Sporulation of *Cercospora arachidicola* as a Criterion for Screening Peanut Genotypes for Leaf Spot Resistance

S. M. Gobina, H. A. Melouk, and D. J. Banks

Graduate student, research plant pathologist, and research geneticist, respectively, Department of Plant Pathology, and Agronomy Department, USDA, ARS, Oklahoma State University, Stillwater 74078.

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ABSTRACT

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The sporulation of *Cercospora arachidicola* on peanut is defined as the number of conidia produced per infected peanut leaflet after 96 hr of incubation at 25 ± 1 C under continuous light (800 lux) and 100% relative humidity. A detached-leaf culture technique was used to determine sporulation on leaflets of nine peanut genotypes. Genotypes that did not differ in numbers of lesions per leaflet, and numbers of conidia per square millimeter of lesion were significantly ($P = 0.05$) different in number of conidia per leaflet. No conidia were recovered from some genotypes even after prolonged incubation of infected leaflets. There was a significant linear correlation between necrotic area and lesions per leaflet on Comet, PI 109839, Florunner, and Tamnut 74. No difference in percent defoliation was observed among genotypes.

Additional key words: *Arachis* spp., *Arachis hypogaea*, disease resistance, early leaf spot, epidemiology.

Early leaf spot, which is caused by the fungus *Cercospora arachidicola* Hori, is one of the most economically important diseases of peanuts (*Arachis hypogaea* L.) worldwide. Control of *C. arachidicola* has usually been accomplished by reducing initial inoculum and subsequent inoculum production either by fungicides, crop rotation, sanitation, and other cultural practices. Fungicides have provided the best control (2,3,14,17,19,20); however, their high cost precludes their use in less developed countries (15). Also, the appearance of fungicide-tolerant strains of *Cercospora* and *Cercosporidium* (5,12,21) reduces the usefulness of fungicides for leaf spot control, and makes the search for resistant cultivars imperative.

All known cultivars are susceptible in varying degrees to *C. arachidicola* (1). However, peanut germplasm potentially useful in breeding programs has been identified (4,9,10,22). Evaluations of *Cercospora* resistance have been based on lesion counts and estimates of the degree of sporulation based on index scales set by the investigators. Based solely on number of lesions produced per leaflet, a peanut genotype may be disqualified from a breeding program, even though further investigation may reveal little or no sporulation on these lesions.

A detached leaf culture technique (13), developed for rapid screening of peanut genotypes for resistance to leaf spot, gives results that are correlated with field evaluations (7). In this paper, the sporulation of *C. arachidicola* on peanut leaflets is proposed as criterion for evaluating peanut genotypes for resistance to early leaf spot, as determined by the product of total area of lesions in square millimeter per leaflet, and number of conidia produced per square millimeter of lesion area.

MATERIALS AND METHODS

Nine peanut genotypes were selected for this study. These were Spanish genotypes Tamnut 74 and Comet; Virginia genotypes Florunner and PI 109839 (the latter a plant introduction from Venezuela described as resistant to *C. arachidicola* [8,22]); the wild species, PI 276233 ([GK 10596], *Arachis* sp., section RHIZOMATOSAE), PI 276235 [GKP 10602] (*A. chacoense* Krap. et Greg. *nom. nud.*, section ARACHIS), and PI 338280 ([HLK 410], *A. stenosperma* Greg. et Greg. *nom. nud.*, section ARACHIS); two hybrids M143 (PI 338280 × PI 276235) and M213 (Chico × [PI 338280 × PI 276235]). There were two trials, but only seven genotypes were used in Trial I due to lack of adequate test material.

Seeds of peanut cultivars Tamnut 74, Comet, Florunner, PI 109839, and PI 338280 were dressed with a mixture (14:1, w/w) of 50% Captan (Chevron Chemical Co., Ortho Div., San Francisco, CA 94150) and 15% Ethrel (Amchem Products, Inc., Agriculture Chemical Division, Ambler, PA 19006). Treated seeds were planted in 16-cm-diameter plastic pots containing a mixture of soil, sand, and finely shredded peat (2:2:1, v/v). Other genotypes were grown from rooted shoot cuttings obtained from greenhouse-grown plants that were several months old. These were potted and placed on a greenhouse bench under conditions favorable to the growth of peanut plants.

