Peanut Science (1978) 5, 109-114

A Method Of Screening Peanut Genotypes For Resistance To Cercospora Leafspot[1]

H. A. Melouk* and D. J. Banks[2]

ABSTRACT

A detached leaf technique was developed for screening peanut genotypes for resistance to leafspot caused by *Cercospora arachidicola*. Compound leaves of four cultivars of peanut (*Arachis hypogaea*) and four wild peanut genotypes were used in this study. Individual leaf petioles, each supported by a foam plug, were immersed in Hoagland's solution in 1 x 14 cm test tubes. Leaves were inoculated with *C. arachidicola* by misting both surfaces with a conidial suspension (2 x 10^4 conidia/ml) using a DeVilbiss atomizer (No. 152). Test tubes with innoculated leaves were placed in racks in a clear polyethylene chamber on a greenhouse bench. Temperatures in the chamber averaged 26 ± 2 C and 31 ± 2 C during night and day, respectively. Relative humidity was maintained between 80 and 90% by hanging wicks of cheesecloth with their bases in water on both sides of the chamber. Lesions appeared on leaves of susceptible peanuts 8 to 10 days after inoculation and leaflet defoliation started 18 to 21 days after inoculation. This screening technique is reproducible, and requires a minimum of leaf tissue, space, and fungal inoculum.

Key Words: Disease resistance, hypersensitive reaction, peanut germplasm, sporulation, early leafspot.

Early leafspot on peanut (*Arachis hypogaea* L.), caused by *Cercospora arachidicola* Hori, is a serious disease worldwide. The disease syndrome includes lesions on the leaves and subsequent defoliation as the lesions increase in size and number. Early leafspot can be controlled by frequent applications of appropriate fungicides, but the most effective ones may enhance development of fungicide-tolerant strains of the pathogen (4, 8). Therefore, an integrated program of managing leafspot diseases of peanut by using resistant cultivars and minimal amounts of fungicides should reduce the rate of tolerant strain development and simultaneously reduce peanut production costs. Concentrated efforts are underway in the Southwest to develop peanut varieties with resistance to Cercospora leafspots (3). This paper reports on a detached leaf technique for preliminary screening of peanut genotypes, under controlled conditions, for resistance to *C. arachidicola*. An abstract report of the method has been published (9).

Materials and Methods

Compound leaves of four peanut cultivars, 'Comet', 'Tamnut', 'Spanhoma', and 'Florunner' (*A. hypogaea* L., section ARACHIS), and four wild species, P. I. number 276233 ([GK 10596], *A.* sp., section RHIZOMATOSAE), 262141, (*A. cardenasii* Krap. & Greg., (*nomen nudum*) section ARACHIS), 276235 (*A. chacoense* Krap. & Greg. (*nomen nudum*) section ARACHIS), and 338280 (*A.* sp., section ARACHIS) grown in greenhouse were used in this study.

The culture of *C. arachidicola* used for inoculation of detached leaves was isolated originally from a leafspot-susceptible peanut cultivar 'Tamnut' grown in Hughes County, Oklahoma in 1976. The fungus was cultured for production of conidia on a peanut-oatmeal-agar medium (10). Conidial suspensions were obtained by flooding 15-20 day old cultures in petri-plates with sterile distilled water, followed by filtration through four layers of cheesecloth to remove most of the mycelial fragments. Compound leaves from test peanut plants were detached and individual leaf petioles, supported by a foam plug, were immersed in Hoagland's solution (6), in 1 x 14 cm test tubes. Both surfaces of the leaves were misted with a conidial suspension (2 x 10^4 conidia/ml) of *C. arachidicola* using a DeVilbiss atomizer (no. 152). Test tubes with inoculated leaves were then placed in racks in a fabricated clear polyethylene chamber on a greenhouse bench. Temperatures in the chamber averaged 26 ± 2 C and 31 ± 2 C during the night and day, respectively. Relative humidity was maintained between 80 and 90% by hanging wicks of cheesecloth with their bases immersed in water on both sides of the chamber.

Twelve compound leaves of each peanut genotype in the test, representing four replications, were inoculated. Non-inoculated leaves served as controls.

