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Evaluation of Resistance to Cercospora Leafspot in Peanut Germplasm Potentially Useful in a Breeding Program[1]

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ABSTRACT

Sixteen peanut lines representing diverse germplasm were evaluated in the greenhouse and field for resistance to Cercospora leafspot caused by *C. arachidicola* Hori. In greenhouse tests, Spantex and Starr developed consistently fewer lesions than NC 3033 and AC 3139 following leaf inoculation with *C. arachidicola*. In field evaluations, with naturally occurring inoculum, the reverse was true. Defoliation of the lines was compared in chlorothalonil sprayed and nonsprayed field plots. 'NC 3033', 'NC 5' and 'AC 3139' were more resistant to defoliation than 'Starr', 'Tennessee Red', 'Spantex', 'Argentine' and 'Spancross'. Spancross had an intermediate number of lesions at all sample dates but had the highest defoliation. Plants grown continuously in the greenhouse tended to develop more lesions than did plants grown outside for 2 weeks before inoculation. The reduction in lesion number was most striking on NC 3033.

A disease index indicative of the interaction between number of lesions per leaf and precent defoliation showed that NC 3033, NC 5 and AC 3139 were more resistant than Spantex, Argentine, Starr and Tennessee Red in field tests. The defoliation ratio technique was very reliable in field tests and had less variability than counting number of lesions or lesion size. Visual estimation of percent leaves infected, however, was a rapid, efficient method for evaluation when large number of plants per entry are available. Entries most useful as parents in breeding for resistance to Cercospora leafspot are NC 3033, NC 5, and AC 3139.

Additional keywords. *Arachis hypogaea* L., *A. cardenasii*, *A. chacoense*, *Cercospora arachidicola*, groundnut.

Cercospora leafspot of peanut (*Arachis hypogaea* L.) is caused by *Cercospora arachidicola* Hori (early leafspot) and *Cercosporidium personatum* (Berk, and Curt.) Deighton (syn. *Cercospora personata*) (late leafspot). the perfect stages of these fungi were described in 1938 and named *Mycosphaerella arachidicola* W.A. Jenkins and *M. berkeleyii* W.A. Jenkins, respectively (6). Cercospora leafspots are considered the most serious diseases of peanut (5) and are found wherever peanuts are grown, with *C. arachidicola* being more common than *C. personatum* (12). Yield losses are estimated to be from 15 to 50% in many areas of the world (5). In the United States the average annual loss for 1951 to 1960 was estimated at 10% (11).

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Although Cercospora leafspot diseases of peanut are successfully controlled by fungicides in the United States, the cost is significant and may be higher, relative to total production costs, in other peanut-producing areas (1). Screening for disease resistance has been intensively carried out by many workers and different levels of resistance have been reported in certain varieties (8, 9). Both tolerant and resistant varieties have been reported in the world germplasm of peanut (2, 3). Some plant introductions were resistant to *C. arachidicola* in greenhouse and field tests (9). However, controversy arose when Gibbons and Bailey (4) found no lesions on *Arachis repens* Handro, *A. glabrata* Benth., and *A. hagenbeckii* Harms in the field but Abdou et al. (1) obtained lesions in the greenhouse. With *A. villosulicarpa* Hoehne, Gibbons and Bailey (4) observed lesions and considerable defoliation; whereas, Abdou et al. (1) found it to be immune.

Intensive effort in North Carolina and Virginia have been undertaken to develop varieties resistant to Southern corn root worm *(Diabrotica undicimpunctata howardi)*, two spotted spider mite *(Tetramychus urticae)*, Cylindrocladium black rot *(Calonectria crotalariae)*, and southern stem rot *(Sclerotium rolfsii)*. A primary goal in this breeding effort is to incorporate Cercospora resistance into breeding lines useful in developing multiple pest resistance. This study compared the disease response of selected peanut varieties and accession lines to Cercospora leafspot both in the field and greenhouse and evaluated various screening methods.

