Field Evaluation of Peanut Genotypes for Resistance to Infection by *Aspergillus parasiticus*[1,2]

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ABSTRACT

Fourteen peanut genotypes *(Arachis hypogaea)* were evaluated for resistance to *Aspergillus parasiticus* infection in 1983 and 1984 in rain-shaded field microplots where water stress conditions were simulated, and in unshaded microplots under normal rainfall conditions. A brown color mutant of *A. parasiticus* (ATCC 24690) was grown on cracked corn and the colonized corn was spread on the soil in each microplot. Soil moisture and soil and air temperature were monitored. Infection of peanut pegs, pods, kernels and tap and fibrous roots was enhanced by low soil moisture. Infection of kernels differed among genotypes; all other tissues were found to be infected at moderate to high frequency and no genotype differences were observed.

Genotypes J-11 and Lampang were characterized as resistant to *A. parasiticus* under both dry (1983) and moist (1984) field conditions. Although percentage infection of kernels varied with genotype, ranking of genotypes reported to have drought resistance was consistent under both dry and moist field conditions. Thirty-four peanut genotypes, including those tested in field microplots, were also evaluated for dry seed resistance to *A. parasiticus* infection in the laboratory. Genotype J-11 and PI 337409 were highly resistant. Except for J-11, there was no correlation between genotype rankings for resistance to dry seed infection and resistance under field conditions.

Key Words: *Arachis hypogaea,* resistance, *Aspergillus parasiticus,* microplots, drought tolerance.

Field infection of seed by *Aspergillus flavus* Link ex Fries and subsequent aflatoxin accumulation may be associated with drought stress, overmaturity or damage to the peanut pod by pathogenic fungi, insects, mites, and possibly nematodes (5,6,8,11,19). Drought stress may increase susceptibility to fungal invasion by decreasing the moisture content of the pod and seed or by greatly lowering the physiological activity of the peanut.

A survey of peanuts in North Carolina in 1968 revealed a high correlation between preharvest *A. flavus* infection and drought stress occurring after peanuts were formed but before they were dug (6). Hill *et al.* (1983) observed that extensive colonization of peanut fruit by the *A. flavus* group of fungi was favored by hot, dry conditions; most associated microorganisms did not grow or grew only weakly because temperature or water availability or both, became limiting (8).

The microflora of the peanut pod is determined by the microflora of the surrounding soil which in turn is influenced by the makeup and structure of the soil, organic matter, moisture, temperature and other environmental, physical and biological factors (6). The mycoflora of the seed is determined by the effect of the above factors and certain inherent characteristics of each microbial species making up the peanut pod microflora (6). The activity of many of these fungi is noticeably reduced under conditions of low moisture (drought); and osmophilic fungi, such as *A. flavus* and *A. parasiticus* Speare increase rapidly to dominate the mycoflora of the soil, pod and, eventually, the seed of even undamaged pods (5).

Colonization of the peanut fruit by soilborne fungi may occur through the flowers (7,22). The infection process is probably similar to infection of corn ears through the silks (18). Griffin *et al.* (1976) suggested that *A. flavus* does not always establish a successful systemic infection following flower inoculation (7). They found that conidia of *A. flavus* in soil may germinate adjacent to developing peanut fruit and infect pods, particularly following injury of the fruit.

It is generally assumed that seed of unblemished, intact immature and mature pods are rarely invaded by *A. flavus* and other fungi before digging under most favorable growing conditions (6). Damage to the testa provides opportunities for rapid and direct invasion of the seed. If favorable temperatures and relative humidity are present at the time of damage, the fungus can grow rapidly and produce aflatoxins.

