TABLE I

Effect of β-Ionone on Growth and Aflatoxin B_1 Accumulation in Shake Liquid Cultures of *Aspergillus parasiticus*

β-Ionone added (μL/L)	Dry wt (g)[b]	Aflatoxin B_1 (ng/mL)[b]
0	1.92	9528
10	1.93	10200
50	1.50	11240
100	1.23	2496
200	1.29	1568
250	1.02	1368
300	0.79	176
400	0.84	280
500	0.71	16
1000	0.74	2

[a]Culture flasks contained 100 mL medium.
[b]Numbers are averages from 4 flasks/treatment.

of growth were noticeable beginning at 50 μL/L of medium. Concentrations above 250 μL/L had little further effect on growth. The primary effect of β-ionone on growth in shake culture seemed to be on the rate of growth; however, sporulation of *A. parasiticus* in shake or submerged culture is inhibited and was not measured. Concentrations of 100 μL and above of β-ionone/L inhibited aflatoxin accumulations whereas 10 and 50 μL/L slightly stimulated aflatoxin production. This shows that the ability of the toxigenic strain of *A. parasiticus* to produce aflatoxin is not necessarily linked to growth; but aflatoxin synthesis may be positively correlated with the asexual reproductive process.

Other investigations on the effects of β-ionone on fungi have not been concerned with asexual morphogenesis of the fungi imperfecti or aflatoxin production. Carotenogenesis is stimulated by β-ionone in *Phycomyces blakesleeanus* (4) and *Blakeslea trispora* (5) Carotenogenesis is inhibited by β-ionone in *Verticillium agaricinum* (6) *Rhodotorula rubra* (7) and *Actinomyces chrysomallus* var. *carotenoides* (8).

The effect of β-ionone on carotene synthesis in the *A. flavus* growth should be investigated. The cause of the effects of β-ionone on growth and aflatoxin production in the *A. flavus* group is unknown and certainly should be investigated.

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Reducing Aflatoxin Contamination in Peanut Genotypes by Selection and Breeding

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ABSTRACT

The potential for developing agronomically suitable cultivars using peanut genotypes that exhibit resistance to seed colonization by aflatoxin-producing strains of *Aspergillus* species is explored. Some factors found to be associated with the nature of resistance to seed colonization by the toxin-producing fungi are cell structure, cell arrangement, permeability, waxy surface, tannin content and amino acid components of the seed testae. The practical implications of developing resistant cultivars are presented in data for yield, value and seed quality for 6 advanced peanut lines that were developed by breeding and selection from crosses.

INTRODUCTION

Aflatoxin contamination of peanuts is a vital concern to the peanut industry. Prevention, removal and inactivation are 3 approaches to coping with the aflatoxin problem in peanuts. However, the best approach to controlling the aflatoxin contamination is to develop cultivars that are resistant to toxin-producing strains of *Aspergillus*. Several workers have reported that certain peanut genotypes are resistant to seed colonization by aflatoxin-producing strains of *Aspergillus* spp (1-4) or to the production of aflatoxin in the seed following contamination by the fungus (5-7). In a study to determine the percentage of susceptible samples from 28 F_3 generation families from crosses between resistant and susceptible genotypes when 30-100% seed was infected after laboratory inoculation, it was concluded that the genetic and environmental influences were interacting to produce variation in seed colonization by the *Aspergillus* fungus (8). This variation allows the breeder to make progress in selecting for resistance in the segregating population of crosses. Further studies in the F_2 generation of seed from plants of crosses between peanut genotypes varying in *A. flavus* seed susceptibility levels gave evidence that crosses between certain genotypes could produce genetic variation greater than that attributed to additive genetic effect. Therefore, selection for resistance from peanut crosses is possible.

