Evaluation of Post-harvest Aflatoxin Production in Peanut Germplasm with Resistance to Seed Colonization and Pre-harvest Aflatoxin Contamination

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ABSTRACT

Contamination of peanut (*Arachis hypogaea* L.) with aflatoxin produced by species of *Aspergillus* remains a problem for the U.S. peanut industry. Several peanut genotypes were reported to be resistant to *in vitro* seed colonization by *Aspergillus flavus* Link ex Fries (IVSCAF), to field seed colonization by *A. flavus* (FSCAF), or to preharvest aflatoxin contamination (PAC), but few to production of aflatoxin *per se*. Cotyledons of 39 peanut genotypes reportedly resistant to IVSCAF, FSCAF, or PAC, and eight susceptible to PAC were evaluated in four tests for their ability to support aflatoxin production after inoculation with *A. flavus*. Cultivars Perry and Gregory were used as checks in each test. Seed cotyledons were separated, manually blanched, inoculated with conidia of *A. flavus*, placed on moistened filter paper in petri dishes, and incubated for 8 d at 28 C. Dishes were arranged on plastic trays enclosed in plastic bags and stacked with PVC spacers between trays. Incomplete block designs were used for all tests. In each test, none of the genotypes examined was completely resistant to aflatoxin production, but significant genotypic variation was observed in the amount of total aflatoxin accumulated in seeds. Genotypes previously reported to be resistant to IVSCAF, FSCAF, or PAC exhibited differential abilities to support aflatoxin production. PI 590325, PI 590299, PI 290626, and PI 337409 supported reduced levels of aflatoxin, and their degree of resistance was consistent across tests. Fungal growth was highly correlated with aflatoxin production in three tests. The results from this study suggested that there were no absolute relationships of aflatoxin production resistance with IVSCAF, FSCAF, or PAC resistance, but that it should be possible to identify a genotype with high IVSCAF, FSCAF, or PAC resistance and reduced capacity for aflatoxin production by *A. flavus*.

Key Words: *Arachis hypogaea* L., *Aspergillus flavus* Link ex Fries, groundnut, breeding.

Aflatoxin contamination of peanut (*Arachis hypogaea* L.) is a serious worldwide problem resulting either from preharvest infection or contamination during storage under improper conditions. Aflatoxins are toxic and extremely carcinogenic secondary metabolites produced primarily by the fungi *Aspergillus flavus* Link ex Fries and *A. parasiticus* Speare (Diener *et al.*, 1982). Late season drought, high temperature, and insect damage contribute to high levels of pre-harvest contamination, while improper harvest and storage practices lead to high levels of post-harvest aflatoxin contamination (Cole *et al.*, 1995). Aflatoxin contamination has had a tremendous impact on the peanut industry. In the U.S., farmer stock peanut lots containing visible *A. flavus* growth are excluded from the edible market and must be crushed for oil (Sands, 1982). Aflatoxin contamination costs the farmer, buying point, and sheller segments of the southeastern U.S. peanut industry more than $25 million annually (Lamb and Sternitzke, 2001). Reduction of aflatoxin contamination of peanuts grown and sold in the U.S. remains a high priority of the U.S. peanut industry.

Elimination of aflatoxin from the human food chain is a goal of many countries. Management of aflatoxin in peanuts is complex. Besides adopting certain cultural, harvest, and storage practices, resistant cultivars should be an effective and low-cost part of an integrated aflatoxin management program. Four types of resistance to *Aspergillus* have been defined: resistance to *in vitro* seed colonization by *A. flavus* (IVSCAF), field resistance to seed colonization by *A. flavus* (FSCAF), preharvest resistance to aflatoxin contamination (PAC), and resistance to aflatoxin production.

Mechanisms of IVSCAF resistance may be related to the combinations of physical and chemical characteristics of the seed testa. This type of resistance depends upon the testa being complete and undamaged. The conditional nature of this type of resistance limits its utility under field conditions. Resistance of peanut pods to *A. flavus* invasion also appears to be associated with undamaged shells and the presence of antagonistic microflora in the shell (Kushalappa *et al.*, 1976). Resistance to preharvest contamination was reportedly associated with drought tolerance (Holbrook *et al.*, 2000). Peanut genotypes with resistance to IVSCAF or FSCAF, and germplasm with reduced levels of PAC have been reported (Mehan and McDonald, 1980; Mehan *et al.*, 1991; Cole *et al.*, 1995; Holbrook *et al.*, 1998). Difference in the ability of peanut cotyledons to support aflatoxin production has received increasing attention. Although no germplasm resistant

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to aflatoxin accumulation has been found, genotypes differ in the concentrations of aflatoxin they support during infection by *A. flavus* (Mehan *et al.*, 1986, 1991; Dange and Prasad, 1989; Ghewande *et al.*, 1989). However, adequate resistance to aflatoxin accumulation has not been incorporated into any agronomically desirable peanut cultivar.

Because resistance may operate at the pod surface, on seed testa, and seed cotyledon, different methods have been used for germplasm screening. It would be best if cultivars could be developed with reduced aflatoxin production in addition to IVSCAF and FSCAF resistance. However, there are many conflicting reports on the relationships among different types of resistance. On one hand, poor correlations have been reported between IVSCAF and FSCAF resistance (Kisyombe *et al.*, 1985) or between IVSCAF and PAC resistance (Davidson *et al.*, 1983; Blankenship *et al.*, 1985; Anderson *et al.*, 1995). Those results led to the suggestion that the laboratory assay method (IVSCAF) should be carefully reassessed. On the other hand, a significant positive correlation was found between IVSCAF resistance and FSCAF resistance (Zambetakkis *et al.*, 1981), and between IVSCAF resistance and PAC resistance. Mehan *et al.* (1987) evaluated 11 genotypes, six resistant and five susceptible to IVSCAF, for field resistance to seed colonization and for aflatoxin contamination. Significant positive correlations were found between IVSCAF resistance and FSCAF resistance, and between FSCAF resistance and PAC resistance.

Mehan *et al.* (1989) reported that seven IVSCAF-resistant genotypes had significantly greater field resistance to *A. flavus* and lower aflatoxin production, but several IVSCAF-resistant breeding lines were highly susceptible in the field while IVSCAF-susceptible genotypes U4-7-5 and VRR-245 showed field resistance. They concluded that there was no consistent positive or negative relationship between IVSCAF resistance and FSCAF or PAC resistance.

Poor correlation between IVSCAF resistance and post-harvest aflatoxin production was also observed by Mehan and McDonald (1983). Mehan *et al.* (1982) tested nine peanut genotypes for resistance to seed colonization and aflatoxin production following colonization of scarified, surface-sterilized seeds by three aflatoxigenic strains of *A. flavus*. IVSCAF-resistant lines PI 337409 and PI 337394F showed significantly less seed colonization and internal infection than the other genotypes, but did not show reduced levels of aflatoxin production. Wilson *et al.* (1977) found production of aflatoxin in IVSCAF-resistant lines PI 337394F and PI 337409 to be similar to that of IVSCAF-susceptible genotypes PI 339396 and Florunner when seed lots were stored under high humidity.