Certain peanut varieties have been identified as being less susceptible to *A. flavus* and *A. parasiticus* infection and aflatoxin contamination; however, these varieties become contaminated with aflatoxins when grown under nonadapted environmental conditions or when heavily stressed (4,6). Peanut varieties with seedcoat resistance attributed to specific morphological factors have been identified and some of these factors are: compact arrangement of seed coat cells, slower water uptake rates of seeds following drying, smaller hila with minimum of exposure of parenchyma cells and more uniform deposition of wax on the seedcoat (6). Peanut pods with resistance to common soilborne fungi have surface cells that are more compact, have a higher concentration of lignin and phenolic compounds within these cells, have a dense layer of lignified sclerenchyma cells within the central region of the pod, and have sufficient strength to resist cracking under normal handling procedures (6).

The purpose of this study was to characterize resistance in peanut to *A. parasiticus* by comparing percent of tissue infected for pegs, pods, roots and kernels from 14 genotypes grown under field conditions. Genotypes selected for testing in field microplots were also evaluated in laboratory tests for dry seed resistance so that the relationship between the two types of resistance could be compared prior to initiating a breeding strategy for developing resistant cultivars.

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Materials and Methods

Evaluation of pegs, pods, roots and kernels in microplot tests

One hundred sixty-eight field microplots at the Upper Coastal Plain Research Station near Rocky Mount, NC were used in this study. Each microplot consisted of a fiberglass cylinder (80 cm diameter and 60 cm high) buried to a depth of 45-50 cm (1). Soil type was a Shubuta gravelly sandy loam (21) and was treated with metam sodium (95L/ha rate) prior to May, 1983 to reduce variation in infection frequencies resulting from indigenous pathogen populations.

The rectangular array of microplots was divided into four equal quadrants (blocks), each containing 42 microplots. Moisture stress was induced in Blocks 1 and 4 (oriented diagonally) by covering the plots with 1 m^2 polyethylene rain shields (covers) supported by four stakes 1 m above the ground (21). Blocks 2 and 3 were not covered with rain shields and were irrigated once a week with 20 liters of water per plot or when moisture stress induced wilting of plants.

Fourteen peanut genotypes were chosen for field evaluation in the microplots (Table 1). Genotypes J-11 (Junagagh-11) and PI 337409 are reported to possess dry seed resistance to *A. flavus* infection (9,12,13,15,16,17). Genotype NC 8C is reported to have partial resistance to Cylindrocladium black rot of peanuts in North Carolina (24). Chico was used as a representative of early maturing peanut. Genotype 47-16 Senegal, 69-101 Senegal and 57-422 Senegal have drought resistance (Dr. J. C. Wynne, personal communication). Genotype US 26 was included as a susceptible check based on tests at the ICRISAT Center in India. (9). Florigiant, NC 6 and NC 7 are recommended peanut cultivars in North Carolina. Lampang is a commercial cultivar in Thailand. Chalimbana and Mani Pintar II are recommended peanut cultivars in Malawi (14).

Table 1. Mean[a] percent peanut pod, peg, tap root and fibrous root infection with *Aspergillus parasiticus* from combined rain shaded and unshaded microplot readings at Rocky Mount, NC in 1983 and 1984.

	1983 Isolation Frequency			
Genotype	Pods	Pegs	Tap roots	Fibrous roots
Chalimbana	79.3	74.0	98.0	85.3
Mani Pintar II	82.3	66.0	94.8	81.3
U.S. 26	82.8	67.8	90.8	70.8
NC 8C	85.8	54.8	90.5	80.3
69-101 Senegal	87.3	66.5	96.3	77.5
47-16 Senegal	87.8	65.8	92.3	77.5
Florigiant	88.3	65.8	91.3	80.8
NC 7	89.3	64.3	91.3	86.0
57-422 Senegal	91.0	73.8	87.0	78.8
J-11	91.5	76.3	94.3	76.8
NC 6	91.8	65.3	89.3	68.3
PI 337409	93.0	66.5	98.8	75.0
Chico	95.3	65.5	93.3	58.3
Lampang	95.3	68.0	92.0	68.8
Mean	88.63	67.17	92.86	76.11
	1984 Isolation Frequency			
47-16 Senegal	28.8	42.2	75.8	46.5
U.S. 26	36.8	23.8	52.5	26.5
PI 337409	38.5	34.8	65.0	35.0
Lampang	38.5	39.3	68.0	40.5
Mani Pintar II	40.8	39.0	72.8	59.0
69-101 Senegal	41.3	47.0	71.8	50.3
Chalimbana	42.8	35.0	71.5	43.3
Chico	44.3	25.3	69.5	28.3
Florigiant	44.5	45.8	74.0	47.3
NC 8C	45.0	34.5	78.3	52.8
57-422 Senegal	47.3	32.0	74.5	48.8
J-11	49.0	35.5	62.0	34.8
NC 7	49.8	31.3	77.0	36.5
NC 6	50.5	39.5	65.5	36.0
Mean	42.71	36.07	69.87	41.83