Many environmental and biological conditions in and around the peanut fruit may influence the *A. flavus* invasion of the peanut fruit. The incidence of *A. flavus* invasion is influenced by the amount and population of *A. flavus* in the soil (9), the type of plant residue in the soil (10), and crop rotation practices (11,12). Several workers have noted that drought stress before digging peanuts is associated with aflatoxin contamination (13-17). Diener and Davis (18) presented evidence that peanut pods are most vulnerable to infection by *A. flavus* when seed moisture is between 12 and 30%. Such seed moisture in combination with other

conditions conducive to growth and invasion of the fungus exist during drought periods prior to harvest and during windrow drying after digging. Also, there is evidence of greater incidence of aflatoxin contamination among overmature seed and seed from dead plants (19-24). Damage to peanut fruit by organisms and from mechanical injury before and after harvest increases the incidence of *A. flavus* invasion (25-32). During the period of fruit development, there are competitive and antagonistic interactions between *A. flavus* and other microflora. Although there is evidence that *A. flavus* is inhibited by certain microorganisms (33-35), this practical control method needs considerable study.

POTENTIAL FOR BREEDING FOR RESISTANCE

Inherent ability of certain peanut genotypes to resist or reduce *A. flavus* invasion, aflatoxin contamination of seed or pods, or both, has been reported. In separate reports in India in 1967, Kulkarni et al. (36) and Suryanarayana-Rao and Tulpule (37) reported a peanut cultivar with resistance to aflatoxin production by *A. flavus*. Accessions of these genotypes were obtained and evaluated by Doupnik and Bell (38) and Mixon and Rogers (39), but the resistance was not substantiated. Further studies were made by Nagarajan and Bhat (40) using the US 26 cultivar previously reported to be resistant by Suryanarayana-Rao and Tulpule (41), and an Indian cultivar, TMV-2. They found that 5 isolates of *Aspergillus* spp produced aflatoxin on laboratory-inoculated seed of US 26, but at a lower level than that on TMV-2. Nagarajan and Bhat (42) discussed the possibility that interaction between genotypes and *A. flavus* isolates may cause variation in the infection potential and the subsequent elaboration of aflatoxin.

Priyadarshini and Tulpule (43) found a correlation of the glucosamine content with *A. parasiticus* fungal growth; but no correlation was found with aflatoxin content. They concluded that peanut genotypes support different levels of fungal growth, which may or may not be associated with aflatoxin production.

Mixon and Rogers (44) screened and reported a range of colonization among seed from a large number of peanut genotypes by the fungal isolates *A. parasiticus* NRRL 2999 and *A. flavus* NRRL A 13794. By using this procedure, the seed colonization of 2 accessions (PI 337394 and PI 337409) was less than colonization of many genotypes. These genotypes were released as a source of germplasm and used as a genetic source of *A. flavus* resistance (45). Using a modification of the procedure of Mixon and Rogers, Bartz et al. (46) found several peanut lines that were more tolerant to seed colonization by *A. flavus* than other genotypes. In another report, Bartz et al. (47) noted a variation in the colonization of different peanut genotypes evaluated at different digging dates and sampling times for each of 4 seasons; however, repeated tests of some lots of seed showed similar *A. flavus* infection.

In laboratory and field studies near Daron, Senegal (48), *A. flavus* infection of the pod and seed indicated that growth rate differences for the fungus were associated with structural differences of the pod and differences in drought stress before harvest. In field tests, 2 genotypes, EH 301-13 and EH 349 bis, had less pod infection, and, in the laboratory, 7 genotypes had no seed infection compared to other genotypes. In other studies under drought stress (49), the fungal invasion of pod and seed differed among genotypes and plants seeded at different dates. Even though no significant correlation was found between fungal invasion of seed in the laboratory and the field samples, there was a significant correlation between pod and seed invasion by the fungus in the field.

To determine the influence of pod inoculation and subsequent mycelial penetration of the seed in the laboratory by the *Aspergillus* fungus, Kushalappa et al. (50) found a wide variation in the percentage seed colonization among peanut breeding lines. They suggested that differences in pod susceptibility were due to the presence of antagonistic microflora. Seed from many lines previously found to be tolerant to seed colonization were less frequently contaminated than susceptible lines, even though the pod was colonized. Also, peanut lines with only minor pod colonization had fewer colonized seed.

In a heritability study using the frequency distribution of *A. flavus* colonization of seed from F_1 and F_2 peanut plants of reciprocal crosses between a resistant and susceptible genotype, a high degree of heritability was found (51). This information was encouraging, but efforts to maintain resistant selections in successive generations from crosses over several seasons when environmental conditions fluctuate were not always successful.