It was reported that resistance to IVSCAF, FSCAF, and aflatoxin production may be influenced by different genes (Utomo *et al.*, 1990). Conflicting results could be due to presence or absence of different genes responsible for different types of resistance or due to differential gene function in different environments. The relationships among various types of resistance need to be better understood to enable breeders to assemble the non-allelic genes influencing different resistance mechanisms.

Although researchers have not been able to identify germplasm combining all types of resistance mechanisms, it is expected that stable high-level resistance will be achieved by accumulating different resistance genes from different sources into one background genotype. The first step in achieving this goal is to collect the sources reported to have resistance to IVSCAF, FSCAF, and PAC, and evaluate their ability to support aflatoxin production. The objective of this study was to evaluate 39 peanut genotypes reportedly resistant to IVSCAF, FSCAF, or PAC, and eight susceptible to PAC (C.C. Holbrook, pers. commun.; Isleib *et al.*, 1994) for their ability to support aflatoxin production.

Materials and Methods

Four experiments were conducted and 47 germplasm lines were evaluated. Large-seeded virginia-type cultivars Perry (Isleib *et al.*, 2003) and Gregory (Isleib *et al.*, 1999) were used as checks (Table 1). In Test 1, 10 lines with PAC scores less than Tifton 8 (Coffelt *et al.*, 1985), and eight lines with PAC scores greater than Tifton 8 were used (C. C. Holbrook, pers. commun.). Tifton 8 was used as a check instead of Gregory in Test 1. Two PAC-resistant lines supporting reduced aflatoxin production from Test 1 and another 11 lines with resistance to IVSCAF or FSCAF were evaluated in Test 2. Breeding line N96074L was included as a third check in this experiment. Eighteen lines with IVSCAF resistance were evaluated in Test 3. The entries of Test 4 were genotypes with reduced levels of aflatoxin content selected from Tests 1, 2, and 3, and two lines, GP-NC WS 2 and N96074L, selected from a previous study in which they showed reduced aflatoxin contamination (unpubl. data). GP-NC WS 2 is an interspecific tetraploid breeding line derived from hybridization between *A. hypogaea* (PI 261942) with *A. cardenasii* Krapov. and W.C. Gregory GKP 10017 (PI 262141) (Stalker and Beute, 1993). *Arachis cardenasii* accession GKP 10017 is a diploid ($2n = 2x = 20$) species that supports only trace levels of aflatoxin production by *A. flavus* (unpubl. data). Tetraploid line N96074L also has *A. cardenasii* GKP 10017 ancestry. Perry, Gregory, and Tifton-8 were used as susceptible checks.

The experimental unit was a group of inoculated seed pieces in a petri dish. Approximately 5 g of dry seeds in each experimental unit were chosen from a particular line. Seed testas were manually removed to eliminate the potential barrier to *A. flavus* growth. The cotyledons of each seed were separated to permit the seed to rest without

Table 1. Genotypes of peanut used in each test for aflatoxin resistance.[a]

Entry	PI no.	Core col. no.[b]	Identity	Resistance type[c]	Entry	PI no.	Core col. no.	Identity	Resistance type[c]
Test 1					**Test 2**				
1	290626	232	Sel. No. 230	PAC	1	590343		Ah 7223	IVSCAF/FSCAF
2	259837	276	Giza Bunch	PAC	2			J11	IVSCAF/FSCAF
3	372318	291	1377.70	PAC	3	337394F		FAV 78	IVSCAF/FSCAF
4	372270	292	3854.69	PAC	4	337409		Rosado	IVSCAF/FSCAF
5	295973	299	No. 718	PAC	5	362144		U4-47-7	IVSCAF/FSCAF
6	196610	329	30-86 Baol	Susceptible	6	590374		UF 71513	IVSCAF/FSCAF
7	153328	336	G 59	Susceptible	7	590332		U4-47-2	FSCAF
8	313129	381	0101	PAC	8	590331		U4-7-25	FSCAF
9	259606	395	No. 45	PAC	9	590353		U4-7-3	FSCAF
10	268845	447	AB13	PAC	10	360862		55-437	IVSCAF/FSCAF
11	288129	511	G 41	PAC	11	363058		55-437	IVSCAF/FSCAF
12	158840	555	Shu-Swi	PAC	12	290626	232	Sel No. 230	PAC
13	431457	593	Manfredi Virginia	Susceptible	13	259606	395	No. 45	PAC
14	475982	595	US 400	Susceptible	14			N96074L	Check
15	468213	602	US 18	Susceptible	15	613600		Perry	Check
16	461440	645	1206	Susceptible	16	608688		Gregory	Check
17	404001	723	PC 19-M-V7-1	Susceptible					
18	203396	394	2201						
19	565463		Tifton 8	Check					
20	613600		Perry	Check					
Test 3					**Test 4**				
1	590327		Ah 6487	IVSCAF	1	590299		C 184	IVSCAF
2	565480		AR-1	IVSCAF	2	564845		GP-NC WS 2	AP
3	565482		AR-3	IVSCAF	3	565479		GFA-2	IVSCAF
4	565483		AR-4	IVSCAF	4	590374		UF 71513	IVSCAF/FSCAF
5	229553		Basse	IVSCAF	5	290626	232	Sel. No. 230	PAC
6	590295		C 116(R)	IVSCAF	6	590295		C 116(R)	IVSCAF
7	590299		C 184	IVSCAF	7	337409		Rosado	IVSCAF/FSCAF
8	590321		Faizpur	IVSCAF	8	590321		Faizpur	IVSCAF
9	565478		GFA-1	IVSCAF	9	590331		U4-7-25	FSCAF
10	565479		GFA-2	IVSCAF	10	259606	395	No. 45	PAC
11	590300		M 395	IVSCAF	11	590325		Monir 240-30	IVSCAF
12	590284		Maria-B	IVSCAF	12	363058		55-437	FSCAF
13	590325		Monir 240-30	IVSCAF	13	337394F		FAV 78	PAC
14	443030		RMP 12	IVSCAF	14	298858		Basse	IVSCAF
15	590352		Var 27 (ICG 4601)	IVSCAF	15			N96074L	AP
16	407492		55-437	IVSCAF/FSCAF	16			J11	IVSCAF/FSCAF
17	565481		AR-2	IVSCAF	17	590343		Ah7223	IVSCAF/FSCAF
18	298858		Basse	IVSCAF	18	565463		Tifton-8	PAC
19	613600		Perry	Check	19	608688		Gregory	Check
20	608688		Gregory	Check	20	613600		Perry	Check

[a]Information based on Isleib *et al.* (1994).

[b]Germplasm lines in the "Core collection" from C.C. Holbrook (pers. commun.).

[c]PAC = Preharvest aflatoxin contamination, IVSCAF = *In vitro* seed colonization by *Aspergillus flavus*, FSCAF = Field seed colonization by *A. flavus*, and AP = Aflatoxin production.

rolling. The seed-halves were surface-sterilized by immersion in a 0.525% (vol:vol) sodium hypochlorite solution (10% vol:vol commercial bleach) for 3 min followed by a rinse in approximately 20 mL of sterile water. The sample was then placed on the surface of four sheets of sterile filter paper moistened with 3 mL sterile water in a 10 cm plastic petri dish. Each piece was inoculated with 50 µL (in Test 1) or 25 µL (in Tests 2, 3, and 4) of a suspension containing approximately 1 $\times 10^6$ mL^{-1} conidia of *A. flavus* strain NRRL 3357 (Natl. Center for Agric. Utilization Res., Peoria, IL).