[a]Mean of two harvest dates (August and September). No significant differences were observed within columns.

Three-day-old germinated seed of the peanut genotypes were transplanted into microplots in the third week of May of 1983 and 1984. Each microplot contained four plants. Recommended peanut practices for North Carolina were followed. Landplaster (gypsum) (U.S. Gypsum Co., 101 S. Wacker Dr., Chicago, IL 60606) was applied (800 kg/ha) to the plants at flowering.

Inoculum for field tests was prepared by growing *A. parasiticus* (10,23) on autoclaved moistened cracked corn at 30 C for 30 days. *A. parasiticus* inoculum was broadcast over the plants when the earliest maturing peanut genotypes were in full flower. In 1983 100 cm^3 of inoculum, and in 1984 50 cm^3 of inoculum was spread over each plot.

In 1983 soil moisture was determined at 22.5 cm depth in 16 microplots (4 plots chosen randomly in each block) once each week using a Troxler Neutron Probe, Scaler-Ratemeter Model 2651 (Troxler Electronic Laboratories, Inc., Research Triangle Park, NC 27709). In 1984 a Neutron Probe, Depth Moisture Gauge Model 3222 (Troxler Electronic Laboratories, Inc., Research Triangle Park, NC 27709) was used in the same plots to record soil moisture at 20.0 cm depth once each week throughout the season (2).

In 1983, soil temperature was recorded at 5 cm depth in eight microplots (two plots in each block) using mercury thermometers. Air temperature and precipitation records were obtained from a weather station located within 200 m of the microplot sites. In 1984 soil temperature was monitored hourly at 5 cm depth using a thermocouple probe and recorded using a micrologger (Campbell Scientific CR-21 Micrologger, Campbell Scientific, Logan, UT 84321).

All plants in 4 plots per block were removed and assayed in August, September and October. Peanut pods, pegs, tap roots and fibrous roots from each plant at the first and second harvests were cut into ten 1-cm long pieces, surface sterilized in 0.5% sodium hypochlorite for 30 sec, blotted dry and pressed into malt salt agar (MSA) in petri plates (3). The MSA contained 6.0% sodium chloride and was adjusted to pH 6.0 with 4.0 N sodium hydroxide. Plates containing plant tissue were placed in plastic bags and incubated at 25 C for 8 days. The third harvest date in October was scheduled to correspond with the physiologic maturity of the peanut. Fresh pods were oven dried at 40 C for 4 days and then shelled by hand. Seed moisture was determined before plating (25). Ten kernels from each of the four plants per microplot were surface sterilized with 0.5% sodium hypochlorite for 30 sec and kernels were placed in each of two MSA petri plates. Plates were placed in plastic bags, sealed, and incubated at 25 C for 8 days.

Evaluation of Dry Seed for Resistance to Infection

All genotypes evaluated for resistance in field studies were also evaluated in laboratory tests for dry seed resistance. Seeds were tested for indigenous, internal microflora prior to laboratory inoculation tests. Twenty full sized and undamaged seed for each genotype were soaked for 1 min in sterile deionized water. The water was drained off, replaced by 0.5% sodium hypochlorite for 3 min, and seed were rinsed with three changes of sterile deionized water. Five seeds of each genotype were aseptically plated on potato dextrose agar (PDA) in 9 cm diameter petri plates. Plates were placed in plastic bags and incubated at 25 C for up to eight days. Identification of the seed contaminants was made starting on the third and continued through the 8th day after plating. Identification of mycroflora was based on fungal spores and mycelium. Only the gram stain was used for bacterial characterization.

