Comparison of Aflatoxin Production in Normal- and High-Oleic Backcross-Derived Peanut Lines

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ABSTRACT

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The effect of the high-oleate trait of peanut on aflatoxin production was tested by comparing normal oleic lines with high-oleic backcross-derived lines. Seeds were blanched, quartered, and inoculated with *Aspergillus flavus* conidia, placed on moistened filter paper in petri dishes, and incubated for 8 days. In one experiment, dishes were stacked in plastic bags in a Latin square design with bags and positions in stacks as blocking variables. High-oleic lines averaged nearly twice as much aflatoxin as normal lines. Background genotype had no significant effect on aflatoxin content, and interaction between background genotype and oleate level was not detected. In a second experiment, dishes were arranged on plastic trays enclosed in plastic bags and stacked with PVC spacers between trays. Fungal growth and aflatoxin production were greater than in the first experiment. Background genotype, oleate level, and their interaction were significant. The mean of high-oleic lines was almost twice that of normal lines, but the magnitude of the difference varied with background genotype. Special care should be taken with high-oleic lines to prevent growth of *Aspergillus* spp. and concomitant development of aflatoxin contamination.

Additional keywords: *Arachis hypogaea*

Aflatoxin contamination of peanut (*Arachis hypogaea* L.) results from growth of toxigenic strains of *Aspergillus flavus* Link ex Fries and *A. parasiticus* Speare. Reduction of aflatoxin contamination of peanuts grown and sold in the United States remains a high priority of the U.S. peanut industry. Practices recommended to prevent aflatoxin contamination (20) include maintenance of near-optimum water relations in the crop through irrigation; control of pod- and seed-feeding insects; optimal timing of digging and harvest; avoidance of mechanical damage during cultivation, harvest, and postharvest handling; rapid postharvest drying; and maintenance of low temperature and relative humidity (RH) in storage. In spite of these precautions, damage and contamination can still occur. Resistant cultivars would be an effective and low-cost component of an integrated aflatoxin management program. Three mechanisms of resistance to *Aspergillus* have been identified: resistance to seed colonization (usually tested in vitro),

resistance to preharvest infection, and resistance to aflatoxin production.

Mixon and Rogers (14) proposed that peanut cultivars resistant to seed colonization by toxigenic *Aspergillus* species would be an effective means of reducing aflatoxin contamination. Mixon (9–13) released germ plasm lines and one cultivar, Sunbelt Runner, exhibiting this characteristic. Likewise, scientists at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) screened large numbers of lines for resistance to in vitro seed colonization by *A. flavus* (IVSCAF), and IVSCAF-resistant improved lines have been developed and released (8). However, several concerns about the utility of IVSCAF resistance have been raised in the United States. Lines reported to be IVSCAF-resistant were found to exhibit as much as or more preharvest contamination than IVSCAF-susceptible cultivars (2) and to develop more aflatoxin contamination under storage at high RH (21). The apparent failure of IVSCAF resistance to hold up under field conditions has prompted the U.S. peanut industry to seek germplasm with resistance to preharvest aflatoxin contamination for the past 10 years. Because of this emphasis, there has been little effort expended in identifying genotypes that support reduced levels of aflatoxin production when subjected to postharvest conditions highly

conducive to fungal growth and aflatoxin synthesis.

Linoleic acid has been variously reported to stimulate or inhibit production of aflatoxin via lipoxxygenase activity in vitro (1,3–5). Fabbri et al. (4) observed that aflatoxin production is increased by lipoperoxides of polyunsaturated fatty acids. In contrast, Doehlert et al. (3) reported that addition of either lipase, linoleic or linolenic acid to the soybean homogenate inhibited germination of *A. flavus* conidia, whereas oleic acid had no effect compared with untreated homogenates. Zeringue et al. (23) found that inhibition of aflatoxin production by volatile compounds emitted by ground corn (*Zea mays* L.) kernels was associated with elevated levels of linoleic acid in their seed oil.

Interest in the fatty acid composition of seed oil in peanut was due originally to its role in flavor and shelf life (16). Researchers at the University of Florida discovered a natural variant with an elevated level of oleic acid and a correspondingly reduced level of linoleic acid. This trait improves the oxidative stability of the oil and extends the shelf life of products made from high-oleic peanuts. Current objectives of all U.S. peanut breeding programs include development of cultivars with elevated levels of oleic acid and depressed levels of linoleic acid. The first high-oleic cultivar was released in 1995 (6); numerous others have been released since or are currently under development. There is a strong negative correlation between levels of oleate and linoleate in peanut genotypes. In this paper, we will refer to the “high-oleic” trait because that is the common term in the peanut industry, but the reader should be aware that the trait could as easily be called the “low-linoleate” trait.