A detached-leaf culture technique was used in this experiment (13). The third expanded leaf was detached from 6- to 8-wk-old seedlings and from several 1-mo-old plants grown from cuttings. Detached shoots were used instead of leaves from PI 276235, PI 276233, and M143 because of their short petioles. Petioles or shoots were supported by foam plugs (13) and inserted into test tubes (16 × 150 mm) containing Hoagland's solution (11). Design of the experiment was a randomized block with genotypes as treatments, and replicates as blocks. There were 16 leaves or shoots per treatment and four replicates in Trial I and 12 leaves or shoots per treatment with three replicates in Trial II.

A single-spore isolate of *C. arachidicola* used in this experiment was obtained from infected plants grown in a greenhouse at Stillwater, OK. Conidia were prepared for inoculum as described by Smith (18), except that leaves of Tamnut 74 were used to prepare the medium, and conidia were suspended (2×10^4 conidia per milliliter) in an emulsion of Amway (Amway Corp., Ada, MI 49301) all-purpose adjuvant (two drops per 100 ml of H_2O). Both surfaces of leaflets were misted with a conidial suspension using a

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DeVilbiss No. 152 atomizer (The DeVilbiss Company, Somerset, PA 15501). Tubes in racks were placed in fabricated clear polyethylene moisture chambers (one replicate per chamber) on greenhouse benches. Relative humidity, recorded with a hygrothermograph placed in the chamber, was maintained at 100% by wetting burlap bags placed at the bottom of each chamber. Temperatures inside the chamber ranged from 21 to 32 C throughout Trial I, and in Trial II ranged from 22 to 32 C in the first 2 wk and from 20 to 26 C during the third and final week. The lower temperatures in each range were recorded during the night and the higher ones during the day. Hoagland's solution was replenished as needed.

Lesions per leaflet were counted 3 wk after inoculation. Where shoots were used, only leaves corresponding to the third expanded leaf at the time of detachment were rated. Leaflets with lesions were incubated in petri-dish moist chambers for 96 hr under continuous light (800 lux), provided by 40W Cool-White Econ-o-watt fluorescent tubes (Westinghouse Electric Corp., Dallas, TX 75247), at 25 ± 1 C. Lesions on leaflets were examined under a dissecting microscope for sporulation. Conidia were washed from surfaces of four leaflets with 2 ml of distilled water containing Amway all-purpose adjuvant (two drops per 100 ml of H_2O), and numbers of conidia in suspensions were determined with a hemacytometer. Lesions on leaflets were excised and surface areas determined with a Li-Cor model 3100 area meter (Lambda Instruments Corporation, Lincoln, NE 68504). Sporulation of *C. arachidicola* on peanut was calculated as number of conidia produced on an infected peanut leaflet under the incubation conditions stated above.

Data were analyzed following standard procedures for analysis of variance. Duncan's new multiple range test was used to distinguish mean difference between peanut genotypes. A standard procedure for a straight-line linear model for bivariate data was used to obtain linear correlations.

RESULTS

Trial I. Mean numbers of leaf spot lesions per leaflet (Fig. 1) were different ($P = 0.05$) among peanut genotypes. Lesions on leaflets of peanut cultivars Comet, Tamnut 74, Florunner, and PI 109839 appeared 11–13 days after inoculation, while on PI 276235, PI 276233, and M143 they appeared 13–15 days after inoculation. There were no differences in mean numbers of lesions per leaflet among Florunner, PI 276235, PI 276233, and M143. Mean necrotic lesion area per leaflet were significantly greater on Tamnut 74 and Comet than on PI 109839 or Florunner (Fig. 1).

Number of conidia per square millimeter of necrotic area among peanut genotypes ranged from 0 to 674 (Fig. 2). No conidia were recovered from lesions on PI 276235, PI 276233, or M143 even after incubating leaflets for 10–13 days, at which time leaflets had started to deteriorate. Sporulation of *C. arachidicola* on Tamnut 74 was significantly greater ($P = 0.01$) than on Florunner, and also greater on Comet and PI 109839 ($P = 0.05$) than on Florunner. There were no significant differences in sporulation among Tamnut 74, Comet, or PI 109839. However, significantly more conidia were produced per leaflet on Tamnut 74 and Comet than on PI 109839 or Florunner (Fig. 2).

Linear regression of total necrotic lesion area per leaflet (Y) in square millimeters on number of lesions (X) per leaflet was calculated for Tamnut 74, Comet, Florunner, and PI 109839.