A color mutant of *A. parasiticus* (ATCC 24690) was used for laboratory evaluation of the genotypes. Inoculum spore suspensions were prepared by flooding 10-day-old cultures, grown on PDA at 25 C, with sterile deionized water containing 5% (v/v) polyoxyethylene (22) sorbitan monooleate (Tween 80) (Fisher Scientific Co., Chemical Manufacturing Division, Fair Lawn, NJ 07410). The spore suspension was adjusted to 4.0 x 10^6 conidia per mL.

One hundred and thirty-five seeds of each genotype were selected for laboratory evaluation. Seed moisture content of the peanuts to be tested was determined by the Proximate Analysis procedure (25). Sound seed of uniform size and shape with intact testa were surface-sterilized by soaking in 0.1% mercuric chloride for 2 min, followed by four rinses in sterile deionized water. Seed were hydrated by soaking in sterile deionized water for 15 min.

Seed of each genotype were aseptically placed in sterile 9 cm diameter petri plates (three replicate plates per genotype, 45 seeds per plate). One mL of the spore suspension was added to each petri plate and distributed by gently swirling. The petri plates were incubated in a moist chamber at 25 C for 8 days. Percentage of infected seed and amount of fungal sporulation on the seed was recorded on day 8 (0 = no sporulation; 1 = sparse sporulation; 2 = moderate sporulation; 3 = dense sporulation) (9). Seed with no apparent surface sporulation were split open and examined for concealed infection. Tests were repeated twice. The General Linear Models procedure (20) was used for

analysis of variance and Fisher's least significant differences were calculated. Differences referred to in the text were significant to the 5% or lower level of probability.

Results

Field Tests

In rain shaded and unshaded microplots at Rocky Mount, NC in 1983, a mean of 4.0% of Lampang kernels were infected with *A. parasiticus* (Fig. 1). Genotypes J-11, Mani Pintar II, and 47-16 Senegal had *A. parasiticus* infection ranging from 7.0-9.0%. The remainder of the genotypes had kernel infection ranging from 13.0-28.0% (Fig. 1).

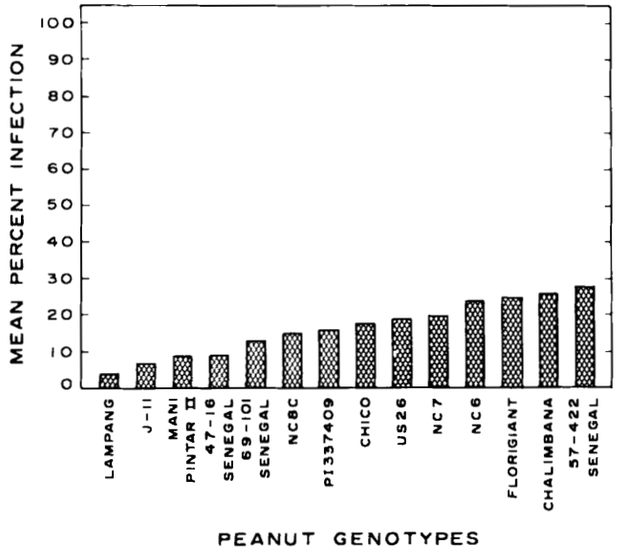

Fig. 1. Percent *Aspergillus parasiticus* kernel infection for 14 peanut genotypes tested in rain shaded and unshaded microplots at Rocky Mount, NC in 1983. The value for each genotype is a mean of four plots (plots rain shaded microplots and two unshaded microplots).