Petri dishes in the same replication were arranged on a plastic tray. Trays were enclosed individually in large plastic bags with short sections of PVC pipe used as spacers between stacked trays. The trays were rotated in vertical position each of the 8 d of incubation at 28 C. In Tests 1, 3, and 4, a 4 × 5 triple rectangular lattice design with two repetitions of block arrangements was used (Cochran and Cox, 1957). The 20 dishes in each of those replicates were arranged on a tray in four rows and five columns with columns as blocks. In Test 2, a balanced 4 × 4 square lattice design was used. The 16 petri dishes in each of those replicates were arranged in a single layer on a plastic tray in four rows and four columns with columns as blocks. Petri dishes were checked daily, and sterile water was added as needed to keep the filter paper near saturation, but without free water being evident at the paper surface.

After 8 d of incubation, samples were removed from the incubator and rated separately for fungal growth, green color, and development of "fluffy" colonies on a proportional scale of 0 (no growth, green color, or fluffy colonies) to 10 (dense mycelium on all quarters, dark green color, all fluffy colonies). Samples then were dried for 1 d at 60 C, and another 3 d at 50 C, then ground to a friable meal in a coffee mill and stored in scintillation vials until analyzed for aflatoxin content in the NCSU Mycotoxin Lab.

Aflatoxin was extracted from a 2 g ground sample with acetonitrile-water (21:4 vol:vol) in a 5:1 ratio of extractant volume to sample weight. The extract was purified using a Mycosep 224 column (Romer Labs, Union, MO). Aflatoxin was measured by fluorescence high performance liquid chromatography as the post-column-generated bromide derivative (Traag *et al.*, 1987; Trucksess *et al.*, 1994). HPLC analysis was conducted using a Brownlee SPHERI-5 ODS, 5µm 100 × 4.6 mm column fitted with a Brownlee NewGuard RP-18, 7 µm 15 × 3.2 mm column (Perkin-Elmer Corporation, Norwalk, CT). The mobile phase was 4/1/1 water/acetonitrile/methanol, containing 10 mg potassium bromide/L and 100 µL nitric acid/L, pumped at 2 mL/min with a LC-6A Solvent Delivery Module (Shimadzu Scientific Instruments, Columbia, MD). Samples were injected with a SIL-9A Automatic Sample Injector (Shimadzu Scientific Instruments, Columbia, MD) and detected using a RF-551 Fluorescence Detector (Shimadzu Scientific Instruments, Columbia, MD) with excitation at 360 nm and emission at 440 nm. Post-column derivatization was carried out using a KOBRA-cell post-column bromination unit (Vrije Universiteit, Amsterdam, The Netherlands) and 110 V power supply (Lamers & Pleuger, Den Bosch, The Netherlands). Data were recorded and calculations relative to pure aflatoxin standard were performed using a microcomputer equipped with SS-420 analog/digital converter boards and the EXCHROM 6.2 chromatography software system (Scientific Software, Inc., San Ramon, CA).

Aflatoxins B1 and B2, and total aflatoxins were measured by fluorescence high performance liquid chromatography as the post-column-generated bromide derivative (Traag *et al.*, 1987; Trucksess *et al.*, 1994). Aflatoxin data were log-transformed [$Y' = \ln (Y + 0.5)$] to stabilize error variance, and raw and transformed data were subjected to analysis of variance by the general linear model procedure (PROC GLM) of SAS version 8.2 (SAS Institute, Cary, NC). Data were analyzed initially using the incomplete block experimental design structure, but if there were no significant block effects, they were re-analyzed as randomized complete block designs. All models included the effects of trays. Means were separated by Fisher's protected t-test. Means of the transformed data were "back-transformed" with the inverse of the transformation function ($Y = e^{Y'} - 0.5$) to present aflatoxin values in parts per billion (ppb).

Results and Discussion

Test 1. The genotypes evaluated in this experiment included 10 lines with PAC scores less than Tifton 8, and eight lines with PAC scores greater than Tifton 8. Tifton 8 and Perry were used as checks (Table 1). Tray and block had no effect on any trait. Blocks were not included in the analysis of variance. The coefficient of variation was reduced after log transformation of values for aflatoxin B1, B2, and total aflatoxin (Table 2). All genotypes supported extensive fungal growth and high concentration of aflatoxin, although significant differences among entries were observed for all traits measured as were found in previous studies. There was no apparent association between the reported level of PAC and the level of aflatoxin accumulation in this test. However, based on the log-transformed total aflatoxin data, the genotypes with the lowest aflatoxin content were PI 259606, PI 290626, PI 288129, and PI 268845 (Table 3). These four genotypes were among the 10 reported to be resistant to PAC (C.C. Holbrook, pers. commun.). None of the other lines that were reported to be either resistant or susceptible to PAC showed reduced levels of aflatoxin contamination in this test. It is not clear why some PAC-resistant lines accumulated less aflatoxin while others supported high concentrations of aflatoxin. It might be

Table 2. Mean squares from analysis of variance of fungal growth and aflatoxin production traits for two checks and 18 lines selected on the basis of PAC resistance in Test 1.

Source	df	*A. flavus* Growth	*A. flavus* Color	*A. flavus* Fluffy	Aflatoxin B1	ln (B1+0.5)	Aflatoxin B2	ln (B2+0.5)	Aflatoxin (B1+B2)	ln (B1+B2+0.5)
		------ 0-10 rating[a] ------			ppb		ppb		ppb	
Tray	5	0.24	0.21	0.46	90,3599,817	0.7434	1,828,761	0.9319	977,307,288	0.7494
Genotype	19	6.46**	6.71**	0.88*	4,071,877,796**	2.1325**	10,331,829**	4.0069**	4,481,318,724**	2.1813**
Error	95	0.76	0.73	0.42	503,846,929	0.7168	1,224,251	0.8688	547,674,728	0.7220
CV (%)		9.5	9.3	74.4	40.1	7.9	52.9	12.9	40.3	7.9

[a]Proportional rating scale from 0 (no growth, green color, or fluffy colonies) to 10 (dense mycelium on all quarters, dark green color, or all fluffy colonies) in one-point increments.

†,*,**Denote mean squares significant at the 10%, 5%, and 1% levels of probability, respectively.

because PAC resistance can involve mechanisms other than the capacity of cotyledonary tissue to support aflatoxin production, e.g., tolerance to drought. The results suggested the possibility of finding germplasm with resistance to both preharvest contamination and reduced ability to support aflatoxin production. Tifton 8 is a drought-resistant cultivar and reportedly accumulated less aflatoxin in the field than Florunner and Tifrun (Wilson *et al.*, 1990). It is often used as a control in PAC studies. However, Anderson *et al.* (1995) observed that Tifton 8 accumulated a large amount of aflatoxin in their field study. In this experiment, Tifton 8 supported the second highest level of aflatoxin contamination among the genotypes tested, suggesting that drought resistance factors, which are important to preventing PAC, are not important to post-harvest aflatoxin production.

Genotypic means for aflatoxin B1, B2, and total aflatoxin and their log-transformed values were significantly correlated with fungal growth and color ratings ($0.75 \leq r \leq 0.79$, $P < 0.01$). PI 290626 and PI 259606 both had the least visible fungal growth and the lowest aflatoxin contamination.

