina, S.M., and H. A. Melouk. 1981. Effect of temperature on the sporulation and conidial germination of *Cercospora arachidicola*. Phytopathology 71:876 (abstr.).
5. Oso, B.A. 1972. Conidial germination in *Cercospora arachidicola* Hori. Trans. Br. Mycol. Soc. 59:169-172.
6. Ricker, M.D., M.K. Beute, and C.L. Campbell. 1985. Components of resistance in peanut to *Cercospora arachidicola*. Plant Dis. 69:1059-1064.
7. Shew, B.B., M.K. Beute, and J.C. Wynne. 1988. Effect of temperature and relative humidity on expression of resistance to *Cercosporidium personatum* in peanut. Phytopathology 78:493-498.
8. Sommartya, T., and M.K. Beute. 1986. Temperature effects on germination and comparative morphology of conidia for Thai and USA isolates of *Cercosporidium personatum*. Peanut Sci. 13:67-70.
9. Stalker, H.T., and M.K. Beute. 1993. Registration of four leafspot-resistant peanut germplasm lines. Crop Sci. 33:1117.
10. Subba Rao, P.V., J.L. Renard, and D. McDonald. 1993. Preliminary investigations on variability in *Cercospora arachidicola* Hori. Int. *Arachis* Newslet. 13:15.
11. Subba Rao, P.V., J.L. Renard, F. Waliyar, D. McDonald, and R. Schilling. 1993. Variation in symptoms of *Cercospora arachidicola* isolates on some groundnut genotypes. Oléagineux 48:243-250.
12. Waliyar, F., J.P. Bosc, and S. Bonkoungou. 1993. Sources of resistance to foliar diseases of groundnut and their stability in West Africa. Oléagineux 48:283-287.
13. Waliyar F., D. McDonald, P.V. Subba Rao, and P.M. Reddy. 1993. Components of resistance to an Indian source of *Cercospora arachidicola* in selected peanut lines. Peanut Sci. 20:93-96.
14. Waliyar F., B.B. Shew, H.T. Stalker, T.G. Isleib, R. Sidahmed, and M.K. Beute. 1994. Effects of temperature on stability of components of resistance to *Cercospora arachidicola* in peanut. Phytopathology 84:1037-1043.

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