***Aspergillus* Colonization and Aflatoxin Contamination in Peanut Genotypes with Reduced Linoleic Acid Composition**

C. Corley Holbrook, Research Geneticist, USDA-ARS, Tifton, GA 31793; **David M. Wilson**, Professor, Department of Plant Pathology, University of Georgia, Tifton 31793; **Michael E. Matheron**, Professor, Department of Plant Pathology, University of Arizona, Yuma 85364; **John E. Hunter**, Proctor & Gamble Co., Cincinnati, OH 45239, Retired; **David A. Knauff**, Associate Dean for Academic Affairs, University of Georgia, Athens 30602; and **Daniel W. Gorbet**, Professor, Agronomy Department, University of Florida, Marianna 32446

ABSTRACT

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Aspergillus flavus and *A. parasiticus* can contaminate several agricultural crops with the toxic fungal metabolite aflatoxin. Previous research has indicated that resistance may be conferred by altering the fatty acid composition of these crops. Recently, peanut breeding lines with reduced linoleic acid content have been developed. The purpose of this study was to examine the effect of reduced linoleic acid composition on preharvest aflatoxin contamination of peanut. Seven breeding lines with relatively low linoleic acid and two check genotypes were grown in a randomized complete block design with 10 replicates for 4 years in Georgia and for 3 years in Arizona. The plots were inoculated with a mixture of *A. flavus* and *A. parasiticus* about 60 days after planting and subjected to drought and heat stress for the 40 days immediately preceding harvest. Differences were observed in only one environment. Low linoleic acid composition had no measurable effect on preharvest aflatoxin contamination in peanut when data were combined across years and locations. Products of the lipoxygenase pathway that have been shown to affect aflatoxin biosynthesis in vitro may not be present in sufficient quantities in peanut.

Aspergillus flavus and *A. parasiticus* can colonize seed of several agricultural crops (3). This can result in the contamination of the edible yield from these crops with the toxic fungal metabolite aflatoxin. The development of resistant crops would be a powerful tool in reducing this problem. Research has indicated that resistance may be possible by altering the fatty acid composition of these crops. Several in vitro studies have shown that fatty acid composition might directly or indirectly affect aflatoxin biosynthesis (2,4,5,12).

Fabbri et al. (5) proposed that aflatoxin production is increased by lipoperoxides of polyunsaturated fatty acids. Passi et al. (12) observed that the addition of linoleic acid hydroperoxides to in vitro *A. parasiticus* cultures enhanced the aflatoxin output by about 30 times after 6 days of incubation, and by about 250 times after 12 days of incubation. The amount of fungal growth was not affected.

Others have observed an inhibitory ef-

fect of hydroperoxides of linoleic acid. Doehlert et al. (4) found that if soybean homogenates were supplemented with high amounts of either lipase, linoleic, or linolenic acid, volatiles that inhibit fungal spore germination were generated. Oleic acid had no effect compared with untreated homogenates. Hexanal, a product of the lipoxygenase pathway with known antifungal activity, was the major volatile generated from lipase-treated homogenates. Burrow et al. (2) observed that 13S-hydroperoxy linoleic acid significantly decreased aflatoxin production when introduced into growth media at 24-h intervals.

A peanut line has been identified with 2% linoleic acid (11). The line is more saturated than standard cultivars, which average 25% linoleic acid. The low linoleic acid trait has been incorporated into high-yielding, agronomically acceptable breeding lines (9) and cultivars (7).

If feasible, altering fatty acid composition would be an attractive approach to managing aflatoxin contamination in peanut. Fatty acid composition is much cheaper to measure than aflatoxin contamination, it is subject to much less environmental variability, and it is simply inherited (10). The objective of this study was to examine seven breeding lines with relatively low linoleic acid and two check genotypes under drought and heat stress conditions to determine if altered fatty acid

composition would influence aflatoxin contamination of peanut.

MATERIALS AND METHODS

Seven peanut breeding lines were selected for evaluation of *Aspergillus* colonization and preharvest aflatoxin contamination based on their low linoleic acid composition. The cultivar Florunner and the germ plasm line Tifton 8 were included as checks. Seeds were planted in Yuma, Arizona, on 25 May 1993, 4 May 1994, and 6 May 1996 in double-row plots (1.5 × 1.8 m) at four seeds per 30-cm linear row. Genotypes were arranged in a randomized complete block design with 10 replicates in each trial. All plants were maintained with adequate moisture, using flood irrigation.

The same genotypes were evaluated at the Coastal Plain Experiment Station Gibbs Farm in Tift County, Georgia. Genotypes were planted on 21 June 1993, 28 April 1994, 5 May 1995, and 17 May 1996 in single-row plots, 1.5 m long, at four seeds per 30-cm linear row in a randomized complete block design with 10 replicates. Plants were maintained with standard cultural practices and irrigation.