Materials and Methods

A culture of *Cercospora arachidicola* isolated from 'Florigiant' peanut in Northampton County, North Carolina, was used in greenhouse inoculation tests. Stock cultures of the fungus were maintained on oatmeal agar medium by transferring the fungus to fresh medium at 7-10 day intervals. After 7-10 days incubation under continuous light, cultures were flooded with 20 ml of distilled water and the conidia brushed free with a camel's hair brush. The resultant suspension was then filtered through 2 to 3 layers of cheesecloth, and the conidial concentrations were determined with a hemacytometer. Water was added to standardize inoculum and suspensions were amended with 2 to 3 drops of the surfactant Tween 80 per 100 ml.

In the first test eight leaflets on each plant and 5 plants of each variety were sprayed with ca. 0.1 ml conidial suspension at a concentration of 15,000 conidia per ml using a DeVilbiss atomizer. The experiment was repeated using conidial suspensions of 10,000 and 5,000 per ml. Inoculated plants were covered with a plastic bag and placed in a mist chamber for eight days. The temperature in the mist chamber averaged 25 to 30 C.

Plants were removed from the mist chamber and leafspots were counted 9 to 14 days after inoculation.

Sixteen peanut lines were tested both in the greenhouse, using 3 inoculum concentrations, and in 1975 in field plots in a randomized complete block design with 8 plot replications. Plots were 2 rows wide and 50 feet in length. Four replications were sprayed with 6 applications of chlorothalonil (tetrachloroisophalonitrile) (16 oz ai/acre) beginning July 1 and 4 replications received no fungicide treatment. Nine entries of *Arachis hypogaea* subspecies *hypogaes* Krap. et Rig. were as follows: Florigiant, 'Florunner', 'Early Bunch', 'NC-Fla 14', 'NC 2', 'NC5', 'NC 3033', 'AC 3139' and 'AC 3196'. Seven entries of *Arachis hypogaea* subspecies *fastigiata* Waldron were as follows: 'Argentine', 'New Mexico Valencia', 'Spancross', 'Spanhoma', 'Spantex', 'Starr', and 'Tennessee Red'.

Ten peanut lines were tested in the field in 1976. Five entries (Florigiant, Early Bunch, NC 2, NC 5 and NC 3033) had been tested previously and five entries ('Avoco 11', 'GK-3', 'NC 6', P.I. 262129 and 'Va 72R' were tested for the first time. Plot design was the same as 1975 except only 4 replications were used and no fungicide treatment was applied.

In addition four peanut lines from 1975 tests (NC 3033, NC-Fla 14, Spantex and Starr), a plant introduction (P.I. 109839), a wild species [*A cardenasii* Krap. et Greg. (PI 262141)] and the triploid F_1 from the cross between 2 wild species [*A. cardenasii* x *A. chacoense* Krap. et Greg. (PI 276235)] were tested in the greenhouse. Two weeks before inoculation in the greenhouse, one-half of the total number of plants from each entry were placed outside to expose leaves to a natural environment. All plants were returned to the greenhouse and eight leaflets from each plant were inoculated as previously described with a conidial suspension of 15,000 conidia per ml.

Several non-inoculated leaves were removed from the upper portion of each of these plants. A few drops of acetone were immediately dropped onto the surface of the leaf, and a small piece of 0.005 clear acetate paper and a glass slide were placed over it. The glass slide was inverted and the leaf was gently pressed onto the acetate paper. After the acetone dried the leaf was slowly removed while the leaf impression remained on the acetate paper attached to the slide. Numbers of stomates per mm^2 were counted and measurements of the stomatal apertures were made with a binocular microscope at a magnification of 500X.

Disease evaluations in 1975 were made at three different times in field tests (7/23, 8/21, and 9/24, representing date 1, 2, and 3, respectively) by the following four methods: 1) *Defoliation ratio:* The lower limbs of 10 randomly selected plants/plot were chosen and measurements made from the base of the limb to the first leaf were divided by the total limb length. Tags were placed on each limb so that individual limbs could be followed through the season. 2) *Lesion count:* Five leaves were chosen randomly from each of ten plants per replication and the number of lesions on each leaflet were counted. 3) *Leaf area infected:* The percent leaf area infected was estimated using an 'intensity grade scale' developed by Sulaiman and Agashe (10). A pictorial diagram having illustrations representing 1%, 5%, 5-10%, 10-25%, 25-50%, and above 50% of leaf area infected was used as a standard for assigning values. 4) *Visual estimation of percent foliage having lesions:* Observations were made by two individuals looking at all the plants in the rows and approximating percent leaves with spots. Scores were made in multiples of 10% and averaged. In 1976, field plots were evaluated only once (9/29) using the visual estimate of percent foliage having lesions.