Test 2. All genotypes except the checks evaluated in this test were reportedly resistant to FSCAF. Some were also reportedly resistant to PAC or IVSCAF (Table 1). Perry and Gregory were included as susceptible checks. Blocks were not a significant source of variation for growth, color, and fluffy ratings, so they were not included in the analysis of variance for those traits. Genotype effects were significant for each trait (Table 4). A wide range of variation was detected among the entries for all traits measured (Table 5). Cultivar Gregory produced significantly less aflatoxin than cultivar Perry. Based on log-transformed total aflatoxin data, all 15 lines produced numerically less aflatoxin than Perry (eight were significantly less), and seven produced numerically less than Gregory (PI 290626 and PI 337409 were significantly less). PI 290626 also had low growth and color score ratings among entries in this experiment, confirming the result from Test 1.

PI 337409 has been reported as a source of stable resistance to IVSCAF (Mixon and Rogers, 1973), FSCAF (Zambettakis *et al.*, 1981; Kisyombe *et al.*, 1985), and PAC (Azaizeh *et al.*, 1989). It has been extensively used in breeding programs to develop cultivars with resistance to IVSCAF (Mixon, 1983; Rao *et al.*, 1995). However, Anderson *et al.* (1995) reported that PI 337409 was susceptible to PAC in the field. PI 337409 was also observed to support substantial aflatoxin following infection by aflatoxigenic strains of *A. flavus* (Mehan *et al.*, 1982) and in storage under high humidity (Wilson *et al.*, 1977).

J11 was found to have stable high-level IVSCAF resistance and FSCAF resistance in multilocational field trails in India (Mehan *et al.*, 1987) and in the U.S. (Kisyombe *et al.*, 1985). Mehan *et al.* (1987) observed a reduction in seed colonization and aflatoxin contamination in undamaged seed under natural field conditions. However, J11 failed to show resistance to aflatoxin contamination in the field studies of Anderson *et al.* (1995). J11 has also been reported to be highly resistant to post-harvest aflatoxin production by aflatoxigenic strains of *Aspergillus* (Dange *et al.*, 1989). However, Mehan *et al.* (1982, 1986) reported that IVSCAF-resistant genotype J11 supported large amounts of aflatoxin production when scarified seeds were inoculated with aflatoxigenic strains of *Aspergillus* and incubated in conditions favorable for fungal growth and aflatoxin production. In the present study, J11 supported moderate levels of aflatoxin production among the entries tested. No significant correlations between fungal growth and aflatoxin production were observed in this experiment.

Test 3. The entries in this experiment were germplasm previously reported to have resistance to IVSCAF (Table 1). Blocks were not a significant source of variation for growth, color, fluffy rating, transformed aflatoxin B1, transformed B2, and transformed total aflatoxin, so they were not included in the analysis of variance for those traits. Genotype effects were significant for all traits measured (Table 6). The two checks were not significantly different but Gregory accumulated numerically less aflatoxin than Perry. Based on the log-transformed

Table 3. Mean for *A. flavus* NRRL 3357 growth and aflatoxin production in inoculated, incubated mature seeds of plant introductions selected on the basis of PAC resistance in Test 1.[a]

Genotype[b]	*A.flavus* Growth	*A.flavus* Color	*A.flavus* Fluffy	Aflatoxin B1	ln (B1+0.5)	Back-transformed B1	Aflatoxin B2	ln (B2+0.5)	Back-transformed B2	Aflatoxin (B1+B2)	ln (B1+B2+0.5)	Back-transformed (B1+B2)
	---- 0-10 rating[c] ----			ppb		ppb	ppb		ppb	ppb		ppb
PI 290626 (CC 232)‡	6.8^{ab}	6.7^{a}	0.8^{a-d}	20,509^{ab}	9.7995^{ab}	18,025	460^{ab}	5.9486^{ab}	383	20,969^{ab}	9.8208^{ab}	18,412
PI 259837 (CC 276)‡	10.0^{g}	10.0^{f}	1.5^{d}	93,754^{fg}	11.4304^{e}	92,078	4692^{i}	8.4239^{g}	4554	98,446^{ghi}	11.4790^{f}	96,668
PI 372318 (CC 291)‡	10.0^{g}	10.1^{f}	1.5^{d}	83,810^{fg}	11.3205^{de}	82,491	3779^{hi}	8.2095^{fg}	3675	87,589^{f-i}	11.3649^{ef}	86,242
PI 372270 (CC 292)‡	9.8^{fg}	10.0^{f}	0.8^{a-d}	70,704^{ef}	11.1260^{cde}	67,915	2240^{d-g}	7.6740^{d-g}	2151	72,944^{d-g}	11.1575^{e-f}	70,091
PI 295973 (CC 299)‡	9.0^{d-g}	9.0^{cde}	0.5^{ab}	51,292^{cde}	10.7471^{b-e}	46,494	1641^{b-f}	7.3046^{e-f}	1487	52,933^{cde}	10.7802^{b-f}	48,058
PI 196610 (CC 329)	7.8^{bc}	7.9^{b}	0.5^{ab}	44,935^{bcd}	10.5663^{b-e}	38,805	1218^{a-e}	6.9144^{b-e}	1006	46,153^{bcd}	10.5936^{b-f}	39,877
PI 153328 (CC 336)	8.9^{def}	8.9^{cd}	0.8^{a-d}	36,371^{abc}	10.4142^{bcd}	33,329	945^{abc}	6.6760^{bcd}	793	37,316^{abc}	10.4383^{b-e}	34,141
PI 313129 (CC 381)‡	9.8^{fg}	9.8^{def}	0.7^{abc}	52,626^{cde}	10.6900^{b-e}	43,913	2153^{c-g}	7.3568^{c-g}	1566	54,779^{cde}	10.7263^{b-f}	45,539
PI 259606 (CC 395)‡	6.7^{a}	6.7^{a}	0.8^{a-d}	12,225^{a}	9.3438^{a}	11,427	252^{a}	5.4703^{a}	237	12,477^{a}	9.3645^{a}	11,667
PI 268845 (CC 447)‡	9.4^{d-g}	9.4^{c-f}	1.0^{bcd}	30,914^{abc}	10.2073^{abc}	27,101	771^{ab}	6.4614^{abc}	639	31,685^{abc}	10.2310^{abc}	27,750
PI 288129 (CC 511)‡	9.9^{fg}	9.8^{def}	1.2^{bcd}	71,689^{efg}	9.9477^{ab}	20,904	2425^{efg}	6.4767^{abc}	649	74,114^{e-h}	9.9763^{ab}	21,511
PI 158840 (CC 555)‡	9.9^{fg}	9.9^{ef}	1.2^{bcd}	97,148^{g}	11.4720^{e}	95,992	3893^{hi}	8.2494^{fg}	3825	101,041^{i}	11.5112^{f}	99,824
PI 431457 (CC 593)	8.8^{cde}	8.8^{bc}	0.8^{a-d}	37,617^{abc}	10.5136^{b-e}	36,812	1252^{a-e}	7.0430^{cde}	1144	38,870^{abc}	10.5454^{b-f}	38,002
PI 475982 (CC 595)	8.6^{cd}	8.6^{bc}	0.2^{a}	40,701^{bc}	10.5738^{b-e}	39,095	1329^{a-e}	7.1372^{cde}	1257	42,030^{bc}	10.6059^{b-f}	40,373
PI 468213 (CC 602)	10.0^{g}	9.9^{ef}	1.0^{bcd}	68,649^{def}	11.0907^{cde}	65,557	2950^{gh}	7.9518^{efg}	2840	71,599^{def}	11.1334^{e-f}	68,418
PI 461440 (CC 645)	9.9^{fg}	9.9^{ef}	0.2^{a}	76,615^{efg}	11.1712^{cde}	71,057	2880^{fgh}	7.8005^{efg}	2441	79,495^{e-i}	11.2060^{def}	73,570
PI 404001 (CC 723)	9.9^{fg}	10.0^{f}	1.3^{cd}	69,607^{def}	11.1006^{cde}	66,214	2871^{fgh}	7.8851^{efg}	2657	72,478^{d-g}	11.1410^{e-f}	68,937
PI 203396 (CC 394)	9.7^{efg}	9.8^{def}	0.5^{ab}	32,713^{abc}	10.3316^{bc}	30,685	1145^{a-d}	6.9559^{b-e}	1049	33,857^{abc}	10.3656^{bcd}	31,746
Tifton 8 (PI 565463)	10.0^{g}	10.0^{f}	1.2^{bcd}	96,845^{g}	11.4519^{e}	94,075	4085^{hi}	8.2348^{fg}	3769	100,930^{hi}	11.4920^{f}	97,929
Perry (PI 613600)	8.8^{cde}	8.9^{cd}	1.0^{bcd}	30,684^{abc}	10.2303^{abc}	27,729	853^{ab}	6.6457^{bcd}	769	31,537^{abc}	10.2577^{a-d}	28,500
Mean	9.2	9.2	0.9	55,970	10.6764	50,485	2092	7.2410	1845	58,062	10.7095	52,363