Genotype J-11 had no kernels infected with *A. parasiticus* in the rain shaded microplots but had 13.0% kernel infection in the unshaded microplots (Fig. 2). Mean kernel infection of Lampang was low, 3.0% and 4.0%, in rain shaded and unshaded microplots, respectively. Genotypes 47-16 Senegal, Mani Pintar II, 69-101 Senegal, NC 8C and PI 3347409 had infection percentages of the kernels ranging from 11.0-18.0 and 7.0-15.0 in rain shaded and unshaded microplots, respectively (Fig. 2). Percent infection of kernels from genotypes US 26, NC 7, Chico, Florigiant, 57-422 Senegal, NC 6 and Chamlimbana ranged from 27.0-48.0 and from 2.0-25.0 in rain shaded and unshaded microplots, respectively (Fig. 2).

Aspergillus parasiticus mycelium was observed growing from fresh pods, pegs and tap and fibrous roots at all harvest dates. A high percent (80-100%) of pods of the 14 genotypes were infected with *A. parasiticus* in field microplots in 1983 (Table 1; Fig. 3). Recovery of *A. parasiticus* was highest from tap roots and pod pieces. Tissue such as pegs and fibrous roots had lower ($P=0.05$) isolation frequency than lignified peanut tissue such as tap roots and pods (Table 1). Percent of tissues infected was lower ($P=0.05$) in unshaded plots at the first sampling date but was not different at subsequent sampling dates in either 1983 or 1984.

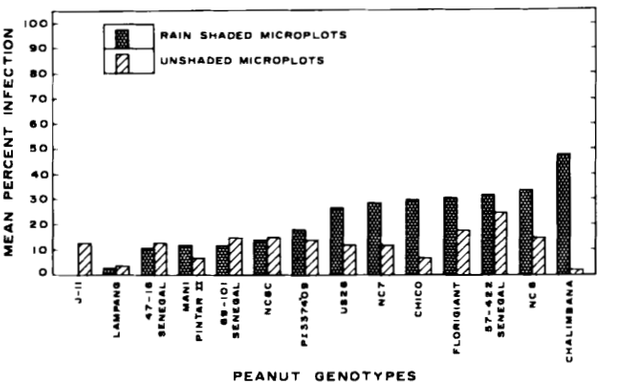

Fig. 2. Percent *Aspergillus parasiticus* kernel infection for 14 peanut genotypes grown in rain shaded microplots and unshaded microplots at Rocky Mount, NC in 1983. Value for Fisher's LSD ($P=0.05$) was 15.5.

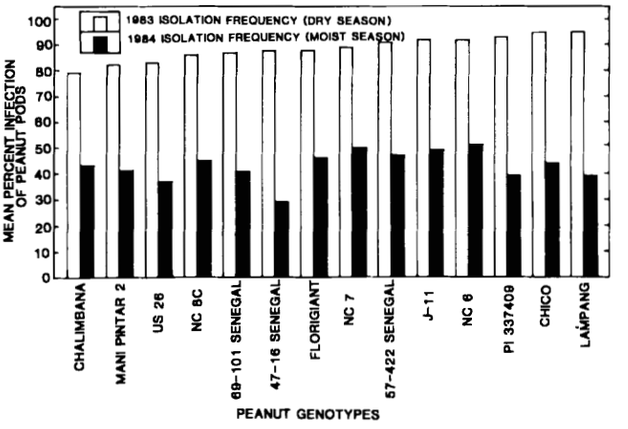

Fig. 3. Percent *Aspergillus parasiticus* peanut pod infection for samples run in August (first harvest) and September (second harvest) from Rocky Mount, NC in 1983 and 1984. A value for each genotype is a mean of sixteen plants, four plants came from each microplot in each replicate. Infection percentages differed ($P=0.05$) between years but did not differ among genotypes within years.

In 1984 mature kernels of Florigiant, J-11, Lampang and 47-16 Senegal had no *A. parasiticus* infection. The remaining genotypes had variable infection ranging from 2.0-10.0% when data were combined for rain shaded and unshaded microplots.