Results

Effect Of Inoculum Density - In greenhouse tests, leaf inoculations with 15,000 conidia per ml resulted in most spots. At this concentration, cultivars

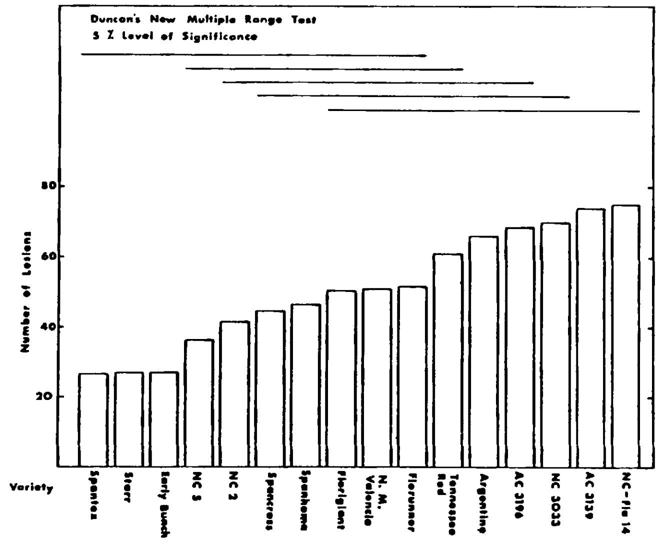

Fig. 1. Average number of lesions per leaf on 16 peanut entries inoculated with 1.5 X 10^4 conidia per ml in greenhouse tests.

Spantex, Starr, and Early Bunch had fewer lesions (P = 0.05) than NC 3033, AC 3139, and NC-Fla 14 (Fig. I). The average number of lesions ranged from 26.7 for Spantex to 75.3 for NC-Fla 14.

Inoculation at a concentration of 10,000 conidia per ml resulted in fewer lesions. Florunner and AC 3139 had more lesions (P = 0.05) than the other entries tested. The average numbers of lesions ranged from 5.4 for Starr to 37.5 for Florunner. It was also noted that Starr and Spantex again developed fewer lesions.

Even fewer lesions developed on each entry inoculated with 5,000 conidia per ml. AC 3139 had more lesions (P = 0.05) than Argentine which in turn had more than the other entries. The average number of lesions ranged from 1.9 on Spantex to 19.8 on AC 3139.

Effect Of Weathering On Leafspot Incidence - The average number of lesions on leaves of plants grown continuously in the greenhouse in these tests was 76.7, with a range from 104.4 for NC 3033 to 26.6 for *A. cardenasii* X *A. chacoense*. *A. cardenasii* X. *A. chacoense* had fewer lesions (P = 0.01) than all other entries. The group of plants exposed to weather had fewer lesions (P = 0.01) than plants grown continuously in the greenhouse (Fig. 2). Lesion numbers averaged 63.96 with a range from 83.8 for NC-Fla 14 to 18.5 for *A. cardenasii* X *A. chacoense*. The two week weathering treatment resulted in a 28 percent reduction in number of lesions developing on leaves of NC 3033.

Stomatal Aperture Sizes and Stomata Numbers Among Peanut Cultivars - Among greenhouse grown plants, the mean stomatal apertures of *A. cardenasii X A. chacoense* and *A. cardenasii* were