[a]Means followed by the same letter are not significantly different ($P < 0.05$) by t-test.

[b]Lines released in the U.S. identified by genotype name, others identified by plant introduction number.

[c]Proportional rating scale from 0 (no growth, green color, or fluffy colonies) to 10 (dense mycelium on all quarters, dark green color, or all fluffy colonies) in one-point increments.

‡Denotes lines with low PAC values as reported by C.C. Holbrook (pers. commun.).

Table 4. Mean squares from analysis of variance of fungal growth and aflatoxin production traits for two checks and 14 FSCAF and PAC resistance lines in Test 2.

Source	df	*A. flavus* Growth	*A. flavus* Color	*A. flavus* Fluffy	Aflatoxin B1	ln (B1+0.5)	Aflatoxin B2	ln (B2+0.5)	Aflatoxin (B1+B2)	ln (B1+B2+0.5)
		------ 0-10 rating[a] ------			ppb		ppb		ppb	
Tray	4	1.94	3.59*	0.20	465,415**	0.9652*	188**	0.4952	483,321**	0.9593*
Block (Tray)	15				191,567†	0.7041*	70	0.9777*	198,535†	0.7059*
Genotype	15	7.34**	7.40**	1.21**	296,492**	1.2379**	131**	1.8336**	308,605**	1.2460**
Error	60 (45)	1.19	1.33	0.49	116,265	0.3227	48	0.5093	120,568	0.3225
CV (%)		14.0	14.7	92.1	54.6	9.2	69.8	35.7	54.7	9.2

[a]Proportional rating scale from 0 (no growth, green color, or fluffy colonies) to 10 (dense mycelium on all quarters, dark green color, or all fluffy colonies) in one-point increments.

†,*,**Denote mean squares significant at the 10%, 5%, and 1% levels of probability, respectively.

total aflatoxin data, 16 genotypes accumulated less aflatoxin than the checks, but only six of them (PI 590299, GFA-2, AR-2, PI 590295, PI 590321, and PI 590325) showed significant reduction of aflatoxin accumulation compared with Gregory (Table 7).

Genotypic means for aflatoxin B1, B2, and total aflatoxin and their log-transformed data were significantly correlated with fungal growth and color ratings in this experiment ($0.71 \leq r \leq 0.86$ ($P < 0.05$). All genotypes with reduced levels of aflatoxin contamination had low fungal growth scores. Genotypes in this experiment were previously reported to be resistant to IVSCAF. Poor fungal growth was the likely cause of reduced aflatoxin production in this experiment.

Test 4. The entries of Test 4 were genotypes with reduced aflatoxin accumulation in Tests 1, 2, and 3, and two additional lines GP-NC WS 2 and N96074L selected from previous studies in which they showed reduced aflatoxin contamination.

Blocks were a significant source of variation only for growth and color ratings, so they were included in the analysis of variance only for those traits. There was significant variation among the genetic entries for each trait except fluffy score (Table 8). Based on the log-transformed total aflatoxin data, seven test genotypes accumulated significantly less aflatoxin than susceptible checks Tifton-8, Gregory, and Perry. PI 590325, PI 590299, PI 290626, and PI 337409 accumulated the lowest levels of aflatoxin among the entries based on either untransformed or log-transformed data (Table 9). PI 337409 was the second lowest in Test 2. PI 290626 was tested in Tests 1 and 2, and was among the genotypes with the lowest aflatoxin accumulation in each test. PI 590325 and PI 590299 were also among the least aflatoxin-contaminated genotypes in Test 3. Genotypic means for aflatoxin B1, and total aflatoxin and log-transformed B1, B2, and total aflatoxin were significantly correlated with fungal growth and color ratings in this experiment ($0.67 \leq r \leq 0.78$, $P < 0.05$).

This experiment was designed to examine genotypes previously reported with resistance to IVSCAF, FSCAF, or PAC for the ability of seed cotyledons to support aflatoxin production after inoculation with *A. flavus*. Seed coats were removed by hand to eliminate the testa barriers that may potentially prevent *Aspergillus* fungal invasion and development. Thus, the results should be more reflective of the ability of seed cotyledons to support aflatoxin production than results obtained using undamaged seeds. Screening of germplasm to detect lines resistant to aflatoxin production requires a system in which

Table 5. Mean for *A. flavus* NRRL 3357 growth and aflatoxin production in inoculated, incubated mature seeds of plant introductions selected on the basis of resistance to FSCAF or PAC in Test 2.[a]