Detection of *A. parasiticus* in peanut tissues was lower ($P=0.05$) for all 14 genotypes in 1984 than in 1983. Lower infection percentages in 1984 was correlated with higher rainfall than recorded in 1983. (Fig. 4). In 1983 the rain covers maintained low soil moisture in the shaded microplots. In 1984 soil moisture increased in the unshaded microplots from mid-July to the beginning of August and from the end of September to October. Soil moisture increased to a lesser extent in rain shaded plots from July - August but was similar to unshaded plots again in mid-September (Fig. 5). Soil temperature was similar and ranged from 15-35 C in

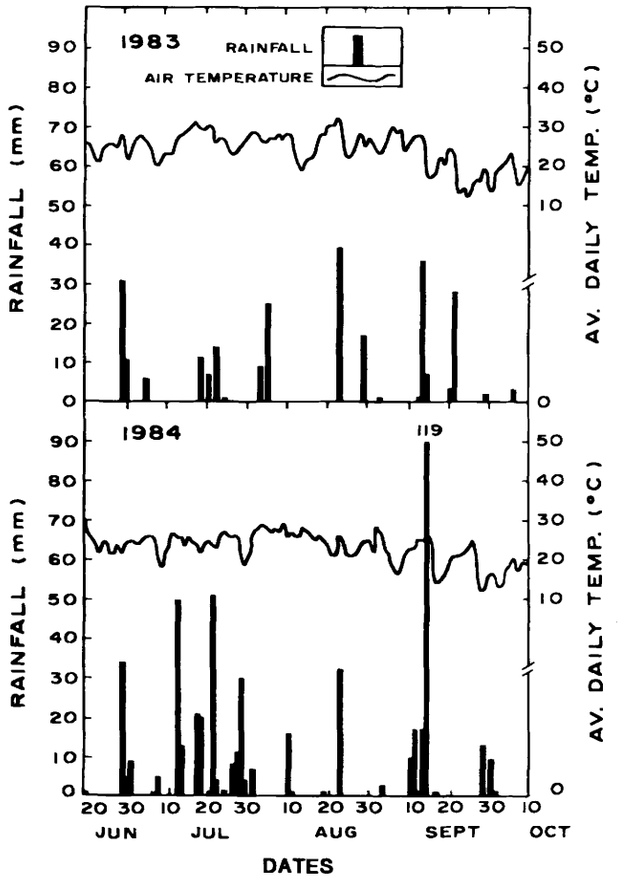

Fig. 4. Rainfall in millimeters and average daily air temperature in C at Rocky Mount, NC in 1983 and 1984. Average daily air temperature is mean of maximum and minimum daily air temperatures.

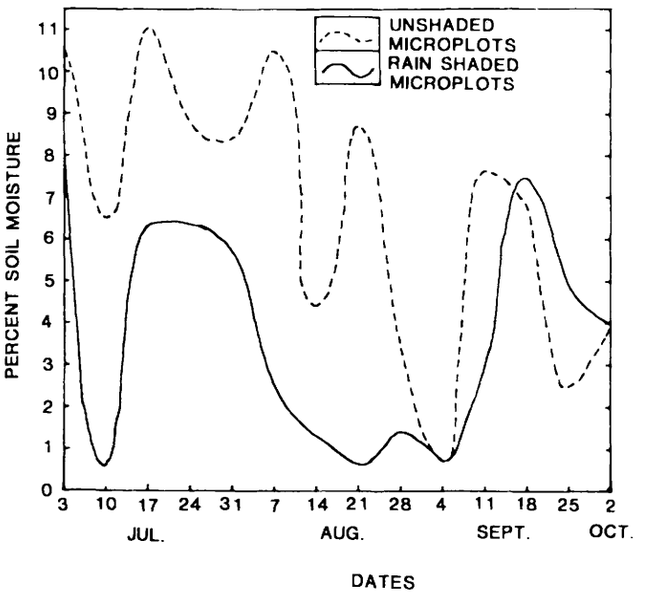

Fig. 5. Soil moisture in unshaded microplots and rain shaded microplots at Rocky Mount, NC in 1984. Moisture values are expressed as a percentage of total soil weight as determined by a Neutron Probe depth moisture gauge. Each moisture value is a mean of eight readings from two replicates, each replicate with four readings. Soil moisture readings were recorded between 8:00 a.m. and 12:00 noon from July to end of August, and between 2:00 p.m. and 6:00 p.m. from end of August to harvest time for peanuts in October 1984.