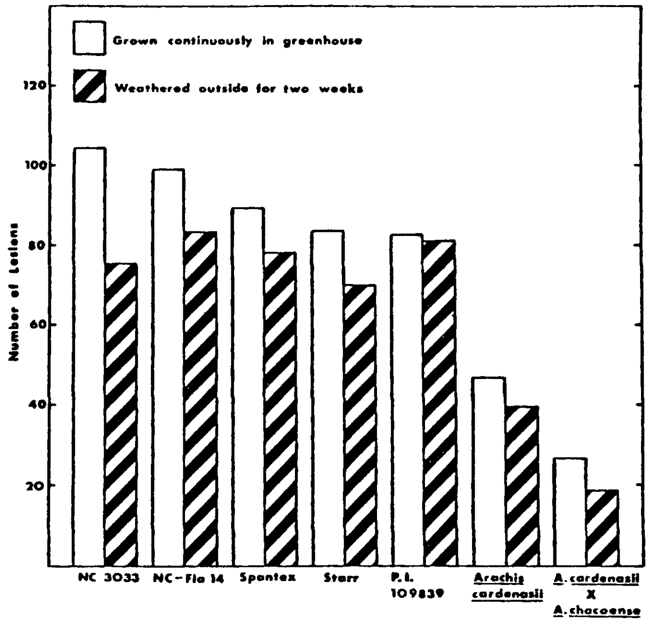

Fig. 2. Effect of weathering plants for two weeks prior to inoculation with 1.5 X 10^4 conidia per ml on average number of lesions per leaf on 7 peanut entries in greenhouse tests.

smaller than other entires on both the upper and lower leaf surfaces. Stomatal apertures on the upper leaf surface of cultivars ranged from 10.6 μm in *A. cardenasii* to 18.7 μm for Starr. Culrivars with intermediate size stomatal apertures were NC-Fla 14 and P.I. 109839, each measuring 14.9 μm. On the lower leaf surface *A. cardenasii X A. chacoense* and *A. cardenasii* had the smallest mean stomatal aperture, each measuring 12.7 μm. NC 3033 had a mean stomatal aperture of 16.1 μm on the upper leaf surface. The average number of stomata per mm^2 on the upper leaf surface ranged from 18.4 in Spantex to 38.6 for *A. cardenasii X A. chacoense.* On the lower leaf surface the average number of stomata ranged from 13.8 in Spantex to 23.6 for NC 3033.

Among plants exposed to weathering for two weeks the mean stomatal aperture of *A. cardenasii X A. chacoense* was smallest on both the upper and lower leaf surfaces. The mean stomatal aperture of the upper leaf surface among entries ranged from 10.3 μm in *A. cardenasii X. A. chacoense* to 20.9 μm for Starr. NC 3033 had a mean stomatal aperture of 14.6 μm on the upper leaf surface. The mean stomatal aperture of the lower leaf surface among entries ranged from 12.5 μm in *A. cardenasii X A. chacoense* to 19.9 μm for Starr and NC-Fla 14. A. cardenasii and NC 3033 had mean stomatal apertures of 14.2 and 13.9 μm, respectively, on the lower leaf surface.

Differences were noted in these tests (P = 0.01) both in mean stomatal apertures and numbers between growth environments, leaf surfaces and cultivars. Smaller (P = 0.01) mean stomatal apertures on leaves of NC 3033 plants exposed to weathering for two weeks was correlated with increased resistance to diseases. Starr, Spantex, and NC-Fla 14 had larger (P = 0.05, P = 0.01, and P = 0.01, respectively) mean stomatal apertures on the upper leaf surface with plants exposed to weathering, but also were more resistant to disease than similar plants grown continuously in the greenhouse. The average number of stomata on the upper surface among cultivars exposed to weathering ranged from 20.8 in NC-Fla 14 to 38 for *A. cardenasii X A. chacoense.* On the lower surface the average number of stomata ranged from 18.0 in NC-Fla 14 to 28.4 for *A. cardenasii X A. chacoense.* Starr, Spantex, and *A. cardenasii X A. chacoense* had a higher (P = 0.01) number of stomata on leaves of plants exposed to weathering.

1975 Field Results: Defoliation ratio - In the sprayed plot, all cultivars had defoliation ratios of less than 0.15 early in the season. By date 2 a considerable increase in defoliation ratio occurred with some varieties having a ratio of more than 0.3 and others more than 0.4. At date 3 most of the cultivars had defoliation ratios of more than 0.6 but NC 3033 had the lowest defoliation ratio of 0.46.