Genotype[b]	*A. flavus* Growth	*A. flavus* Color	*A. flavus* Fluffy	Aflatoxin B1	ln (B1+0.5)	Back-transformed B1	Aflatoxin B2	ln (B2+0.5)	Back-transformed B2	Aflatoxin (B1+B2)	ln (B1+B2+0.5)	Back-transformed (B1+B2)
	----- 0-10 rating[c] -----			ppb		ppb	ppb		ppb	ppb		ppb
PI 590343 (Ah 7223)	7.70^{c-f}	8.30^{def}	0.80^{abc}	661^{a-d}	5.9572^{bcd}	386	11^{a-d}	1.8540^{bcd}	6	672^{a-d}	5.9729^{bcd}	392
J11	7.90^{c-f}	7.80^{cde}	0.60^{abc}	512^{abc}	6.0969^{b-e}	444	6^{abc}	1.7804^{bcd}	5	518^{abc}	6.1095^{b-e}	450
PI 337394F (FAV 78)	6.60^{bc}	6.60^{bc}	0.60^{abc}	409^{ab}	5.8802^{bcd}	357	7^{abc}	1.8998^{bcd}	6	415^{ab}	5.8959^{bcd}	363
PI 337409 (Rosado)	8.40^{efg}	8.40^{def}	0.80^{abc}	302^{ab}	5.3587^{ab}	212	4^{ab}	1.1611^{ab}	3	306^{ab}	5.3714^{ab}	215
PI 362144 (U4-47-7)	8.70^{efg}	8.60^{def}	1.00^{bc}	1047de	6.7840^{ef}	883	21de	3.0496^{e}	21	1067de	6.8028^{ef}	900
PI 590374 (UF 71513)	7.00b^{cd}	7.30^{bcd}	1.20^{cd}	573^{a-d}	6.2006^{c-f}	493	8^{abc}	2.0986^{b-e}	8	581^{a-d}	6.2154^{c-f}	500
PI 590332 (U4-47-2)	9.00^{fg}	8.70^{def}	0.60^{abc}	767^{b-e}	6.6512^{def}	773	9^{abc}	2.3726^{cde}	10	776^{b-e}	6.6640^{def}	783
PI 590331 (U4-7-25)	7.50^{cde}	7.40^{cd}	0.20^{ab}	385^{ab}	5.7229^{bc}	305	4^{ab}	1.1268^{ab}	3	389^{ab}	5.7341^{bc}	309
PI 590353 (U4-7-3)	8.10^{def}	8.20^{def}	0.20^{ab}	937^{cde}	6.8668^{ef}	959	14^{cde}	2.6446^{de}	14	951^{cde}	6.8822^{ef}	974
PI 360862 (55-437)	8.30^{def}	8.50^{def}	0.00^{a}	676^{bcd}	6.3865^{c-f}	593	12^{bcd}	2.4955^{cde}	12	688^{bcd}	6.4043^{c-f}	604
PI 363058 (55-437)	8.40^{efg}	8.20^{def}	0.60^{abc}	402^{ab}	5.8534^{bcd}	348	7^{abc}	1.7458^{bcd}	5	409^{ab}	5.8694^{bcd}	354
PI 290626 (CC 232)	5.90^{ab}	5.90^{ab}	0.40^{abc}	176^{a}	4.9063^{a}	135	2^{a}	0.6032^{a}	1	179^{a}	4.9161^{a}	136
PI 259606 (CC 395)	5.00^{a}	4.80^{a}	0.80^{abc}	717^{b-e}	6.4346^{c-f}	623	12^{a-d}	2.2515^{cde}	9	728^{b-e}	6.4497^{c-f}	632
N 96074L	8.00^{def}	8.00^{cde}	1.20^{cd}	628^{a-d}	6.4192^{c-f}	613	11^{a-d}	2.4634^{cde}	11	639^{a-d}	6.4381^{c-f}	625
Perry (PI 613600)	9.70^{g}	9.50^{f}	2.00^{d}	1198^{e}	6.9935^{f}	1089	23^{e}	2.9484^{e}	19	1221^{e}	7.0115^{f}	1109
Gregory (PI 608688)	9.00^{fg}	9.20^{ef}	1.20^{cd}	601^{a-d}	6.1762^{cde}	481	8^{abc}	1.5248^{abc}	4	609^{a-d}	6.1909^{cde}	488
Mean	7.83	7.84	0.76	624	6.1680	543	10	2.0013	8	634	6.1830	552

[a]Mean followed by the same letter are not significantly different ($P < 0.05$) by t-test.

[b]Lines released in the U.S. identified by genotype name, others identified by plant introduction number.

[c]Proportional rating scale from 0 (no growth, green color, or fluffy colonies) to 10 (dense mycelium on all quarters, dark green color, or all fluffy colonies) in one-point increments.

aflatoxin contamination can be induced reliably and consistently. The screening method used in this experiment has been demonstrated to have good environmental control and relatively low experimental error, especially for log transformed aflatoxin values.

Fungal growth was highly correlated to aflatoxin production in three of four experiments. In previous studies (unpubl. data), correlations between visible fungal growth and aflatoxin production ranged from near zero to high.

Table 6. Mean squares from analysis of variance of fungal growth and aflatoxin production traits for 18 IVSCAF resistance lines and two checks in Test 3.

Source	df	*A. flavus* Growth	*A. flavus* Color	*A. flavus* Fluffy	Aflatoxin B1	ln (B1+0.5)	Aflatoxin B2	ln (B2+0.5)	Aflatoxin (B1+B2)	ln (B1+B2+0.5)
		---- 0-10 rating[c] ----			ppb		ppb		ppb	
Tray	5	6.18*	3.88†	1.55†	284,270	0.6053	245	0.2830	299,223	0.6018
Block (Tray)	24				604,885*		463*		637,568*	
Genotype	19	22.39**	22.82**	2.33**	1,132,601**	3.2036**	590**	3.0015**	1,183,477**	3.2069**
Error	95(71)	2.03	2.00	0.69	324,918	1.0968	225	0.7497	341,096	1.1006
CV (%)		20.7	20.7	74.7	77.6	17.2	104.9	40.5	78.0	17.2

[a]Proportional rating scale from 0 (no growth, green color, or fluffy colonies) to 10 (dense mycelium on all quarters, dark green color, or all fluffy colonies) in one-point increments.

†,*,**Denote mean squares significant at the 10%, 5%, and 1% levels of probability, respectively.

Table 7. Mean for *A. flavus* NRRL 3357 growth and aflatoxin production in inoculated, incubated mature seeds of plant introductions selected on the basis of IVSCAF resistance in Test 3.[a]