both 1983 and 1984. Placement of rain shields over plots had minor effect on soil temperature in both years. For example, soil temperature at 11 AM in unshaded and rain shaded microplots in August, 1983 averaged 29.5 and 28.1 C, respectively (Table 2). Average maximum and minimum soil temperatures for unshaded and rain shaded microplots in August, 1984 were 30.5-24.0 and 30.4-23.4, respectively. In 1984 infection of the peanut genotypes by *A. parasiticus* was low, but more infection occurred in the rain shaded than unshaded microplots.

Table 2. Soil temperature at 5.0 centimeter depth in microplots at Rocky Mount, NC in 1983.

Date (Replicate)		Unshaded microplots[a]	Shaded microplots[a]
July	25	29.5	29.4
	30	29.1	28.4
August	01	30.5	29.8
	06	31.8	29.8
	08	28.9	27.8
	13	30.9	27.9
	15	26.6	24.6
	20	31.0	29.3
	22	29.0	29.0
	27	26.0	25.3
	29	30.5	29.3
September	03	26.5	25.4
	06	36.5	32.0
	11	26.5	25.8
	12	29.3	28.1
	17	18.1	18.0
	20	29.0	27.3
	27	23.3	22.5
October	04	27.5	24.8
	10	18.1	18.4
Mean		27.9	26.7

[a] Mean of four plots. Temperature was determined with mercury thermometers at approximately 11:AM each day.

Laboratory Tests

Genotype PI 337409 had the lowest percent infection and sporulation ratings (Table 3). Genotype J-11 also had low infection and would be considered resistant

Table 3. Percent of peanut seed infected with *Aspergillus parasiticus* after 8 days incubation at 25 C and sporulation scores.

Peanut Genotype	Resistance Rating[a]	Percent seed infected[a]	Sporulation score[b]
Chico	HS	77.0	3.0
U.S. 26	HS	64.0	3.0
Chalimbana	HS	60.0	1.7
Mani Pintar II	HS	59.0	2.7
69-101 Senegal	S	46.0	2.0
NC 6	S	42.0	2.0
47-16 Senegal	S	37.0	1.0
57-422 Senegal	S	37.0	1.0
Lampang	S	32.0	1.0
Florigiant	S	31.1	1.7
NC 7	MR	30.0	1.7
NC 8C	MR	29.0	2.0
J-11	R	15.0	1.0
PI 337409	R	4.5	1.0
LSD		11.2	0.6

[a]Values are average of three separate tests using 135 seed per test. Ratings were based on recommendations by the International Crops Research Institute for the Semi Arid Tropics (ICRISAT) for percent seed infected: 0-15 = resistant, 16-30 = moderately resistant, 31-50 = susceptible and 51-100 = highly susceptible.

[b]Sporulation scores were based on 0-3 scale recommended by the International Crops Research Institute for the Semi Arid Tropics (ICRISAT).

according to the ICRISAT (9) classification. Genotypes NC 8C and NC 7 would be classified as moderately resistant while all other genotypes were either classified as susceptible or highly susceptible. The large seeded confectionery peanut Chalimbana and the oilseed genotype Mani Pintar II were highly susceptible to *A. parasiticus* in these tests.

