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Seed Tolerance in Peanuts (*Arachis hypogaea* L.) to Members of the *Aspergillus flavus* Group of Fungi[1]

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ABSTRACT

An assay of cured, hand-shelled seeds of various peanut genotypes for tolerance to members of the *Aspergillus flavus* group of fungi has been performed in Florida for the years 1971-1974. The assay involved exposing peanut seed at 20-30% moisture to conidia of *A. parasiticus* or *A. flavus* in petri plates and incubating at 25 C. After 1 week, the percentage of the seeds with sporulating colonies of the test fungus was determined. Typically, individual lines or cultivars were evaluated on the basis of the average of three plates. However, second or third assays of the same seed lots were done on 45 occasions during the 4 year period. More than 95% of these repeated assays yielded data similar to those from the original assay. However, different seed lots of the same line also were assayed and did not always yield similar results unless the dates of digging, methods of curing and location of the plantings were the same. Some shifts in susceptibility were quite extreme. One lot of stackpole cured 'Altika' resulted in 12% colonized seeds in the assay but 77% of a windrow-cured seed lot, dug on the same day from the same plot had colonies of the test fungi. No particular change in the harvesting procedure was consistently associated with increases or decreases in apparent susceptibility. Based on tests of all seed lots of 15 commonly grown cultivars during the years 1971-1974. 'Florunner' was the most tolerant cultivar and 'Tifspan' was the most susceptible.

Keywords: Aflatoxin, mycotoxin, disease resistance.

Resistance in peanuts (*Arachis hypogaea* L.) to penetration of the seeds by *Aspergillus flavus* Link or *Aspergillus parasiticus* Speare has been reported (5). This resistance has been based on an assay system where cured, hand-shelled peanut seeds at 5-7% moisture were hydrated to 30% moisture and exposed to conidia of *A. flavus* or *A. parasiticus* in petri plates. After incubation, the percentages of seeds with sporulating colonies of the test fungus were calculated. Classification of different genotypes as resistant (= tolerant) was based on a failure of the test fungus to colonize the seed. Two plant introductions (P. I. 's) were reported to be resistant to penetration by *A. flavus*. (5). Only 5% of the seeds of P. I. 337394 had colonies in five evaluations performed in 4 years, whereas 9% of the seeds of P.I. 337409 were colonized. Cultivars, 'Argentine', Florunner, and 'Wilco I', were used for controls in 2 of the 4 years and averaged 34, 39 and 30% diseased seed, respectively. Although more seeds of Florunner were colonized in these tests than of the other two cultivars, the range of test analyses reported for Florunner was 19 to 66%. This seemed to be an unusually large range which would indicate that resistance to colonization may be a variable characteristic at least with seeds of some peanut lines or cultivars.

A similar assay has been used on seeds of peanut breeding lines and cultivars in Florida during the years 1971-1975. The purpose of the testing was three-fold. First, the assay system itself was being studied to determine if it could accurately evaluate the resistance of peanut seeds to penetration by *A flavus* group fungi (Aff). Second, an accurate estimation of the tolerance of Florida-grown cultivars and breeding lines was needed. Third, the variability of the characteristic itself needed evaluation.

Materials and Methods

Cultural Practices. Most of the various peanut genotypes tested were planted in adjacent or nearby 1.8 X 6.1 m plots within a 2-3 day period unless otherwise indicated. Standard cultural practices were used with all plants. When near or at optimum maturity, the plants were dug with a digger-shaker-inverter and either cured for 2-3 days in a windrow or 4-6 weeks on a stackpole. A CeCoCo plot picker was used to separate the cured pods from the vines. Pods from the windrowed plants were at ca. 20% moisture when picked and then were dried to ca. 10% moisture in a conventional farm-wagon dryer. Pods from the stacked plants did not require additional drying. All pods were handshelled. Damaged, discolored and shriveled seeds were discarded.

The assay system. At least 60 days following curing, the seeds from hand-shelled pods were weighed into three replicates of ca. 15 g each for 1971-1973 and 20 g for 1974. Each replicate was immersed in distilled H_2O for 15 minutes and then placed into a preweighed petri plate. Additional distilled H_2O was added so that the net weight for each plate, plus the weight of the inoculum to be added would equal the enclosed peanut seed at 30% moisture in 1971, 25% moisture in 1972 and 20% moisture in 1973 and 1974. The inoculum was composed of conidia from 2-3 week old cultures of *A. parasiticus* (N.R.R.L. #2999) growing on Czapek's agar. The concentration of the suspensions used was ca. 6×10^6 conidia/ml 5% (1971 and 1972) or 0.5% (1973 and 1974) Tween 20 in distilled H_2O. One ml of the inoculum in 1971-73 and 1/2 ml in 1974 was added to each plate. The plates were closed, swirled gently to distribute the inoculum and stored for 1 week at 25 C. The percentage of seeds colonized by the *A.* flavus group was based on the number of seeds with one or more sporulating colonies of that group of fungi which included those arising from natural infections. All percentage data were converted by the arc sin transformation unless such conversion was not necessary for statistical analysis. Comparisons of percentages of seeds with colonies for the various genotypes were made by the Duncan's New Multiple Range Test only after the presence of significant differences had been established by the one-way analysis of variance test. Only the latter test was used for comparisons involving just two genotypes.