The split-plot analysis (combining data from 3 dates) on defoliation ratio in unsprayed plots gave a significant difference among the cultivars. These results also gave an idea of the level of defoliation for each variety at any given time during the growing season. The varietal reaction in descending order of resistance based on defoliation ratios are as follows: NC 3033, AC 3196, AC 3139, NC 2, Spancross, New Mexico Valencia, Spanhoma, Starr, NC 5, Spantex, Argentine, Early Bunch, Florigiant, NC-Fla 14, Florunner, and Tennessee Red.

The defoliation ratio as previously described may not reflect actual defoliation due to leafspot disease but rather a phenotypic response resulting from several factors. To determine the actual effect of leafspot disease on defoliation among germplasm tested, defoliation ratio values from sprayed plots were subtracted from values in unsprayed plots. When this correction was made, a higher percentage defoliation was detected among the Spanish peanuts than the Virginia-bunch peanuts. NC 3033 had the lowest actual defoliation ratio (less than 0.01), whereas Spancross exhibited the highest actual defoliation ratio of more than 0.17. Actual defoliation ratio values (at date 3) are as follows: NC 3033 (0.007), NC 5 (0.011), AC 3139 (0.024), Early Bunch (0.039), NC-Fla 14 (0.07), Florigiant (0.072), Florunner (0.087), Spanhoma (0.09), AC 3196 (0.092), New Mexico Valencia (0.104), Starr (0.134), Tennessee Red (0.135), Spantex (0.137), Argentine (0.157), and Spancross (0.172).

Number of lesions- Differences (P = 0.05) in number of lesions per leaf among varieties in the unsprayed

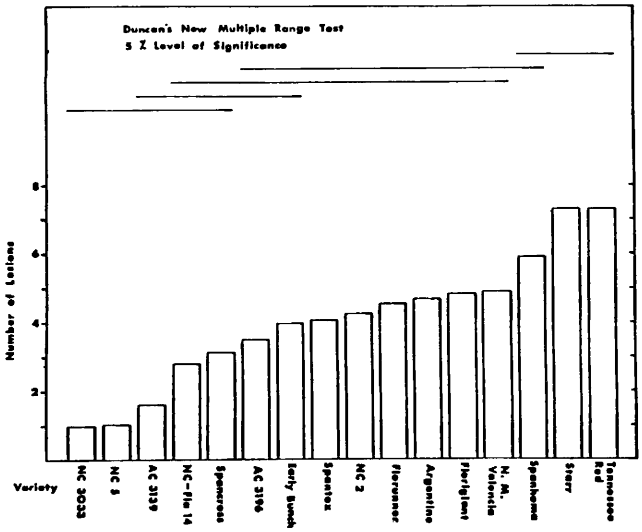

Fig. 3. Average number of lesions per leaf on 16 peanut entries in unsprayed field plots at date 3 (9/24).

plot were observed on most of the entries but NC 3033, NC 5, and AC 3139 had the lowest number of lesions, averaging less than two per leaf (Fig. 3). Starr and Tennessee Red remained constantly high averaging more than 7 lesions per leaf. The number of lesions ranged from 0.95 with NC 3033 to 7.3 with Tennessee Red. The split-plot analysis showed the descending order of resistance as follows: NC 3033, NC 5, AC 3139, NC-Fla 14, AC 3196, NC 2, Spancross, Spantex, Florunner, Early Bunch, Florigiant, Argentine, New Mexico Valencia Spanhoma, Tennessee Red, and Starr.

Defoliation characteristics of germplasm tested did not always correspond to the number of lesions present. At date 3, Spancross had a lower number of lesions than many other cultivars but exhibited the highest percent defoliation due to the disease. NC 3033, NC 5, and AC 3139 all had a lower number of lesions and percent defoliation. Starr and Tennessee Red had the highest number of lesions among cultivars but exhibited a lower percent defoliation than Spancross and Argentine (Fig. 4). The disease

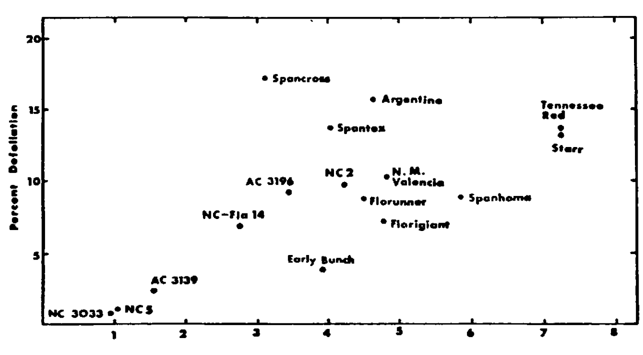

Fig. 4. Position of 16 peanut entries when plotted as the average number of lesions per leaf X average percentage defoliation (sprayed plot ratio subtracted from unsprayed plot ratio) at date 3 (9/24).