Discussion

Although the "wild type" of *Aspergillus* spp. was occasionally recovered from tissues, the brown color mutant (ATCC 24690) isolate of *A. parasiticus* used in these studies was the predominant *Aspergillus* spp. recovered from all tissues during isolations (10,22). The high level of inoculum applied to microplots, however, greatly exceeded the density of naturally occurring populations of *Aspergillus* spp. in North Carolina peanut fields. The density of inoculum was maintained at a high level to ensure a rigorous evaluation of genotype resistance under field conditions.

Prolonged periods of drought and conducive soil temperatures during the 1983 growing season in North Carolina resulted in conditions conducive for evaluating peanut genotypes for resistance to infection by *A. parasiticus*. Genotypes Lampang and J-11 appeared to possess resistance to *A. parasiticus* infection of mature kernels in field tests under both moderate moisture (unshaded plots) and dry (rain shaded plots) growing conditions during 1983 and 1984. Records of soil temperature in 1983 and 1984 indicated that slightly higher temperatures (1.2 C) occurred in the unshaded plots which also had less drought duration than rain shaded plots (Table 2; Fig. 4 and 5). Growing conditions in North Carolina were ideal for peanut in 1984, i.e. rainfall was adequate for plant growth and temperatures were moderate throughout the season. Under these conditions infection by *A. parasiticus* was reduced in all genotypes tested. For example, 81 and 48% of pegs, pods and root tissues sampled were infected with *A. parasiticus* in 1983 and 1984, respectively.

No visible damage within infected tissue was observed during either year of this study. Screening of peanut genotypes for resistance to *Aspergillus* spp. on the basis of frequency of recovery of the pathogen from vegetative pegs, pods and root tissues was not adequate. No correlation was observed between percent vegetative tissue infected by *A. parasiticus* during the growing season and either resistance to infection on mature kernels in the field study, or dry seed resistance in laboratory tests.

The observation in 1983 that almost all subterranean tissues (pegs, pods, fibrous and tap roots) were infected with *A. parasiticus* by the end of the growing season (in all 14 genotypes) suggests that the fungus was present within vegetative tissues but was restricted in its ability to infect the kernels of Lampang and J-11. Under less stressful moisture conditions occuring in 1984, the resistance of Lampang and J-11 kernels to infection by *A. parasiticus* was even more pronounced. Genotype J-11 is a commercial cultivar in India and is reported to be resistant to pod-rotting pathogens (13).

Although the genotypes 47-16 Senegal, Mani Pintar II, 69-101 Senegal, NC 8C and PI 337409 were considered only moderately susceptible to *A. parasiticus*, infection of these genotypes was not enhanced by drought stress. These genotypes may possess environmental tolerance factors which could be used to complement disease resistance components.

Several studies have demonstrated that peanut genotypes can be identified which possess post-harvest kernel resistance to infection by *Aspergillus flavus* group fungi when seeds were dried and subsequently inoculated (12, 16). Unfortunately, studies have also shown that a poor correlation exists between dry seed resistance (post-harvest infection) and field resistance (preharvest infection) when genotypes are grown in field sites under conditions conducive for *Aspergillus* infection (4,6). Several genotypes which appeared to possess moderate levels of dry seed resistance in this study were also highly susceptible under field conditions. However, genotype J-11 was found in these tests to be resistant to both preharvest and post-harvest infection by *A. parasiticus*. Lampang was consistently rated as susceptible in dry seed screening tests but was identified as highly resistant to preharvest infection in field tests for both 1983 and 1984. Genotype PI 337409 showed high resistance to *A. parasiticus* in dry seed screening tests but was susceptible in field tests for kernel infection.

Additional studies are needed to determine if the resistance detected in J-11 and Lampang will protect kernels from infection by populations of *Aspergillus* occuring in other peanut areas of the world. If the resistance is effective in a wide area, breeding work should be initiated to determine heritability of resistance so that cultivars can be improved wherever infection by *Aspergillus* spp. is a problem in peanut production. Care should be taken in additional screening and evaluations to ensure the stability of the resistance response over differing levels of environmental stress, i.e. drought and extreme temperatures.

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