The results of different assays of a particular genotype which were performed in the same year were compared statistically. The number of compared results for the different combinations of harvest date, curing method, production location and seedlot are given in Table 1. Also included in this report were the 4-year assay averages for six Spanish type cultivars, five Virginia type cultivars, four runner-type cultivars, and three resistant

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genotypes (PI337394 F, PI337409 and UF 71513.) The names of the cultivars and the years assayed are given in the results section. The values for each cultivar represent the average of all assays performed with seed of that cultivar in a given year.

Results

Sources of variation. The results from repeated tests of the same lot of seed or from different lots of seed of the same genotype that had received identical handling were remarkably similar (Table 1). Only two of the 45 one-way analyses of variance comparisons were different (P=.05). However, if one or more of the three handling or production factors differed for two lots of seed, the results from the assay of those two lots were different ca. 50% of the time. No particular change in the curing technique, date of harvest or production location were consistently associated with increases in susceptibility.

Table 1. Comparisons[a] of susceptibility[b] to *Aspergillus flavus* group fungi of different samples of peanut seed of the same genotypes from various combinations (different of the same) of harvest dates, curing methods, seed lots and production location.

Harvest date	Curing method	Production location	Seed lot	Comparisons No.	Comparisons Significantly different %
DIFFERENT	SAME	DIFFERENT	DIFFERENT	11	45
SAME	DIFFERENT	SAME	DIFFERENT	6	50
DIFFERENT	SAME	SAME	DIFFERENT	14	57
DIFFERENT	DIFFERENT	SAME	DIFFERENT	14	43
SAME	SAME	SAME	DIFFERENT	6	0
SAME	SAME	SAME	SAME	45	4

[a] Each comparison involved two samples of the same genotype compared by the one-way analysis of variance method at P=.05.

[b] Based on the colonization by A. flavus group fungi of moistened, hand-shelled peanut seed following inoculation with conidia of that fungus.

Table 2. Percentage of seeds of different lots of three peanut cultivars with colonies of *Aspergillus flavus* group fungi following inoculation[a] and incubation for 7 days at 25 C.

Cultivar	Harvest date[b]	Curing method[c]	Seeds with colonies %
Florunner	9/4/73	Windrow	56
	9/4/73	"	53
	8/27/73	"	22
	9/17/73	"	24
	9/7/71	Stackpole	52
	9/14/71	"	16
Florigiant	9/16/74	Windrow	23
	9/16/74	Stackpole	71
Altika	9/30/74	Windrow	77
	9/30/74	Stackpole	12

[a] Addition of 0.5 ml of 5 X 10^6 conidia/ml of A. parasiticus to each of three plates containing 15-20 g of seed. Final moisture content of seed was 30% in 1971 and 20% in 1973 and 1974.

[b] Harvest date corresponds to the date when the peanut plants were dug.

[c] Plants cured 2-3 days in an inverted windrow followed by machine-picking and forced warm-air (37 C) drying, or on a stackpole for 6-8 weeks.

The magnitude of the differences of assay results for two lots of seed of the same line that have been produced or handled differently is illustrated in Table 2. The greatest difference illustrated came from two lots of Altika seed harvested from plants grown in the same plot but which had been cured differently. Seeds from the stackpole-cured plants were relatively tolerant while those from windrow-cured plants were quite susceptible. During the same growing season, windrow-cured Florigiant seed were relatively tolerant as compared to those from plants cured on a stackpole. The results from assays of two lots of Florunner seed that had been harvested on the same date were different from those of one lot dug ca. one week earlier or one dug ca. two weeks later. In a different comparison of two lots of Florunner seed, a one week earlier digging date was associated with a 4-fold increase in the percentage of inoculated seeds with colonies of the test fungus as compared with that from the later digging date.

Tolerance of seeds of different genotypes to members of the A. flavus group. The average percentage of seeds with colonies of the test fungus following inoculation of seeds of 18 genotypes for the years 1971-1974 are presented in Table 3. The 4-year average is the average of all tests. Extensive year to year variation occurred in the test results for certain genotypes. However, in all instances where large shifts occurred, the higher or lower value was from just one assay. Seeds of all Spanish cultivars were heavily colonized by Aff in 1972 assays but except for Tifspan were intermediate in 1973 and 1974.

Table. 3. Percent of cured, hand-shelled and then moistened seed of various peanut genotypes with colonies of *Aspergillus flavus* group of fungi at 7 days following inculation[a] with conidia and incubation at 25 C during the years 1971-1974.