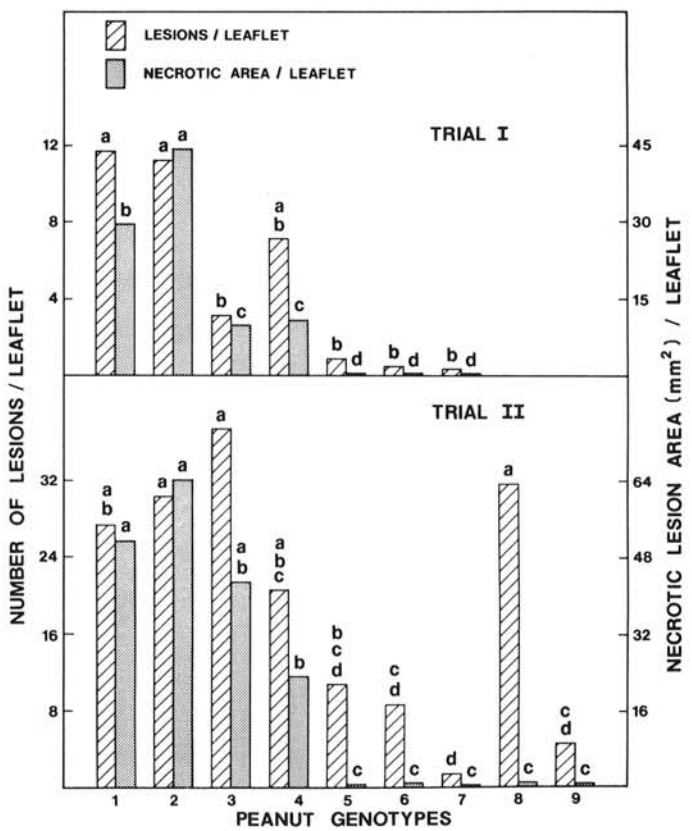

Fig. 1. Mean number of total lesions and necrotic lesion area per leaflet on different peanut genotypes 21 days after inoculation with *Cercospora arachidicola*. Genotypes were given the following numbers: 1 = Tamnut 74; 2 = Comet; 3 = Florunner; 4 = PI 109839; 5 = PI 276233; 6 = PI 276235; 7 = M143 (a hybrid between PI 338280 × PI 276235); 8 = PI 338280 and 9 = M213 (a hybrid of Chico × [PI 338280 × PI 276235]). Bars with same shade followed by the same letter are not significantly different ($P = 0.05$), using Duncan's new multiple range test. Means of four and three replicates in Trials I and II, respectively.

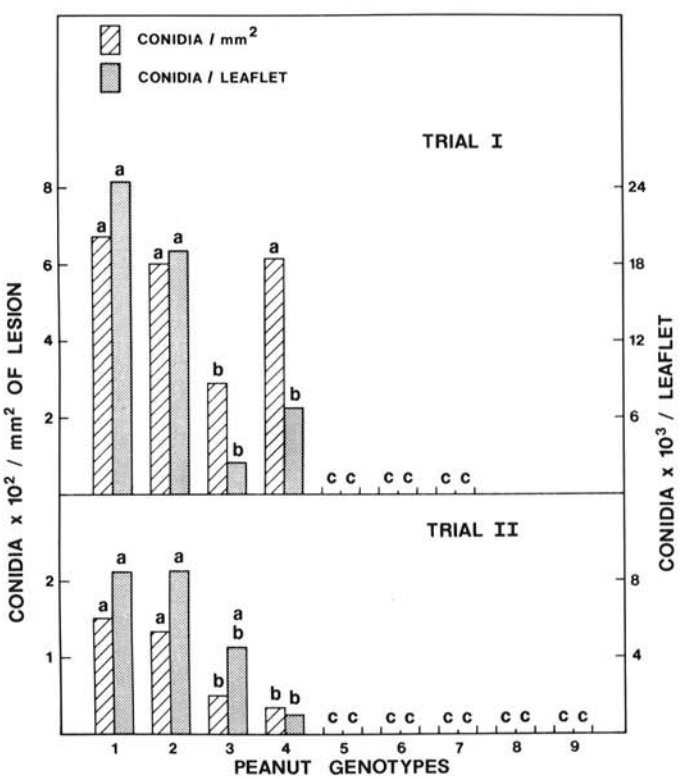

Fig. 2. Number of conidia per square millimeter of lesion and conidia of *Cercospora arachidicola* produced per leaflet on different peanut genotypes 21 days after inoculation. Genotypes were given the following numbers: 1 = Tamnut 74; 2 = Comet; 3 = Florunner; 4 = PI 109839; 5 = PI 276233; 6 = PI 276235; 7 = M143 (a hybrid between PI 338280 × PI 276235); 8 = PI 338280 and 9 = M213 (a hybrid of Chico × [PI 338280 × PI 276235]). Bars with same shade followed by the same letter are not significantly different ($P = 0.05$), using Duncan's new multiple range test. Means of four and three replicates in Trials I and II, respectively.