HEREDITY AND ENVIRONMENTAL FACTORS ASSOCIATED WITH SEED AND POD RESISTANCE

LaPrade and Bartz (52) showed that the testa of whole, intact peanut seed of *A. flavus*-resistant lines were less permeable to diffusion of aqueous stain than susceptible lines. LaPrade et al. (53) and Taber et al. (54) observed that seed of *A. flavus*-resistant lines had more wax accumulation on the testae than the susceptible genotypes. In electron microscopy studies in Texas (55,56) seed of resistant genotypes had thinner and tighter fitting testae with more compact cell structure than susceptible genotypes. Light microscope studies showed that the testae of the resistant genotypes had a uniform waxy coating, smaller hila and a compact palisade-like cell layer (57,58). Zambettakis and Bockelee-Morvan (59) presented evidence indicating differences in testae structures among peanut genotypes, and they presented a method of classification of these differences as a guide for selecting for resistance to *A. flavus*.

The penetration of *A. flavus* into and through the peanut testa was observed by Waliyer and Abadie (60). After germination of the *A. flavus* mycospore in direct association with the intracavitary material, the testae soon was penetrated. Penetration by the fungus was 4 days after inoculation, and lysis by the germ tube was observed at the point of contact within an intracellular substance below the external layer of the testae. The central cavity was invaded by the mycelium 5 days after inoculation and the intracavitary material began to disintegrate by hydrolysis. Extensive invasion was noted on the 6th day, after a mycelial network formed on the surface of the testae.

In Nigeria (61), seed of peanut cultivars with colored testa tended to be more resistant to seed invasion by *A. flavus* than seed with colorless or variegated testa. Data showed that testae tannin inhibited germination of *A. flavus* spores when used as an amendment in nutrient media. However, after the fungus became established, it grew rapidly on the testa or on synthetic media amended with tannins. Inhibition of *A. flavus* by 4 compounds occurring in freshly harvested peanut cotyledons was reported by Lindsey and Turner (62). One of the inhibitory compounds was identified as 5,7-dimethoxyisoflavone (63). Evidence of changes in tannin-like substances in maturing peanuts was reported by Sanders (64) and he suggested that there was a relationship between testae tannin and *A. flavus* invasion of seed. Extracted testae tannins from peanut genotypes with different reactions to seed colonization by *A. parasiticus* indicated a correlation between tannins and seed invasion by the fungus (65).

TABLE I

Percentage Seed Colonization of Advanced Selections and Check Genotypes, 1977-80

Genotype	Inoculated[a] (%)	Uninoculated[b] (%)	Means of inoculated + uninoculated (%)
72118	10.0[a,c]	3.9[a]	7.0[a]
PI 337409 (Res. ck)	11.1[a]	3.8[a]	7.5[a]
7405	11.1[a]	5.3[a,b]	8.2[a]
72120	12.3[a]	4.4[a,b]	8.4[a]
7404	11.8[a]	6.0[a,b]	8.9[a]
72125	21.3[b]	5.5[a,b]	13.4[b]
7109	20.7[b]	8.8[b]	14.8[b]
Florunner (com. ck)	40.5[c]	15.8[c]	28.1[c]
PI 331326 (Sus. ck)	85.8[d]	21.5[d]	53.6[d]
Significant levels and interactions (1977-80)			
Block (BK)	NS	Year (Y)	**
Cultivar (CU)	**	BK X Y	*
BK X CV	NS	CU X Y	**
Inoculation Method (I)	**	I X Y	**
CU X I	**	CU X I X Y	**
BK X Inoc.	NS		

[a]Inoculated with *A. parasiticus*.

[b]Field or incidental contamination with *Aspergillus* sp.

[c]Column means with same letter are not different at 0.05 probability level (DNMR test).

*,**Indicates that data of parameter is significant at .05 and .01 levels of probability.

NS indicates that data for parameter was not significant at .05 level of probability.

In Texas, several peanut accessions with pod tissue containing compact sclerenchyma cell zones and thick parenchyma cells were resistant to fungal penetration (66). Also, certain soluble amino acids extracted from testae of peanut genotypes resistant to *A. flavus* colonization were present in smaller amounts than amino acids from genotypes of susceptible genotypes (67).

