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Evaluation of Detached Leaf Culture for Screening Peanuts for Leafspot Resistance[1]

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ABSTRACT

A detached leaf culturing technique has been proposed as a rapid and simple method for screening peanut (*Arachis hypogaea* L.) genotypes for leafspot resistance. This study was conducted to (a) determine the effect of both leaf age and outdoor plant weathering on infection with *Cercospora arachidicola* Hori as measured by the detached leaf technique, (b) evaluate the resistance to early leafspot of several peanut genotypes in the field and in the greenhouse using the detached leaf technique, and (c) determine the relationship between leafspot resistance measured in the field and the greenhouse.

The age of the leaf had a significant effect on leafspot resistance when evaluated using the detached leaf technique. Younger leaves averaged 11.4 lesions per leaflet compared to 5.6 lesions per leaflet for older leaves. The number of lesions per leaflet was similar for weathered and greenhouse-grown plants. PI 270806, PI 109839, Kanyoma, and PI 259679, four Virginia (ssp. *hypogaea* var. *hypogaea*) types, were the most resistant genotypes evaluated in these tests.

The number of lesions per leaflet caused by early leafspot for the 16 genotypes measured by the detached leaf technique was significantly correlated (r = 0.85) with the same trait measured in the field. PI 109839 had the fewest number of lesions per leaflet in both greenhouse and field tests.

Key Words: *Arachis hypogaea* L., breeding, disease resistance, host resistance.

Early and late leafspot caused by *Cercospora arachidicola* Hori (5) and *Cercosporidium personatum* (Berk. & Curt.) Deighton (6), respectively, are serious diseases of peanuts (*Arachis hypogaea* L.). Although considerable research to develop leafspot-resistant cultivars has been done, there are presently no cultivars wth high levels of leafspot resistance.

Breeding for resistance to leafspots would be greatly facilitated if there was a rapid method to evaluate peanut genotypes for resistance. Such a method could be employed to screen large numbers of genotypes for the purpose of identifying acceptable parental sources of resistance, as well as screening segregating progenies after hybridization.

Melouk and Banks (4) have developed a detached leaf culture technique for rapid screening of peanut genotypes for resistance to leafspot. There are numerous advantages in using their method, *i.e.*, conservation of space, plant material and inoculum, and also a greater control over the environmental conditions to insure optimum disease development (4, 9). One obvious disadvantage is the fact that the detached leaf system is highly artificial, and as a result the disease reaction may not correspond to that on attached leaves. However, as Tuite (9) has pointed out, the artificiality may not be as great as expected and it depends upon the host and pathogen employed. Another disadvantage is the disease reactions obtained in the greenhouse may differ from those found in the field (2). The goal of this study was to evaluate the usefulness of the detached leaf method described by Melouk and Banks (4). Specific objectives were to (a) determine the effect of both leaf age and outdoor plant weathering on infection with *C. arachidicola* as measured on detached peanut leaves, (b) evaluate the resistance of 16 diverse peanut genotypes to early leafspot in the field and in the greenhouse using the detached leaf method, and (c) correlate the results from the detached leaf evaluation with leafspot data collected on these same genotypes in a field study.

Materials and Methods

Sixteen genotypes were evaluated for early leafspot resistance in the field at the Upper Coastal Plain Research Station at Rocky Mount, NC during the 1978 growing season and in the greenhouse at Raleigh, NC during the early spring of 1979. All genotypes were Virginia (ssp. *hypogaea* var. *hypogaea*) type except for PI 259747, PI 350680 and PI 262129 which were Valencias (ssp. *fastigiata* Waldron var. *fastigiata*).

Fifteen fungicide-treated (Captan-Maneb) seeds of each genotype were planted in 10-cm plastic pots containing a 2:2:1 mixture of soil, sand and peat moss for the greenhouse experiment. After seedling emergence, half of the plants were grown outdoors for a period of approximately 5 weeks; the other plants remained in the greenhouse. Temperatures ranged between 13-32 C and 23-32 C for outdoors and greenhouse, respectively. In addition to temperature differences, the plants grown outside were subject to natural rainfall, including occasional heavy showers, and also a thrip infestation but they were not infected by leafspot. At the end of 5 weeks the plants outside were shorter and had fewer and smaller leaves than those grown in the greenhouse.