The number of lesions per leaflet and the number of leaflets defoliated were counted and used as criteria for assessing infection and disease development. Lesions per leaflet on all entries were counted 3 weeks after inoculation, except for P. I. 276233, where they were counted 5 weeks after inoculation. The percentage of leaflet defoliation was recorded 4-6 weeks after inoculation.

Results and Discussion

Leafspot noticeable to the unaided eye (<1.5 mm) began to develop on detached leaves of 'Comet', 'Florunner', 'Tamnut', 'Spanhoma', P. I. 262141 and P. I. 338280, 8 to 10 days after inoculation with *C. arachidicola* (Fig. 1). Only minute lesions (>0.5 mm) began to appear 21 to 25 days after inoculating leaves of P. I. 276233 (GK 10596) (Fig. 2). this differs from the findings of Abdou *et al.* (1), wherein no reaction was noted on P. I. 27633 (GK 10596) after inoculation with *C. arachidicola*. No lesions had developed on inoculated leaves of P. I. 276235 after 6 weeks incubation and termination of the test. Leaflets with lesions (<1.5 mm) began to defoliate at 18-21 days after inoculation. The average number of lesions per leaflet and leaflet defoliation on inoculated leaves are shown in Fig. 3.

More than 90% of the lesions (<1.5 mm) on inoculated leaves exhibited sporulation by *C. arachidicola* after leaves were incubated on moist paper in petri-plates and under continuous fluorescent light (800 lux) at 23-25 C. However, the minute lesions (>0.5 mm) that developed on leaves of P. I. 276233 (GK 10596) failed to exhibit sporulation by the fungus, and *C. arachidicola* could not be isolated from them. This suggests that a hypersensitive reaction to infection by *C. arachidicola* had occurred following inoculation.

[1]Cooperative investigations of Fed. Res., Science and Ed. Admin., USDA, and Oklahoma State University, Journal Article No. 3471, Oklahoma Agricultural Experiment Station, Oklahoma State University, Stillwater, OK 74074.

[2]Research Plant Pathologist and Research Geneticist, respectively, Federal Research, Science and Education Administration, U. S. Department of Agriculture, Department of Plant Pathology and Department of Agronomy. Oklahoma State University, Stillwater, OK 74074.

Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the USDA or by Oklahoma State University, nor imply their approval to the exclusion of other products that may also be suitable.

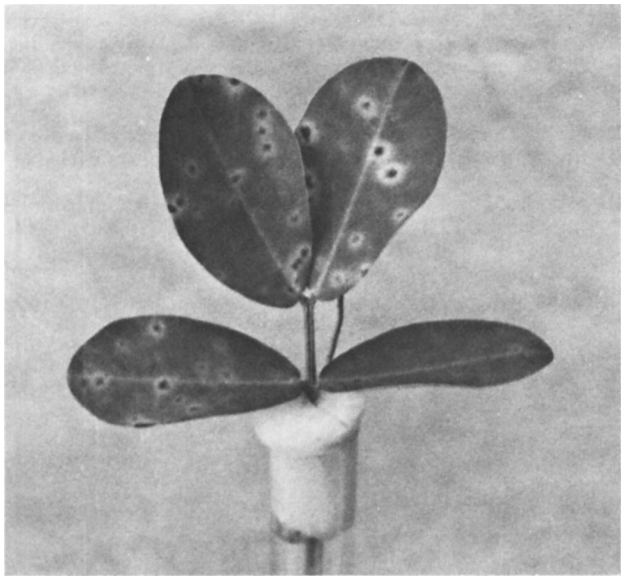

Fig. 1. Compound leaf from peanut cultivar (Tamnut) exhibiting lesions 4 weeks after inoculation with *Cercospora arachidicola*. Leaf petiole was supported by a foam plug and immersed in Hoagland's solution in 1 x 14 cm test tube.

Fig. 2. Hypersensitive reaction of peanut leaflet from P.I. 276233 (GK 10596). Leaflet on right with minute lesions, 6 weeks after inoculation with *Cercospora arachidicola*. Leaflets on left is a non-inoculated control.

Comparable results were obtained when inoculations were repeated 3 times during the year.