Significant positive coefficients of determination (r^2) of 0.36 ($P = 0.05$), 0.76 ($P = 0.001$), 0.64 ($P = 0.001$), and 0.59 ($P = 0.001$) were obtained for Tamnut 74, Comet, Florunner, and PI 109839, respectively. Regression equations were $Y = 0.46X + 23.14$, $Y = 3.60X + 4.16$, $Y = 3.11X - 0.29$ and $Y = 1.37X + 4.61$ for Tamnut 74, Comet, Florunner, and PI 109839, respectively.

Amounts of leaflet defoliation of peanut plant genotypes infected with *C. arachidicola* were determined 21 days after inoculation. There was no defoliation on Comet, Florunner, or PI 276235. Defoliation was 17.2, 10.9, 7.8, and 4.7% on PI 276233, M143, Tamnut 74, and PI 109839, respectively; however, there were no significant differences among these means.

Trial II. Among genotypes, significant differences ($P = 0.05$) occurred in mean numbers of lesions per leaflet and necrotic lesion area (Fig. 1). There was no difference in number of lesions between Tamnut 74, Comet, Florunner, PI 338280, and PI 109839. Mean necrotic lesion area ranged from 0.1 mm^2 on M143 to 64.1 mm^2 on Comet.

Conidia produced per square millimeter of necrotic lesions and number of conidia produced per leaflet were significantly different among genotypes (Fig. 2). More conidia were produced per square millimeter of lesions on Tamnut 74 and Comet than on Florunner and PI 109839. No conidia were recovered from PI 276235, PI 276233, M143, PI 338280, and M213 except from two deteriorating leaflets of PI 338280 (409 conidia per square millimeter of lesion). This may suggest a stronger saprophytic nature of *C. arachidicola* than previously suspected.

Positive significant correlations with coefficient of determinations values (r^2) of 0.49 ($P = 0.01$), 0.49 ($P = 0.01$), 0.46 ($P = 0.05$) and 0.76 ($P = 0.001$) were obtained for Tamnut 74, Comet, Florunner, and PI 109839, respectively. Regression equations were $Y = 1.28X + 16.20$, $Y = 2.31X + 1.68$, $Y = 1.86X - 26.33$, and $Y = 0.84X + 6.14$ for Tamnut 74, Comet, Florunner, and PI 109839, respectively.

DISCUSSION

Greenhouse screening for resistance to *Cercospora* spp. by using the detached leaf culture technique has been shown to be useful, but due to the large variation involved with different methods of disease estimation, no single method is adequate. Visual estimation may be subjective (16). The sporulation of *C. arachidicola*, as defined in this paper, represents an important parameter for estimation of leaf spot severity and is determined by the total necrotic lesion area per leaflet, and number of conidia produced per square millimeter of necrotic lesion. There were no differences among Tamnut 74, Comet, and PI 109839 in mean numbers of lesions per leaflet or conidia produced per square millimeter of lesion; however, total number of conidia produced per leaflet was different ($P = 0.05$) between Tamnut 74 and PI 109839, probably due to differences in lesion area (Trial I). Although PI 109839 was reported to have a high degree of resistance to *C. arachidicola* (8,22), others have reported that it is susceptible (6). We found that numbers of conidia produced per square millimeter of necrotic area on PI 109839 after 96 hr of incubation were two to three times greater than what was previously reported (6).

Temperatures in the chambers dropped to 20 C at night and only reached 26 C in the day during the 3rd wk after inoculation in Trial II. This may have reduced the rate of lesion expansion and delayed lesion maturity, and may explain the low number of conidia per square millimeter of lesion on Tamnut 74, Comet, and PI 109839. Also, the low numbers of conidia per square millimeter recorded on PI 109839 when compared to Tamnut 74 and Comet (Trial II) may indicate a greater effect of low temperatures on PI 109839. Except on Florunner, the sporulation in Trial II followed a similar trend as that in Trial I. Sporulation was generally lower in PI 109839 and was zero for some of the wild genotypes. Genotypes on which *C. arachidicola* sporulates poorly may be resistant by reducing the rate of disease increase (23) under field conditions.

The close relation between number of lesions per leaflet and total necrotic lesion area per leaflet suggests that a fast and objective estimate of necrotic lesion area can be obtained by counting lesions.

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