Five newly opened but fully expanded leaves and five older leaves were removed from each genotype grown outside and inside, making a total of 20 detached leaves from each genotype. The removed leaves were divided into five groups (replications) with four leaves of each genotype per replication. Of these four leaves, one leaf was derived from each of the four environment-leaf age combinations.

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The petioles were inserted into small test tubes (1x7 cm) containing Hoagland's complete nutrient solution (3). Leaves were held in place with foam plugs. The tubes were kept in high humidity chambers, one for each of the fice replications, Each chamber consisted of a wooden base in which holes had been drilled, and the test tubes were placed within these holes. A removable wooden frame above the base of each chamber was built to support a clear, plastic cover. The top and three of the sides were covered with the plastic; cheesecloth was draped over the fourth side. During the study the cheesecloth was kept moist.

The isolate of *C. arachidicola* used was derived from infected leaves collected at Lewiston, NC. Single conidia were transferred to water agar in the manner described by Abdou and Cooper (1), with subsequent transfers on peanut-oatmal-agar medium (7). The inoculum was prepared from the Lewiston culture as outlined by Smith (7). The conidia were suspended (10,000/ml) in water containing Tween 80 (three drops per 100 ml). The conidial suspension was applied to the detached leaves with cotton swabs on the day after the leaves were removed from the plants.

After inoculation, the chambers were periodically misted with water (12-sec spray every 6 min) to keep the relative humidity within the chambers high. During the subsequent 3-4 days the leaves were handmisted with water several times each day in order to maintain a thin film of water on the leaf surface. The nutrient solution within the tubes was replenished as needed by means of a hypodermic syringe. Small lesions were first visible on the leaves 8 days after inoculation.

Three weeks after inoculation the number of lesions per leaflet was recorded for each of the leaves. An analysis of variance was performed with the sources of variation in the model being replications, genotypes, leaf age (young *vs* old), and place (greenhouse *vs* outdoors).

The 16 genotypes were grown in the field in a randomized complete block design with four replications. Plants were grown in two-row plots with 20 plants per plot. Rows were spaced 90 cm apart with 25 cm between plants within rows. Standard cultural practices were performed except no measures were taken to control leafspot. The plants became naturally infected with early leafspot with symptoms first observed during the last week in July. The lesions on 12 leaves were counted for each of three randomly selected plants within each plot. Four leaves were taken from the upper, middle, and lower portions of each plant.

A simple correlation over genotypic means was computed between field and greenhouse data in order to ascertain the relationship between field and detached leaf data.

Results and Discussion

The age of the leaf had a significant (.01 α-level) effect on resistance to early leafspot as measured by the detached leaf method. The younger leaves averaged 11.4 lesions per leaflet compared to only 5.6 lesions per leaflet for the older leaves (Table 1). Weathering of the plants outdoors did not significantly influence the number of lesions that developed on a leaflet, although plants grown outside averaged 8.2 lesions compared to 8.8 lesions per leaflet for plants grown in the greenhouse. A similar trend was found by Hassan and Beute (2) when they compared the leafspot resistance of weathered and nonweathered intact plants.

A significant (.05 α-level) age x place interaction was found for leafspot resistance. The relative difference in leafspot resistance of old and young leaves changed depending on whether the leaves were weathered or not. The younger leaves had relatively fewer lesions than older leaves from plants grown outside in contrast to the number of lesions for old and young leaves from greenhouse-grown plants.

Table 1. Effect of leaf age and growth environment on leafspot resistance.

Age of leaf	Leafspot lesions/leaflet		
	Environment		
	Outside	Greenhouse	$\bar{X}$
Young	10.19a	12.64a	11.42a
Old	6.25b	5.03b	5.64b
	8.84a	8.22a	

Means within groups with same letters are not significantly different at .05 α-level.