RECENT PROGRESS IN SELECTION AND BREEDING

Peanut lines have been developed by pedigree and multiline selection within segregating generations of crosses between peanut lines identified as being resistant to *A. flavus* colonization and agronomic cultivars or advanced peanut lines (72118, 72120, 72125, 7404 and 7405) (Table I). Another genotype (7109) was selected for agronomic performance up to the F_5 generation, then selected for resistance to *A. flavus* colonization beginning with the F_6 and successive generations. A laboratory screening procedure was used to determine the percentage colonization of seed by *A. flavus* strain NRRL A13794 or *A. parasiticus* strain NRRL 2999.

During 4 years (1977-80), the above 6 resistant peanut lines, resistant genotype (PI 337409), susceptible genotype (PI 331326), and commercial cultivar Florunner were grown in nursery plots near Tifton, GA. In this same period (1977-80), the lines 7109, 72118 and 72120 were grown in replicated field tests each year with the Florunner cultivar as a check. For 2 years (1979-80), lines 7109, 72125, 7404 and 7405 and the check cultivar were tested. The randomized test had 6 replications each year. Cultural practices recommended for maximal yields were used. Seed used for laboratory evaluation for *A. flavus* penetration were hand-shelled from hand-picked pods, and dried 5-7 days on inverted field rows of nursery plots.

Duplicate samples of sound, mature seed from 6 plants of each genotype selected at random from nursery plots were evaluated for *A. flavus* colonization in the laboratory. A laboratory screening procedure of Mixon and Rogers (68) was used except the moisture was adjusted to 20% (seed wt

TABLE II

Aflatoxin Content of Seed from Selected Advanced Cultivars and Florunner, Tifton, Georgia

	Total aflatoxin (ppb)[a]			
	Sound-mature kernels from hand-picked samples[b]			Total composite kernels from pods stored dry for ca. 90 days
	Irrigated		Non-irrigated	Non-irrigated
Cultivar	1979	1980	1979	1979
7109	2.5	0.0	3.0	16.0
72118	5.5	0.0	–	–
72120	4.0	0.0	–	–
Florunner	4.0	0.0	2.0	30.0

[a]Analysis courtesy Dr. D. Wilson, Plant Pathology Department, Univ. of Georgia Coastal Plain Station, Tifton, GA.

[b]Data from duplicate samples.

basis; i.e., 4 mL H_2O/20 g seed) and the sample was inoculated with a 1-mL spore suspension of *A. parasiticus*. A similar set of samples was not inoculated as a measure of field and incidental *A. flavus* spp contamination. The percentage of seed with conidia and conidiophore development was recorded as colonized (infected) seed.

Yield, dollar value, aflatoxin determinations and seed quality were obtained from the field tests adjacent to the peanut nursery. The experimental design was a randomized complete block with 6 replications. Peanuts were grown in 2-row 6.1 × 1.8 m plots. Plots were dug, inverted on the row, dried and yield of pods was recorded. Seed data were determined from 500-g pod samples using Federal State Inspection Service procedures.

Aflatoxin determinations were made from peanuts grown in the field tests near Tifton and from farmer plantings grown under drought stress in Terrell County, GA. One-pound, hand-shelled seed samples from hand-picked pods were obtained from 2 replications of an irrigated field test near Tifton, GA, immediately after harvest in 1979, and a non-irrigated test in 1980. Also in 1979 at Tifton, a composite seed sample was analyzed for aflatoxin after field-cured samples were stored for about 90 days. In 1980, seed samples were obtained from field-cured peanuts grown under slight, moderate and severe drought stress in Terrell County, GA. Duplicate aflatoxin analyses for total aflatoxin were made from these drought stress peanuts, the peanut meal and seed extracts.