Because linoleate has been reported to affect the production of aflatoxin in vitro, there has been speculation that high-oleic peanuts would accumulate aflatoxin at levels different from normal-oleic peanuts. The only published work in this area was by Holbrook et al. (7), who reported no difference between the two fatty acid types in preharvest aflatoxin contamination measured on field-grown seeds. The objective of this study was to evaluate the influence of the high-oleate trait on the ability of peanuts to support postharvest production of aflatoxin under laboratory condi-

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tions favoring fungal growth and aflatoxin development.

MATERIALS AND METHODS

High-oleic peanut lines (Table 1) were developed by backcrossing the genes from University of Florida mutant line F435 (15) into eight large-seeded virginia cultivars: NC 7, NC 9, NC 10C, NC-V 11, NC 12C, Gregory, VA-C 92R, and VA 93B. High-oleic lines were selected after two, three, or four backcrosses to the cultivar. Lines were evaluated in two tests using different experimental designs and incubation methods.

For test 1, 100 sound mature kernels (SMK) of each of five high-oleic lines produced under irrigation in the 1999 crop season (lines derived from NC 7, NC 9, NC 10C, NC-V 11, and VA-C 92R) were assayed for fatty acid profile using the technique of Zeile et al. (22). Samples were extracted for 12 h in 1 ml of solvent (chloroform:hexane:methanol, 8:5:2 vol/vol/vol) in stoppered test tubes. Fatty acid methyl esters of the lipid extracts were prepared using sodium methoxide. The samples were analyzed by gas chromatography using an HP 5890 Series II GC (Agilent Technologies, Inc., Wilmington, DE) equipped with an AT-Silar 30 m $\times$ 0.53 mm column (same source). Operating conditions were 1- μ l injection volume, a 20:1 split ratio, and He carrier gas flow of 6 ml min⁻¹. Temperatures were 250, 200, and 275°C for the injector, oven, and FID, respectively. Chromatograms were analyzed using HP ChemStation software.

Fifty high-oleic seeds (average oleate 810 $\pm$ 14 g kg⁻¹, linoleate 34 $\pm$ 1 g kg⁻¹) were chosen from each backcross-derived line for use in the experiment, as were 50 normal-oleic (average oleate about 580 g kg⁻¹, linoleate 280 g kg⁻¹) SMK of the cultivars grown in 1999. Thus there were five pairs of normal- and high-oleic lines, each pair with a distinct background genotype. To avoid problems due to prior contamination of seeds with aflatoxin, all seeds used in the tests were visually inspected for apparent freedom from fungal colonization either on the seed surface or within the lumen. Seeds were not assayed for aflatoxin content prior to conducting the bioassay.

Five seeds were chosen at random from a particular line for each experimental unit. The cotyledons of each seed were separated to permit the seed to rest without rolling. The testa was removed from each seed half to eliminate the potential barrier to *A. flavus* growth. Because many cotyledons of the high-oleic lines cracked at the point where tissue was sampled for fatty acid analysis, each unbroken cotyledon was cut perpendicularly to the main axis of the seed into two roughly equal parts or "seed quarters." The 20 quarters 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 5 ml of sterile water in a 10-cm plastic petri dish. Each piece was inoculated with 25 μ l of a suspension containing approximately 1×10^6 conidia per ml of *A. flavus* strain NRRL 3357 (National Center for Agricultural Utilization Research, Peoria, IL). Groups of 10 samples were stacked, enclosed in plastic bags to prevent desiccation, and placed in an incubator at 28°C. The five pairs of lines were tested in a 10 $\times$ 10 Latin square experimental design; each line appeared once in each bag of 10 units and once at each position. Samples were checked for desiccation on the second day of incubation, and 1 ml of sterile water was added to each to keep the filter paper near saturation. After 8 days, samples were removed from the incubator and rated separately for mycelial 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, or all fluffy colonies) in one-point increments. These growth-related characteristics were found in previous methodological studies (data not shown) to be related to aflatoxin production levels, particularly at rating levels below 5.