More important, however, was the lack of interaction for leafspot resistance of entries with age or place. The performance of an entry was similar regardless of whether young or old leaves or weathered or nonweathered leaves were used for measuring leafspot resistance using the detached leaf technique. Thus either young or old leaves or weathered or nonweathered leaves may be used for the detached leaf technique as long as the choice of material is consistent for each genotype.

The 16 cultivated genotypes were significantly (.01 α-level) different for the number of lesions per leaflet as measured by the detached leaf method in the greenhouse (Table 2). PI 270806, PI 109839, Kanyoma and PI 259679 (four Virginia types) had the fewest lesions per leaflet in the detached leaf study. The 16 genotypes were also significantly different in their response to the early leafspot pathogen in the field study. PI 109839 had the fewest lesions per leaflet. This entry reported to be resistant by Sowell *et al.* (8) was recently released as resistant germplasm (personal communication, R. O. Hammons, Tifton, GA).

Table 2. Leafspot lesions per leaflet measured by the detached leaf technique and in the field for 16 cultivated peanut genotypes.

Genotype	Botanical variety	Leafspot lesions per leaflet*	
		Detached-leaf	Field
PI 262129	fastigiata	14.38a	16.87a
PI 350680	fastigiata	12.16ab	11.14b
NC Ac 3139	hypogaea	10.33abc	9.19bcd
NC 2	hypogaea	9.94abc	10.82bc
PI 269685	hypogaea	9.78abc	5.50d-g
PI 259639	hypogaea	9.09abc	9.11b-e
PI 259747	fastigiata	8.90bc	9.36bcd
PI 162857	hypogaea	8.73bc	8.67b-f
Florigiant	hypogaea	8.59bc	5.10d-g
NC 5	hypogaea	7.84bc	4.15fg
NC 3033	hypogaea	7.41bc	4.32fg
Leafspot X-ray selection	hypogaea	6.70bc	6.53c-g
PI 270806	hypogaea	5.90c	4.66efg
PI 109839	hypogaea	5.86c	2.27g
Kanyoma	hypogaea	5.66c	5.45d-g
PI 259679	hypogaea	5.20c	5.80d-g

*Means within groups with same letters are not significantly different at .05 α-level according to Duncan's multiple range test.

The coefficient for the correlation of entry means in the detached leaf study and the field study (0.85) was significant (.01 α-level). This is in contrast to the results of Hassan and Beute (2) who found a lack of correlation between field and greenhouse results in North Carolina. Their techniques were quite different from those used in the detached leaf method (4) and could explain the discrepancy between these two studies. The significant correlation of leafspot resistance measured in the field and by the detached leaf method showed that the detached leaf technique is useful to screen for leafspot resistance in the greenhouse.

With the simplicity and rapidity of the method devised by Melouk and Banks (4) and the correlation of results from the method with field results, the detached leaf technique should be useful in breeding for leafspot resistance in peanuts. Although this technique may be useful in identifying acceptable parental sources of resistance and in screening to reduce the size of segregating populations requiring field tests, it may also be used in conjunction with recurrent selection to concentrate resistant genes from different genetic sources of resistance. Recurrent selection for leafspot resistance in a population generated from several sources of resistance using the detached leaf method may allow peanut breeders to develop cultivated genotypes with higher levels of resistance. As many as three cycles of selection per year could be completed using the detached leaf technique. Development and use of highly resistant cultivated genotypes as an alternative to using the resistance from the wild diploid species of *Arachis* need attention by peanut breeders.

Using leafspot lesions per leaflet as a sole measurement of resistance may have one major disadvantage. Other mechanisms of resistance such as resistance to defoliation, a longer latent period and reduced sporulation may be overlooked. The detached leaf technique should be useful in studying the latent period and sporulation. Greater attention to mechanisms of resistance other than reduced infection may be needed if leafspot is to be controlled through breeding.

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