A summary of seed colonization percentages for the advanced generation line selection from hybrids is given in Table I. Mean colonization percentages for the samples showed that the resistant lines plus PI 337409 (resistant check) were more resistant to fungal infection than the cultivar Florunner (commercial check) and PI 331326 (susceptible check). Colonization percentages from the inoculated samples were equal to the resistant genotype, PI 337409, for 4 of the inoculated genotypes averaged over the 4-year period (1977-80), or for 5 of the uninoculated genotypes for the same period. It is obvious that the uninoculated samples of all lines had much less colonization than the inoculated samples. The uninoculated treatment is an index of the *Aspergillus* infection resulting from field or prelaboratory contamination. A considerable amount of variation occurred in the percentage colonization as reflected in a highly significant interaction for cultivar by inoculation method, line by year, inoculation by year and line by inoculation by year. Even with the variability, there are enough consistencies exhibited between the resistant and susceptible genotypes to suggest an association between inherent reaction of the genotypes and infection by the

TABLE III

Aflatoxin Content of Seed from 7109 and Florunner Grown under Drought Stress Regimes in Terrell County, Georgia (1980)

	Total aflatoxin (ppb)[a]					
	Slight drought stress		Moderate drought stress		Severe drought stress	
Cultivar	Early planting	Late planting	Early planting	Late planting	Early planting	Late planting
7109	1.5	4.6	280.1	294.4	241.7	465.1
Florunner	2.6	0.5	1.1	2.9	587.8	662.8

[a]Data from average of duplicate analysis of meal and extract samples by Federal State Inspection Service, Albany, GA.

TABLE IV

Yield and Value of Peanut Pods from Advanced Line Field Tests, 1977-80

Genotype	Yield (kg/ha)[a]	Calculated value[b] ($/ha)	Total seed (%)	Sound mature kernels (%)	Seed/ 100 g (no.)	External damage[c] (%)
			1977-80			
7109	5461[a,e]	2707	73.0[b]	69.7[a]	144[c]	1.0[a]
Florunner (ck)[d]	5131[b]	2587	75.0[a]	71.0[a]	177[a]	0.9[a]
72120	5084[b,c]	2536	73.7[a,b]	69.5[a]	181[a]	0.9[a]
72118	4824[c]	2365	72.7[b]	69.2[a]	156[b]	0.9[a]
			1979-80			
7109	5327[a]	2566	73.3[a,b]	67.7[b,c]	149[e]	0.9[a]
Florunner (ck)	4798[b]	2419	74.6[a]	70.6[a]	182[c]	0.8[a]
71125	4723[b]	2315	74.9[a,b]	69.3[a,b]	163[d]	1.2[b,c]
7405	4113[c]	1977	74.6[a]	66.9[c]	215[b]	1.4[a,b,c]
7404	3689[c]	1649	71.1[c]	62.2[d]	258[a]	1.7[a,b]

[a]Yield in kilograms/hectare.

[b]Calculated value based on 1980 market price as established by the Agricultural Stabilization and Conservation Service, USDA, for Runner market type peanuts.

[c]Visible percentage of seed that was rancid, decayed, moldy, or with sprouts, insect or worm damage.

[d]Check cultivar.

[e]Column means followed by the same letter are not significantly different at the 0.05 probability level according to DNMR test.

TABLE V

Levels of Significance from Analysis of Variance[a]

Source of variation	Yield (kg/ha)	Total seed (%)	Sound mature kernels (%)	Seed/ 100 g (no.)	External damage (%)
	1977-80				
Blocks (BK)	NS[b]	NS	NS	*	NS
Cultivars (CV)	**	**	**	**	**
BK X CV	NS	NS	NS	NS	NS
Year (Y)	**	**	**	**	**
BK X Y	NS	NS	NS	**	NS
CV X Y	*	NS	NS	**	NS
	1979-80				
Blocks (BK)	NS	*	NS	**	NS
Cultivars (CV)	**	**	**	**	*
BK X CV	NS	**	NS	NS	NS
Year (Y)	**	**	NS	**	NS
BK X Y	NS	**	NS	NS	NS
CV X Y	*	**	NS	*	*

[a]Information applies to data presented in Table II.

b,*,**Indicates that data of the parameter are significant at .05 and .01 probability levels. NS indicates that data for the parameter are not significant at .05 probability.

fungus.