Genotype[b]	*A. flavus* Growth	*A. flavus* Color	*A. flavus* Fluffy	Aflatoxin B1	ln (B1+0.5)	Back-transformed B1	Aflatoxin B2	ln (B2+0.5)	Back-transformed B2	Aflatoxin (B1+B2)	ln (B1+B2+0.5)	Back-transformed (B1+B2)
	---- 0-10 rating[c] ----			ppb		ppb	ppb		ppb	ppb		ppb
PI 590327 (Ah 6487)	8.17^{hij}	7.83^{fg}	1.67^{d-g}	798^{a-e}	6.5712^{d-h}	714	16^{a-d}	2.4665^{c-g}	11	814^{a-e}	6.5872^{d-g}	725
AR-1 (PI 565480)	5.50^{a-d}	5.33^{a-d}	0.50^{abc}	491^{abc}	5.9046^{a-f}	366	7^{ab}	1.7962^{a-d}	6	498^{abc}	5.9189^{a-e}	371
AR-3 (565482)	7.08^{d-h}	6.83^{def}	1.00^{a-e}	391^{ab}	6.3021^{c-h}	545	5^{a}	2.4834^{c-g}	11	396^{ab}	6.3232^{c-g}	557
AR-4 (565483)	8.67^{h-k}	8.83^{gh}	1.17^{b-e}	1218^{c-f}	6.6319^{d-h}	758	34^{de}	2.7858^{d-g}	16	1251^{c-f}	6.6529^{d-g}	775
PI 229553 (Basse)	6.17^{b-f}	6.00^{cde}	1.00^{a-e}	687^{a-d}	6.0429^{b-g}	421	13^{abc}	2.0385^{b-e}	7	700^{a-d}	6.0598^{b-f}	428
PI 590295 (C 116(R))	6.50^{c-g}	6.33^{def}	0.83^{a-d}	262^{ab}	5.3513^{abc}	210	3^{a}	1.2150^{ab}	3	265^{ab}	5.3648^{abc}	213
PI 590299 (C 184)	4.17^{a}	4.33^{ab}	0.17^{a}	36^{a}	4.7301^{a}	113	-3^{a}	1.0223^{a}	2	33^{a}	4.7513^{a}	115
PI 590321 (Faizpur)	6.75^{c-g}	7.17^{ef}	1.00^{a-e}	391^{ab}	5.5662^{a-d}	261	8^{abc}	1.7156^{abc}	5	399^{ab}	5.5870^{a-d}	266
GFA -1 (PI 565478)	7.58^{fgh}	7.67^{fg}	1.33^{c-f}	523^{abc}	6.0918^{b-g}	442	11^{abc}	2.0933^{b-e}	8	534^{abc}	6.1088^{b-f}	449
GFA -2 (PI 565479)	5.33^{abc}	5.33^{a-d}	0.50^{abc}	418^{ab}	5.0361^{ab}	153	9^{abc}	1.1524^{ab}	3	427^{ab}	5.0530^{ab}	156
PI 590300 (M 395)	6.42^{b-g}	6.50^{def}	1.00^{a-e}	730^{a-e}	6.3867^{c-h}	593	14^{a-d}	2.4377^{c-f}	11	744^{a-e}	6.4048^{c-g}	604
PI 590284 (Maria-B)	5.50^{a-e}	5.67^{b-e}	0.67^{abc}	1381^{def}	5.7764^{a-f}	322	27^{b-e}	2.3673^{c-f}	10	1408^{def}	5.7899^{a-e}	326
PI 590325 (Monir 240-30)	4.17^{a}	4.17^{ab}	0.83^{a-d}	446^{abc}	5.5762^{a-d}	264	11^{abc}	1.8053^{a-d}	6	457^{abc}	5.5975^{a-d}	269
PI 443030 (RMP 12)	9.50^{ijk}	9.67^{h}	1.83^{efg}	1913^{f}	7.4050^{h}	1644	38^{e}	3.4557^{g}	31	1951^{f}	7.4242^{g}	1676
PI 590352 (Var 27)	9.46^{ijk}	9.24^{gh}	2.31^{g}	1351^{def}	6.8033^{fgh}	900	27^{cde}	2.8481^{efg}	17	1377^{def}	6.8222^{efg}	918
PI 407492 (55-437)	8.00^{ghi}	7.67^{fg}	1.00^{a-e}	797^{a-e}	6.4666^{c-h}	643	15^{a-d}	2.2764^{c-f}	9	811^{a-e}	6.4809^{c-g}	652
AR-2 (PI 565481)	4.83^{ab}	4.67^{abc}	0.33^{ab}	79^{a}	5.0552^{ab}	156	-2^{a}	1.1254^{ab}	3	77^{a}	5.0712^{ab}	159
PI 298858 (Basse)	4.17^{a}	3.83^{a}	0.67^{abc}	424^{ab}	5.8279^{a-f}	339	8^{ab}	1.6482^{abc}	5	432^{ab}	5.8415^{a-e}	344
Perry (PI 613600)	9.67^{ijk}	9.67^{h}	2.17^{fg}	1431^{ef}	7.2266^{gh}	1375	27^{cde}	3.2471^{fg}	25	1459^{ef}	7.2449^{fg}	1400
Gregory (PI 608688)	10.00^{k}	10.00^{h}	2.17^{fg}	868^{b-e}	6.8028^{fgh}	900	16^{a-d}	2.8328^{efg}	16	884^{b-e}	6.8213^{efg}	917
Mean	6.88	6.84	1.11	732	6.0778	556	14	2.1406	10	746	6.0953	566

[a]Mean followed by the same letter are not significantly different ($P < 0.05$) by t-test.

[b]Lines released in the U.S. identified by genotype name, others identified by plant introduction number.

[c]Proportional rating scale from 0 (no growth, green color, or fluffy colonies) to 10 (dense mycelium on all quarters, dark green color, or all fluffy colonies) in one-point increments.

The inconsistent relationship between visible fungal growth and aflatoxin production on seed cotyledons of different genotypes indicates that some varieties are less suitable for aflatoxin production than others, irrespective of how well they support growth (Priyadarshini and Tulpule, 1978). In the course of review of this manuscript, it was pointed out that our experimental protocol did not allow the water status of cotyledonary tissue in

Table 8. Mean squares from analysis of variance of fungal growth and aflatoxin production traits for 18 post-harvest aflatoxin resistance lines and two checks in Test 4.

Source	df	*A. flavus* Growth	*A. flavus* Color	*A. flavus* Fluffy	Aflatoxin B1	ln (B1+0.5)	Aflatoxin B2	ln (B2+0.5)	Aflatoxin (B1+B2)	ln (B1+B2+0.5)
		----- 0-10 rating[a] -----			ppb		ppb		ppb	
Tray	5	3.42**	2.84*	0.67	506,939,895**	4.1101**	452,027**	5.6562**	537,006,886**	4.1308*
Block (Tray)	24	2.02*	2.06*							
Genotype	19	7.83**	8.50**	0.73	172,201,244**	2.1739**	208,668**	3.8666**	183,890,050**	2.1931*
Error	71(95)	1.00	1.02	0.64	52,049,951	0.4153	83,870	0.8128	5,328,692,378	0.4202
CV (%)		12.8	13.0	217.9	66.2	7.2	93.8	17.5	66.8	7.2

[a]Proportional rating scale from 0 (no growth, green color, or fluffy colonies) to 10 (dense mycelium on all quarters, dark green color, or all fluffy colonies) in one-point increments.

[†],*,**Denote mean squares significant at the 10%, 5%, and 1% levels of probability, respectively.

Table 9. Mean for *A. flavus* NRL 3357 growth and aflatoxin production in inoculated, incubated mature seeds of plant introductions selected on the basis of post-harvest aflatoxin resistance lines and two checks in Test 4.[a]