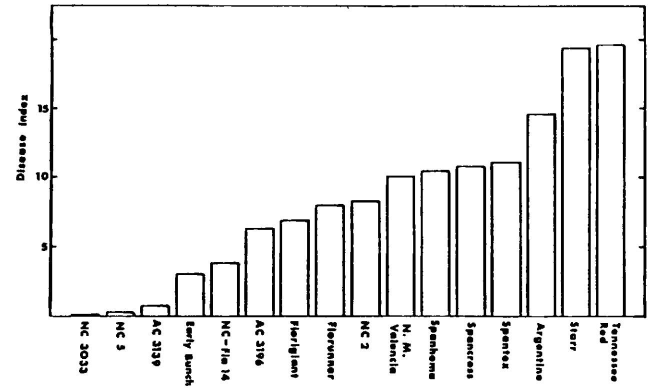

Fig. 5. Disease indices expressed as the product of average number of lesions per leaf and averae percentage defoliation for 16 peanut entries at date 3 (9/24).

index for each variety was determined by disease index in descending order were as follows: NC 3033, NC 5, AC 3139, Early Bunch, NC-Fla 14, AC 3196, Florigiant, Florunner, NC 2, New Mexico Valencia Spanhoma, Spancross, Spantex, Argentine, Starr, and Tennesse Red (Fig. 5).

Leaf area infected - Differences (P = -.01) in the area of leaf infected occurred between the cultivars at all three dates observed. At date 3 (Fig. 6), NC 3033, NC 5, and AC 3139 had only 1.45, 1.53, and 2.6% of the leaf area diseased, respectively. Based on this criterion, the most susceptible cultivars were Starr and Tennessee Red with 16.1 and 19.0% of leaf area diseased, respectively.

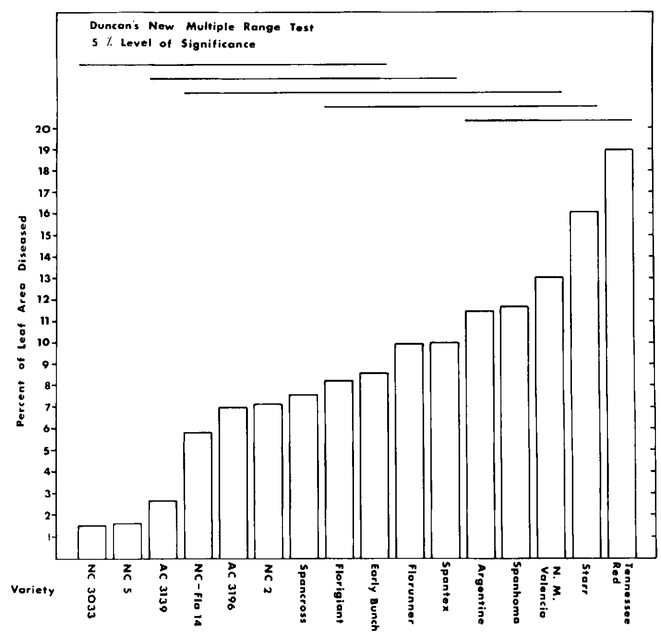

Fig. 6. Comparison of leaf area diseased for 16 peanut entries in unsprayed plots at date 3 (9/24).

Split-plot analysis showed that with respect to the area of leaf diseased, the more resistant cultivars in descending order were: NC 5, NC 3033, AC 3139, NC-Fla 14, NC 2, AC 3196, Spancross, Florigiant, Florunner, Early Bunch, Spantex Argentine, Spanhoma, New Mexico Valencia, Starr, and Tennessee Red.