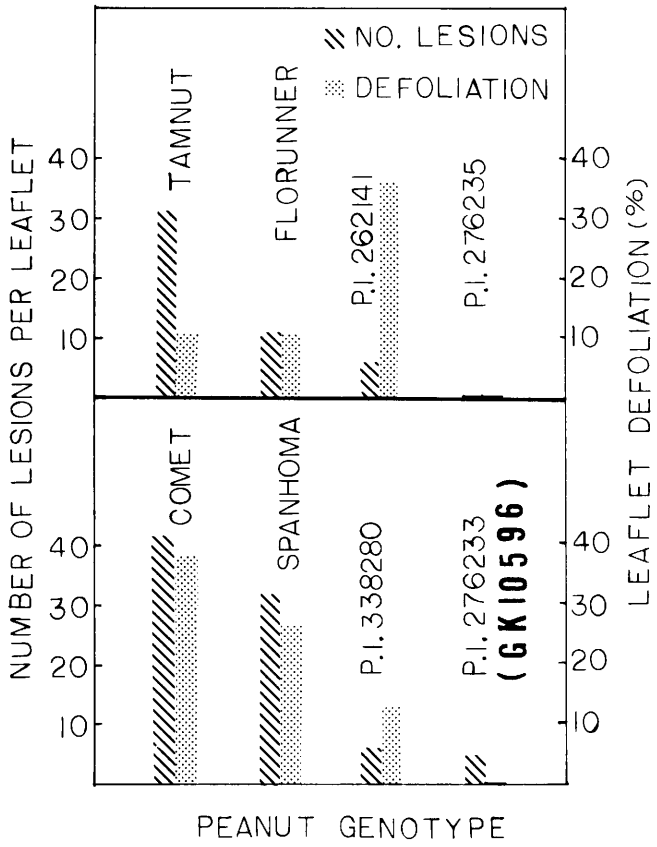

Fig. 3 Reaction of detached leaves from eight genotypes of peanut to inoculation with *Cercospora arachidicola*.

The detached leaf inoculation technique has advantages over inoculation of intact plant. It requires minimal amounts of inoculum of *C. arachidicola*, which grows slowly and sporulates poorly in culture (2, 7, 10, 11). Also, it requires a minimum of leaf tissue and working space. Forty peanut genotypes can be tested at the same time in one m^2 of space in an inoculation chamber. We use this technique for preliminary screenings of large numbers of peanut genotypes and hybrids for resistance to *C. arachidicola*. The technique, however, is not a substitute for field evaluation, because reactions in the field may differ from those in the greenhouse (5).

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* (Beck & Curtis) Deighton in *Arachis* species. Peanut Sci. 1:6-11.

2. Abdou, Y, A-M, and W. E. Cooper. 1974. Effect of culture medium and light on sporulation of two peanut leaf spotting fungi, *Cercospora arachidicola* Hori and *Cercosporidium personatum* (Beck & Curtis) Deighton. Peanut Sci. 1:11-18.

3. Banks, D. J., and H. A. Melouk. 1977. Breeding for peanut leafspot resistance. Okla. Agric. Exp. Stn. Prog. Rep. P-754. p. 4.

4. Clark, E. M., P. A. Backman, and R. Rodriguez-Kabana. 1974. *Cercospora* and *Cercosporidium* tolerance to benomyl and related fungicides in Alabama peanut fields. Phytopathology 64:1476-1477.

5. Hassan, H. N., and M. K. Beute. 1977. Evaluation of resistance to *Cercospora* leafspot in peanut germplasm potentially useful in a breeding program. Peanut Sci. 4:78-83.

6. Hoagland, D. R., and D. I. Arnon. 1950. Calif. Agric. Exp. Stn. Circ. 374. 32 p.

7. Jenkins, W. A. 1938. Two fungi causing leaf spot of peanuts. J. Agr. Res. 56:317-332.

8. Littrell, R. H. 1974. Tolerance in *Cercospora arachidicola* to benomyl and related fungicides. Phytopathology. 64:1377-1378.

9. Melouk, H. A., and D. J. Banks. 1977. Screening for resistance of peanut genotypes to Cercospora leafspot by a detached leaf technique. Proc. Am. Peanut Res. and Ed. Assoc. 9:14 (Abstr.)

10. Smith, D. H. 1971. A simple method for producing *Cercospora arachidicola* conidial inoculum. Phytopathology 61:1414.

11. Woodroof, N. C. 1933. Two leaf spots of the peanut (*Arachis hypogaea* L.) Phytopathology. 23:627-640.

Accepted August 14, 1978