Genotype	Percent of seeds with colonies[b] 1971	1972	1973	1974	Average
Tifspan	74	96	82	62	79
Spancross	75	93	44	39	63
Comet	75	88	52	38	63
Starr	--	92	50	38	60
Argentine	--	85	37	13	45
Spanhoma	--	91	43	34	56
Florigiant	71	57*	55*	41**	56
Altika	43***	19***	24	45*	33
NC-Fla 14	66	71*	75**	42**	64
NC-17	90	--	48	12	50
NC-5	69	--	--	42	56
Florunner	34*	22**	39***	25*	30
Early Runner	26	49	39	27	35
Dixie Runner	14	35	64	14	32
Florispan	62	48	--	10	40
337394 F	-	27*	21	10	25
337409	-	24**	21	13	21
UF 71513	4	13	18**	4***	10

[a] 1.0 ml in 1971 and 0.5 ml in 1973-1974 of a 6 X 10^6 conidia/ml suspension of A. parasiticus were added to each of 3 plates per treatment.

[b] Without asterisk = average percentage of seeds in three plates with visible sporulating colonies of A. parasiticus following one week at 25 C; with one asterisk two tests of three plates were averaged; with two asterisks three tests and three asterisks four tests.

The 4-year average for all Spanish types was 62%, for all Virginia types 51%, and for all runner types

34%. The difference between the averages for the Spanish or Virginia types and the runner types was significant at P=.05. However, the difference between the Spanish and Virginia types was not significant. Seeds of the three "tolerant" breeding lines were not colonized as much as were those of the 15 cultivars. However, the differences between Florunner, the lowest ranking cultivar (=most tolerant) and the two P.I. lines were not significant at P=0.05. On the other hand, the 4-yr. test average for UF 71513 seeds was significantly lower, P=0.01, than those of the two P.I. lines and Florunner, respectively.

Discussion

Tolerance in peanut seed to Aff was an extremely variable characteristic in the tests reported here. Some of the lines that were tolerant in one assay were susceptible in another. Shifts in the susceptibility of the different genotypes studied were not predictable. Changes in the harvest date, production location or curing method were associated with significant changes in susceptibility about 50% of the time.

Tolerance to Aff however, did seem to be a varietal characteristic. A Spanish type, Tifspan, was consistently one of the more susceptible cultivars in each year's test, and at the end of the 4-year period was the most susceptible cultivar tested. Florunner was the most tolerant cultivar, but none of the Florunner seed lots ranked as the most tolerant in any single year. Altika, the most tolerant of the large-seeded Virginia types, was statistically as tolerant as Florunner but was much more variable. Although the most tolerant lots of Altika averaged 12, 12 and 13% colonization as compared to 13, 16 and 19% for Florunner. Other lots of Altika yielded 79, 77 and 60% as opposed to 52, 52 and 56% for Florunner. Thus Florunner proved on the average, to be as tolerant as Altika.

Although tolerance to Aff seems to be a varietal character, it is, unfortunately, too variable to be used readily in peanut breeding programs. For example, the resistance of individual plants within a population cannot be measured with certainty nor can that of a small population of plants since several seed lots representing different harvesting situations are needed for an accurate determination of tolerance.

The variability of tolerance to Aff in peanuts seems to be associated with the curing environment. Changes in the latter could be assumed to have occurred in every comparison where the susceptibility differed. The curing environment includes climatic and biotic components. The climatic component involves temperature, rainfall, dew, etc., while the biotic component involves the microbiological community present in the soil and in the air. Because of the large number of possible combinations and sequences of these components, the curing environment involving two groups of plants of the same line would be similar only if the digging date, curing method, and the field location were similar for each group.

There are several possible explanations for the variation in tolerance to Aff among seed lots of a given genotype. One possibility is that some lots of seed had heavy natural infestations of Aff. In these assays no attempt was made to distinguish between colonies arising from latent infections of the seed and those arising from the inoculation. The genotypes were tested simply for tolerance to these fungi whether they came from inoculum in the test or from latent infections. Most genotypes tested came from the same field in any particular year, and thus should have been exposed to the same amount of natural inoculum. Of course, seeds of lines that were dug on different dates, cured in different ways or planted in different locations or years may have been exposed to different levels of naturally occurring Aff. However, enough uninoculated seeds have been observed during the course of these assays and in other tests to assure us that latent infections cannot be the only cause for variation in susceptibility.

Another possibility was that seeds from susceptible seed lots were damaged in some manner. Mechanical damage to seed has been related to increased susceptibility (2). However, the seeds used in these assays were always hand-shelled and carefully examined for blemishes or damaged testa. Those with any signs of damage were discarded before being inoculated. In addition, those that split open during the incubation period were not counted when the percentage of seeds with colonies was determined. While grossly damaged seeds were eliminated before inoculation or during the counting of the seeds with colonies, minute cracks may have excaped detection. This sort of damage would have predisposed such seeds to infection as pin prick wounds and carborundum abrasions have been reported to be sites for invasion on otherwise tolerant seeds (4).

A third explanation for variation in resistance to Aff involves the microflora that are naturally present in the peanut testa (2). Some of these fungi can antagonize while others can enhance the growth of *A. flavus* (1, 6). A tolerance of intact pods to Aff of certain peanut lines has been suggested to be caused by the presence of antagonistic fungi (3). This explanation would fit all of the situations reported here where two seed lots of the same line differed in susceptibility as any change in the curing environment would probably affect the number and type of fungi present not only in the pod but also in the seeds.

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