Visual Estimation - The more susceptible Spanish, Valencia, and Virginia runner types increased in disease severity at a much faster rate than more resistant entries. At date 3 (Fig. 7) two distinct groups of cultivars can be recognized. The more resistant group of cultivars were visually estimated to have 40% or less leaflets infected; whereas, the more susceptible group of cultivars had more than 60% leaflets infected. NC 5 and NC 3033 have less than 20% leaflets infected and the most susceptible cultivar is Tennessee Red with 92.5% leaflets infected.

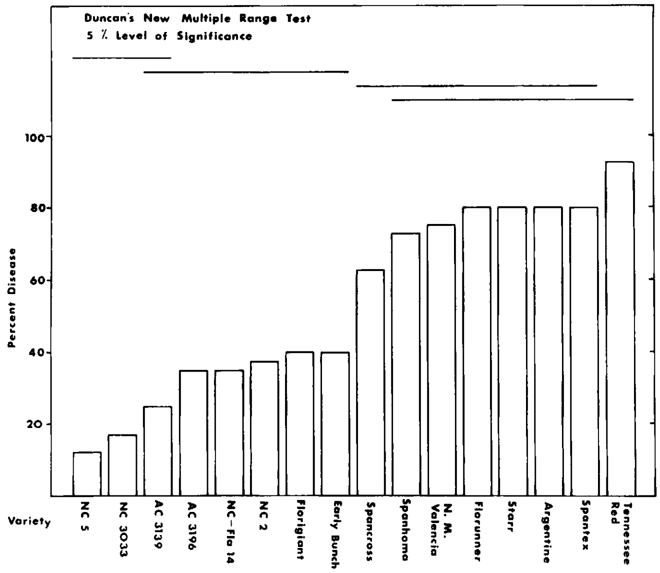

Fig. 7. Visual estimation of percent leaflets having lesions for 16 peanut entries in unsprayed plots at date 3 (9/24).

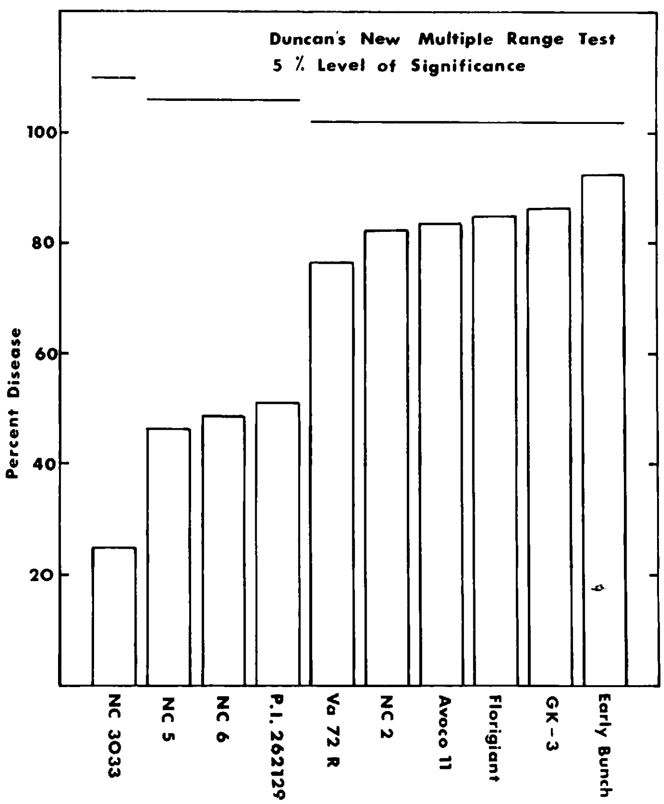

Fig. 8. Visual estimation of percent leaflets having lesions for 10 peanut entries in unsprayed plots in 1976.

Split-plot analysis in time of visual estimates of percent leaflets with lesions indicated that cultivars ranked in ascending order of susceptibility are: NC 5, NC 3033, AC 3139, NC 2, NC-Fla 14, Early Bunch, Florigiant, Spancross, Spanhoma, Florunner, New Mexico Valencia, Spantex, Starr, Argentine, and Tennessee Red.

1976 Field Results - Visual estimation showed that NC 3033 had a significantly (P = 0.05) lower incidence of leaf spot, than all other varieties tested (Fig. 8). NC 5, NC 6, and P.I. 26219 had significantly lower disease incidence than Va 72R, NC 2, Avoco II, Florigiant, GK-3, and Early Bunch.