Genotype[b]	*A. flavus* Growth	*A. flavus* Color	*A. flavus* Fluffy	Aflatoxin B1	ln (B1+0.5)	Back-transformed B1	Aflatoxin B2	ln (B2+0.5)	Back-transformed B2	Aflatoxin (B1+B2)	ln(B1+B2+0.5)	Back-transformed (B1+B2)
	--- 0-10 rating[c] ---			ppb		ppb	ppb		ppb	ppb		ppb
PI 590299 (C 184)	6.41^{abc}	6.15^{a-d}	0.50	4,707^{a}	8.0192^{ab}	3,038	114^{abc}	4.1279^{ab}	62	4,821^{a}	8.0396^{ab}	3,101
GP-NC WS 2 (PI564845)	8.69^{f-i}	8.73^{ghi}	0.67	13,652^{c-h}	9.4862^{gh}	13,176	349^{a-g}	5.7850^{efg}	325	14,001^{b-g}	9.5108^{gh}	13,504
GFA -2 (PI 565479)	8.42^{e-h}	8.22^{e-h}	0.00	7,650^{a-d}	8.6700^{b-f}	5,825	234^{a-f}	5.0710^{b-f}	159	7,885^{abc}	8.6973^{b-f}	5,986
PI 590374 (UF 71513)	8.59^{e-i}	8.50^{f-i}	0.50	8,557^{a-e}	8.9652^{c-g}	7,825	176^{a-e}	5.0619^{b-f}	157	8,733^{a-d}	8.9852^{c-g}	7,984
PI 290626 (CC 232)	5.78^{ab}	5.60^{ab}	0.00	4,589^{a}	8.1174^{ab}	3,352	79^{a}	4.1134^{ab}	61	4,668^{a}	8.1355^{ab}	3,413
PI 590295 (C 116(R))	5.72^{ab}	5.71^{ab}	0.00	13,489^{b-h}	9.1268^{e-h}	9,198	461^{d-h}	5.5078^{c-g}	246	13,950^{b-g}	9.1544^{e-h}	9,455
PI 337409 (Rosado)	7.91^{d-g}	7.71^{e-h}	0.00	4,424^{a}	8.2719^{bc}	3,912	85^{ab}	4.2370^{ab}	69	4,510^{a}	8.2897^{abc}	3,982
PI 590321 (Faizpur)	6.87^{bcd}	6.87^{b-e}	1.00	5,334^{ab}	8.5066^{a-e}	4,947	149^{a-d}	4.8959^{b-e}	133	5,484^{ab}	8.5335^{a-e}	5,082
PI 590331 (U4-7-25)	7.37^{cde}	7.35^{c-f}	0.00	5,518^{abc}	8.4167^{a-e}	4,522	115^{abc}	4.4853^{bc}	88	5,634^{ab}	8.4364^{a-e}	4,611
PI 259606 (CC 395)	6.12^{abc}	6.12^{abc}	0.33	6,782^{a-d}	8.3357^{a-d}	4,170	242^{a-f}	4.7048^{bcd}	110	7,025^{abc}	8.3631^{a-d}	4,286
PI 590325 (Monir 240-30)	5.47^{a}	5.45^{a}	0.00	4,512^{a}	7.8802^{a}	2,644	112^{abc}	3.2274^{a}	25	4,624^{a}	7.8993^{a}	2,695
PI 363058 (55-437)	8.64^{e-i}	8.48^{f-i}	0.33	16,797^{e-h}	9.4936^{gh}	13,274	483^{e-h}	5.9201^{efg}	372	17,280^{d-g}	9.5221^{gh}	13,657
PI 337394F (FAV 78)	8.46^{e-h}	8.65^{f-i}	1.00	11,066^{a-g}	9.2717^{fgh}	10,632	247^{a-f}	5.4793^{c-g}	239	11,313^{a-f}	9.2940^{fgh}	10,872
PI 298858 (Basse)	7.41^{c-f}	7.44^{d-g}	0.33	10,243^{a-f}	9.0589^{d-g}	8,594	265^{a-g}	5.3403^{c-g}	208	10,509^{a-e}	9.0829^{d-g}	8,803
N96074L	8.83^{ghi}	8.93^{hij}	0.17	14,405^{d-h}	9.5199^{gh}	13,628	437^{c-h}	5.9904^{fg}	399	14,842^{c-g}	9.5490^{gh}	14,031
J11	8.00^{d-g}	8.01^{e-h}	0.00	14,931^{d-h}	9.2777^{fgh}	10,697	354^{a-g}	5.5247^{d-g}	250	15,285^{c-g}	9.3010^{fgh}	10,948
PI 590343 (Ah7223)	9.47^{hi}	9.68^{ij}	0.33	18,915^{gh}	9.3566^{fgh}	11,575	710^{h}	5.8220^{efg}	337	19,625^{fg}	9.3865^{fgh}	11,925
Tifton-8 (PI565463)	8.88^{ghi}	8.93^{hij}	0.83	14,241^{d-h}	9.3270^{fgh}	11,237	412^{b-h}	5.6810^{d-g}	293	14,653^{c-g}	9.3535^{fgh}	11,539
Gregory (PI 608688)	8.93^{ghi}	8.79^{ghi}	0.67	17,801^{fgh}	9.6943^{gh}	16,224	581^{gh}	6.1339^{g}	461	18,383^{efg}	9.7232^{gh}	16,701
Perry (PI 613600)	9.93^{i}	10.18^{j}	0.67	20,406^{h}	9.8258^{h}	18,505	566^{fgh}	6.1813^{g}	483	20,972^{g}	9.8519^{h}	18,994
Mean	7.80	7.78	0.37	10,901	8.9311	8,849	309	5.1645	224	11,210	8.9554	9,078

[a]Mean followed by the same letter are not significantly different ($P < 0.05$) by t-test.

[b]Lines released in the U.S. identified by genotype name, others identified by plant introduction number.

[c]Proportional rating scale from 0 (no growth, green color, or fluffy colonies) to 10 (dense mycelium on all quarters, dark green color, or all fluffy colonies) in one-point increments.

the various experimental units to equilibrate before inoculation, possibly leading to differences in the time of incubation at high water activity. However, it is unlikely that the slight differences in effective incubation time caused the significant differences in fungal growth and aflatoxin production observed in this study.

None of the genotypes examined in any experiment was completely resistant to aflatoxin accumulation, but significant genotypic variation was observed in the amount of total aflatoxin production in seeds after inoculation with a toxigenic strain of *A. flavus*. Genotypes that were previously reported to be resistant to IVSCAF, FSCAF, or PAC exhibited differential abilities to support aflatoxin production; some lines supported reduced levels of aflatoxin while others supported high levels. Lines showing reduced aflatoxin accumulation in this experiment exhibit more than one form of resistance. These results agreed with the conclusion by Mehan *et al.* (1982) and Mehan and McDonald (1983) and emphasized that there were no consistent relationships between resistance to aflatoxin production and resistance to IVSCAF, FSCAF, or PAC. However, the consistent and reduced levels of aflatoxin production in PI 590325, PI 590299, PI 290626, and PI 337409 suggested that it is possible to identify genotypes with high resistance to IVSCAF, FSCAF, or PAC and reduced capacity for aflatoxin production.

Are the differences in aflatoxin production observed in these experiments sufficient to warrant a breeding effort? The experimental protocol used in these studies maximizes aflatoxin production by providing environmental conditions highly favorable to fungal growth and aflatoxin production. In ideal commercial peanut storage, none of these conditions would exist. However, although one can argue its importance relative to FSCAF or PAC, post-harvest aflatoxin does occur, so these conditions are met at some isolated points in peanut storage. The tolerances for aflatoxin contamination in commercial lots reflect a vast majority of "clean" peanuts mixed with a few contaminated ones. A significant reduction of the level of contamination in the colonized seeds could make the difference between meeting or exceeding the limits on contamination of the whole. It is unlikely that any one of the several methods proposed for management of aflatoxin will completely eliminate the problem from commercial peanuts. It is more likely that a combination of methods will be necessary to achieve meaningful reduction of contamination on a large scale. The genetic aspects of aflatoxin management should not be neglected.

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