Table II presents total aflatoxin contents of peanut samples from selected lines and the Florunner cultivars. The aflatoxin contents from the field tests at Tifton were all at low levels and indicated no apparent differences between the lines and Florunner. Also, total aflatoxin from seed samples from 1980 drought stress fields in Terrell County, GA (Table III), indicated that the resistance of 7109 for *Aspergillus* spp found in the laboratory may not hold for long periods of drought stress in the field. Therefore, the laboratory resistance for lines selected using the laboratory procedure may only be effective for short periods (5-8 days). This could be important immediately after harvest when peanuts might be subjected to conditions that are highly conducive to contamination by the toxin-producing fungus.

Yield, market value and seed quality data for the advanced lines and the Florunner cultivar are presented in Table IV. Breeding line 7109 exceeded Florunner (check cultivar) in yield and market value, and equaled the check in sound-mature seed and seed damage in the 4-year summary of data. As shown in the 1979-80 data, the total seed (shelling %) is usually less than Florunner, but the seed size is much larger (smaller count/100 g). The other genotypes selected for resistance lacked one or more favorable characteristics, but crossing or backcrossing might improve them. There were interactions (Table V) of cultivar by year for yield and seed/100 g in the 1977-80 summary, and for yield, total seed, seed/100 g and damage in the 1979-80 summary.

Research has indicated that the genetic resistance to *Aspergillus* spp thus far reported is effective for a short period of time (5-8 days). The combined use of (a) a genetically resistant cultivar, (b) preventive measures such as crop rotation and good cultural practices (including irrigation) to prevent plant stress and maintain rapid growth of healthy plants, and (c) safe harvesting and rapid drying procedures of peanuts dug at optimal maturity will reduce aflatoxin contamination by *Aspergillus* spp group of fungi in peanuts. Although the genetic resistance now available will not eliminate aflatoxin contamination, it is believed that resistant cultivars that will effectively reduce aflatoxin levels in peanuts may be developed.

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Effect of Drought on Occurrence of *Aspergillus flavus* in Maturing Peanuts

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ABSTRACT

Florunner peanuts were grown in experimental plots with soil moisture and soil temperature modified during the last third of the growing period to produce drought, drought with cooled soil, irrigated and irrigated with heated soil treatments. Twice each week, beginning 97 days after planting, random samples were harvested and maturities of individual pods were determined without destroying pod integrity. The nature and quantity of the microflora associated with the pods and kernels were subsequently assessed. Drought and lower soil temperature resulted in maturity distributions containing higher proportions of immature pods. On peanuts with no visible damage to the pod or kernel, colonization by *Aspergillus flavus* was more frequent in immature than mature kernels. Drought stress increased the incidence of *A. flavus* and irrigation decreased it, except when soil temperatures were modified. *A. flavus* infestation was greatly increased at all maturity levels by pod damage.

INTRODUCTION

Peanuts without obvious damage can be invaded by *Aspergillus flavus* and contaminated with aflatoxin in the field before digging. Although the exact circumstances have not yet been fully delineated, severe, prolonged drought stress during the last 4-6 weeks of the growing season favors invasion of peanuts by *A. flavus* (1-3). The relationship between high *A. flavus* invasion in pods and kernels of peanuts and severe drought was noted in South Africa (4). In Texas, peanuts grown under drought conditions contained more aflatoxin before digging than peanuts grown under irrigation (2). The geographical distribution of rainfall and of farms which produced segregation-3 peanuts in North Carolina suggested that drought after peanuts are formed, but before they are dug, is conducive to their infection with *A. flavus* before digging (1). Data from irrigation experiments indicated that the incidence of kernels with visible *A. flavus*, insect damage and aflatoxin were related to drought conditions before digging (1). Timing of the drought period affects the occurrence and extent of *A. flavus* infection. Dickens et al. (1) found that irrigation during the last 2 months of the growing season was just as effective in reducing aflatoxin contamination as was irrigation throughout the growing season. Reduced metabolic activity due to a decrease in pod moisture content under drought conditions has been suggested to increase the susceptibility of peanuts to fungal invasion (5). Several investigators have reported that *A. flavus* activity was restricted above 30% and below 10% kernel moisture content (5-8). Diener et al. (9) found a higher incidence of