Discussion

Greenhouse inoculation tests have shown that Spanish-type cultivars Starr and Spantex have consistently fewer lesions at all the three inoculum concentrations than Virginia-type entries NC 3033, AC 3139, and NC-Fla 14. However, in field tests Starr had more lesions at all evaluation dates and Spantex exhibited an intermediate number of lesions. In inoculation studies to compare the susceptibility of plants grown continuously in the greenhouse with greenhouse-grown plants which had been exposed to a natural environment (weathered outside) for 2 weeks before inoculation, more lesions were developed on leaves of plants grown continuously in the greenhouse. NC 3033 had most lesions among plants grown continuously in the greenhouse but was consistently the most resistant in field tests and had fewer lesions (P = 0.01) when exposed to weathering for two weeks prior to inoculation in greenhouse tests. This phenomenon suggests that environmental factors in field situations may increase resistance of certain peanut selections to Cercospora leaf spot. A similar phenomenon may explain differences between the greenhouse evaluations of Abdou et al. (1) and field studies conducted by Gibbons and Bailey (4) for cultivars that are not resistant in greenhouse tests but perform well in field production.

Gibbons and Bailey (4) correlated resistance in field-grown *Arachis* species to size of stomatal apertures. Species with stomatal apertures of less than 12 um had no lesions and species with larger mean stomatal apertures developed more lesions corresponding to the increase in size of stomatal aperture. In these tests using greenhouse grown plants, *A. cardenasii X A. chacoense* had the smallest mean stomatal aperture (10.56 μm) on lesions formed. *Arachis cardenasii* with a mean stomatal aperture of less than 12 μm also had fewer lesions. Starr which had a bigger stomatal aperture than NC 3033 did not have as many lesions as NC 3033. Among greenhouse-grown plants exposed to weathering for two weeks, *A. cardenasii X A. chacoense* and NC 3033 had smaller mean stomatal aperture on the upper surface compard to plants grown continuously in the greenhouse and also fewer lesions. However, P.I. 109839, NC-Fla 14, Spantex, and Starr had larger mean stomatal aperture on the upper surface compared to plants grown continuously in the greenhouse but had fewer lesions. Thus, while stomatal size changes do occur as the result of changes in growth environments, stomatal size does not appear to be the mechanism for increased resistance in these entries.

Visual estimation of disease in these tests showed less variation than defoliation ratio, number of lesions, and percent of leaf area diseased, respectively. Visual estimation may be subjective in nature as stated by Pedrosa (7) but if the technique is properly described and standardized it can be used for a particular disease by any researcher. Furthermore, it is a fast method. A large number of seeds of each entry must be available, however, to establish replicated plots with 100 feet of row. A more detailed evaluation using the defoliation ratio, number of lesions, and the average lesion size may be necessary for small seed lots or segregrating populations. The defoliation ratio technique was very reliable in these tests and had less variability than counting number of lesions or lesion size. Similar results were obtained by Pedrosa (7) who found that percent defoliation had less variability.

Greenhouse screening may not identify the potentially useful cultivars. Moreover, in screening for resistance it is best not to rely on only one method of evaluation. Cultivars that develop fewer lesions may not be useful if they have extensive defoliation. For instance, Spancross and Argentine varieties had only a moderate number of lesins but had much defoliation, whereas Tennessee Red and Starr had very high numbers of lesions but had less defoliation than Spancross and Argentine in these tests. A disease index calculated from number of lesions X percentage defoliation is useful because it considers both parameters. On the basis of disease index NC 3033, NC 5, and AC 3139 are clearly more resistant than the rest of the cultivars.

In this study several cultivars appear resistant according to the various methods employed. Although NC 3033 does not show resistance to leafspot in the greenhouse, it is the most resistant under all methods of evaluation in the field. It had the lowest defoliation ration, percentage defoliation due to the disease, number of lesions on each leaf, and the lowest percent leaf area diseased. We consider NC 3033, NC 5 and AC 3139 to be potentially valuable parents in breeding programs for resistance to *C. arachidicola*.

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