Inoculum of *A. flavus* (NRRL 3357) and *A. parasiticus* (NRRL 2999) was prepared by the organic-matrix method (16) and introduced into test plots to ensure the presence of sufficient aflatoxin-producing fungi in the peanut pod zone. Conidia of *A. flavus* or *A. parasiticus*, each from a 10-day culture, were suspended in sterile distilled water (10 ml per 114 g of corn) and used to inoculate sterile moisture-equilibrated (25% moisture) cracked corn. The corn was incubated at 25 to 30°C for 3 days.

Each double-row plot in Arizona was treated with 57 g of corn infested with *A. flavus* and 57 g of corn infested with *A. parasiticus* on 26 July 1993, 18 July 1994, and 15 July 1996. Drought stress was induced by terminating flood irrigation on 17 August 1993, 14 August 1994, and 9 August 1996. Thereafter, irrigation was applied with subsurface perforated tubing 35 cm beneath the rows. Four hours of drip irrigation was applied when plants showed significant symptoms of drought stress (8). Pods were harvested on 14 October 1993, 29 September 1994, and 10 October 1996,

Corresponding author: C. C. Holbrook
E-mail: holbrook@tifton.cpes.peachnet.edu

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dried, and sent to the Coastal Plain Experiment Station for processing.

Each single-row plot in Georgia was inoculated with 28 g of corn infested with *A. flavus* and 28 g of corn infested with *A. parasiticus* on 25 August 1993, 30 June 1994, 5 July 1995, and 16 July 1996. Drought and heat stress were induced by covering the entire test area with a mobile greenhouse (Atlas Greenhouse System Inc., Alapaha, GA) on 20 September 1993, 1 August 1994, 8 August 1995, and 19 August 1996. Pods were dug 15 November 1993, 24 September 1994, 28 September 1995, and 14 October 1996, hand-picked from the plant, and dried.

Seeds from undamaged pods were stored at -20°C until processing to prevent post-harvest accumulation of aflatoxin. Peanuts were shelled with a Penco (Peerless Engineering Company, Chula, GA) peanut sheller and ground in a household food processor for 1 min. Aflatoxin concentration was measured on a 100-g subsample by the immunoaffinity column fluorometer

method (15). The fluorometer was calibrated from 0 to 400 ng/g. If the initial analysis indicated a concentration above 400 ng/g, the sample extract was diluted 1:10 and reanalyzed. If the reanalyzed sample indicated a concentration above 4,000 ng/g, an additional 1:10 dilution and analysis was performed.

Fifty pods were surface-disinfested with 0.5% sodium hypochlorite and aseptically shelled for analysis of infection by species of the *A. flavus* group and *A. niger*. Half-shells (100) and seeds (100) were placed in petri dishes containing malt extract agar containing 10% NaCl by weight and incubated at 30°C . After 7 days, the material was examined for green conidial heads of *Aspergillus* spp. of the *A. flavus* group and black conidial heads of *A. niger* to determine the percent infection.

Data were analyzed by the general linear model procedure of SAS (SAS Institute, Cary, NC), and genotypic means were compared by Fisher's protected least significant difference (LSD). Unless other-

wise stated, all differences referred to in the test were significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

All of the low linoleic peanut lines had less preharvest aflatoxin contamination than Florunner when tested in Arizona in 1993 (Table 1). Five of these lines also had numerically less aflatoxin contamination than Florunner when tested in Georgia in 1993, but these differences were not significant. Aflatoxin contamination levels in both locations for 1994 were low, and none of the entries showed a difference in aflatoxin levels compared with Florunner. Higher levels of contamination were observed in 1995 and 1996, but no significant differences in aflatoxin contamination were observed.

Four additional breeding lines with reduced linoleic acid were examined in a separate set of tests in Arizona and Tifton in 1994 and 1995 (data not presented). These tests also showed no significant effect of reduced linoleic acid composi-

Table 1. Preharvest aflatoxin contamination in peanut genotypes when grown under drought stressed conditions in Georgia and Arizona

Entry	Aflatoxin contamination (ng/g) ^a							
	Georgia				Arizona			
	1993	1994	1995	1996	1993	1994	1996	Mean
F1250 ^b	4	24	2,800	958	653	12	18,559	3,447
Florunner	77	10	4,054	468	5,968	16	14,027	3,028
F1341 ^b	144	11	2,592	273	333	18	13,071	2,503
F1344 ^b	44	31	1,040	1,206	46	12	12,727	2,124
Tifton 8	8	47	915	42	91	10	12,195	2,047
F1348 ^b	12	15	3,985	932	210	327	9,391	1,976
F1334 ^b	11	399	2,419	1,455	186	10	10,082	1,972
F1316 ^b	203	9	2,737	397	64	12	5,895	1,310
F1315 ^b	20	13	2,504	1,221	88	33	4,136	1,173
LSD ^c (0.05)	NS ^d	167	NS	NS	2,656	NS	NS	NS

^a Each value is the mean of 10 replicates.

^b Genotypes with low linoleic acid content.

^c Least significant difference.

^d Entry effect in ANOVA was not significant.

Table 2. *Aspergillus* colonization in seeds and shells of peanut genotypes when grown under drought stressed conditions in Georgia (1993 and 1994) and Arizona (1994)

Entry	<i>Aspergillus</i> colonization (%)							
	Georgia				Arizona			
	Seeds ^a		Shell ^b		Seeds ^a		Shell ^b	
	<i>A. flavus</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>A. niger</i>
F1250	1	6	13	22	7	0	52	17
F1315	2	1	14	8	12	2	50	10
F1316	3	4	13	22	7	0	55	20
F1334	5	1	16	8	1	0	35	14
F1341	1	2	8	10	7	1	59	24
F1344	2	0	13	11	4	2	42	29
F1348	1	2	9	14	6	1	55	22
Florunner	3	2	9	10	12	0	66	18
Tifton 8	5	2	29	16	15	0	64	18
LSD ^c (0.05)	NS ^d	3	10	10	8	NS	22	NS

^a Each value is the mean percentage of seeds colonized, determined by plating a 100-seed subsample from 10 replicates for 2 years (Georgia) or 1 year (Arizona).

^b Each value is the mean percentage of half-shells colonized, determined by plating a 100-half-shell subsample from 10 replicates for 2 years (Georgia) or 1 year (Arizona).

^c Least significant difference.

^d Entry effect in analysis of variance was not significant.

tion on preharvest aflatoxin contamination.

When the data are viewed in total, there appears to be no effect of low linoleic acid content on preharvest aflatoxin contamination in peanut. The significant differences observed in Arizona in 1993 may have been due to the large inherent variability of aflatoxin contamination in peanut. Three of the 10 replicates of Florunner had much higher aflatoxin contamination than all other plots in this test.

Several in vitro studies have shown that fatty acid composition might directly or indirectly affect aflatoxin biosynthesis (2,4,5,12). In contrast, we observed no effect of altered fatty acid composition on preharvest aflatoxin contamination in peanut. The previously observed effects of fatty acid composition on aflatoxin biosynthesis appeared to be related to substances formed during the oxidation of polyunsaturated fatty acids. The lack of significant differences in the present study may be due to a low level of lipid oxidation in peanut prior to harvest regardless of varying endogenous levels of linoleic acid. However, other degradatory enzymes (e.g., lipase) are probably not limiting, since it has been reported that *A. flavus* secretes enzymes with abundant lipase activity when invading oil-seed hosts (13). Gardner et al. (6) postulated that lipase secreted by an invading fungus would trigger the lipoxygenase pathway in soybean, resulting in the generation of volatile aldehydes.

Doehlert et al. (4) showed that if soybean homogenates were supplemented with high amounts of lipase, volatiles were generated that inhibited fungal spore germination. Headspace analysis indicated that hexanal, a product of the lipoxygenase pathway with known antifungal activity, was the major volatile generated. The lipoxygenase (LOX I) isozymes in soybean react with linoleic acid to form the 13-hydroperoxide from which hexanal would be generated by the action of hydroperoxide lyase.

The lack of an effect of reduced linoleic fatty acid composition on preharvest aflatoxin contamination in peanut may be due to a different lipoxygenase pathway in peanut. Burrow et al. (2) demonstrated that the 13-hydroperoxy linoleic acid significantly decreased aflatoxin production, whereas 9-hydroperoxy linoleic acid (formed by LOX II isozymes) did not decrease aflatoxin production. Sung and Jeng (14) observed that LOX II activity was

much higher than the activity of LOX I in accelerated aged peanut seed. For whatever reason, reducing linoleic fatty acid composition does not appear to be a viable approach to reducing preharvest aflatoxin in peanut.

Conflicting information is in the literature on the relative susceptibility of the two checks included in this study. Wilson et al. (17) reported that the germ plasm line Tifton 8 had some resistance to preharvest aflatoxin contamination. However, Anderson et al. (1) did not observe a reduction in PAC in Tifton 8 compared with Florunner. The present study supports the findings of Anderson et al. (1).

None of the low linoleic peanut lines exhibited different levels of colonization of shells or seeds by *A. flavus* group fungi compared with Florunner when tested in Georgia (Table 2). All these genotypes were also at least as susceptible as Florunner to colonization of shells and seeds by *A. niger* in the Georgia tests.

The patterns observed in colonization of shells or seeds in Georgia were not observed when this material was tested in Arizona (Table 2). Two of the low linoleic lines (F1334 and F1344) exhibited significantly less colonization of seeds and shells by *A. flavus*. This may be due to differences in environmental variables (temperature and humidity) between the two locations, or to a difference in fungal population dynamics between the two locations. Wilson et al. (18) observed poor survival of *A. parasiticus* in Arizona. The developing pods in the Georgia tests were challenged by a mixture of *A. flavus* and *A. parasiticus*, whereas those in the Arizona test were challenged primarily by *A. flavus*.

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