In test 2, 25 seeds of each of the eight high-oleic lines (average oleate 814 $\pm$ 14 g kg⁻¹, average linoleate 29 $\pm$ 7 g kg⁻¹) and eight corresponding normal lines were used in the test. The seeds were field-grown under irrigation in the 2000 crop season. Sample preparation and inoculation procedures were the same as for test 1, but the eight pairs of lines were tested in a balanced 4 $\times$ 4 square lattice design with five replicates. The incubation procedure was altered as follows. The 16 petri dishes in each replicate were arranged in a single layer on a plastic tray in four rows and four columns with columns as blocks. The trays were then enclosed individually in large plastic bags. The five trays were stacked in the incubator. Short sections of PVC pipe were inserted between adjacent trays in the

stack to bear the weight and avoid downward pressure on the petri dishes' lids. The trays were rotated in vertical position each of the 8 days of incubation at 28°C.

Following incubation, samples were dried for 1 day at 60°C and for another 3 days at 40°C, then ground to a friable meal in a coffee mill and stored in scintillation vials until analyzed for aflatoxin content by high-pressure liquid chromatography in the NCSU Mycotoxin Lab in the Department of Poultry Science. Aflatoxin was extracted from a 2-g ground sample with acetonitrile-water (84 + 16 vol/vol) in a 5/1 extractant volume/sample weight ratio. 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 (18,19). Aflatoxins B1, B2, and total aflatoxin were measured. Aflatoxin data were log-transformed [$Y' = \ln(Y + 0.5)$] to stabilize error variance and subjected to analysis of variance by general linear model procedure (PROC GLM) of SAS version 8.2 (SAS Institute, Cary, NC). 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 values in parts per billion.

RESULTS AND DISCUSSION

Means for both untransformed and transformed aflatoxin values are presented because, although the log transformation resulted in lower coefficients of variation and greater precision of comparison, regulatory agencies do not perform such transformations prior to calculating mean aflatoxin content in samples taken from peanuts in trade. There were some discrepancies in rank between means of untransformed data and back-transformed values of means of transformed data. The latter were generally lower than the former.

Although it would not be possible to assert with complete confidence that none of the peanut samples had any aflatoxin contamination prior to conducting the experiment, there is indirect evidence that a priori contamination was not a problem. None

Table 1. Pedigrees of high-oleic lines developed by back-crossing the Florida high-oleic genes into large-seeded virginia-type cultivars.

Line	Background genotype	Parentage ^y	Pedigree ^z
N00091ol	NC 7	NC 7*5 / F435	BC4F1-03-11: F04
N00092ol	NC 9	NC 9*5 / F435	BC4F1-01-03: F04
N00083ol	NC 10C	NC 10C*4 / F435	BC3F1-01-22: F05
N00087ol	NC-V 11	NC-V 11*4 / F435	BC3F1-02-01: F05
N00089ol	VA-C 92R	VA-C 92R*4 / F435	BC3F1-01-10: F05
N00095ol	NC 12C	NC 12C*3 // NC 9*2 / F435	BC2F1-01-01: F04
N00098ol	Gregory	Gregory*3 // NC 9*2 / F435	BC2F1-01-03: F04
N00102ol	VA 93B	VA 93B*3 // NC-V 11*2 / F435	BC2F1-02-11: F04

^y Purdy et al.'s method for illustrating parentage is used (17).

^z Pedigrees indicate the BC_nF₁ and BC_nF₂ plants selected and the current generation of the test material.

of the seeds used for the test had any visibly obvious fungal colonization either on the exterior surface of the seed or within the lumen between cotyledons. There was no aflatoxin G1 or G2 detected in any sample, indicating that there was no contamination due to *A. parasiticus*. All seeds were produced in irrigated fields, minimizing the risk of preharvest aflatoxin contamination.

Test 1. Pronounced position effects were observed in growth and color of mycelium in stacked experimental units with the most vigorous growth and darkest color developing in the upper units. Fungal growth and color development appeared to be depressed in the lower units. We observed that the petri dishes at lower levels became sealed shut because of the weight of the dishes on top of them. This may have resulted in insufficient air supply in the

lower dishes. There was weak to moderate positive correlation ($0.395 \leq r \leq 0.712$) of the growth-related ratings for mycelial growth and color with aflatoxin contents, raw or transformed. Inspection of the data on individual experimental units showed that units with growth or color ratings less than 5 had low aflatoxin production levels, while those with ratings greater than 5 exhibited a range of aflatoxin production from low to high. Position effects were detected ($P < 0.0001$) for all traits rated or measured (Table 2). In each case, means were greater for units higher in the stack and less for units lower in the stack (data not shown), justifying the use of the Latin square design. Bag effects were detected for growth score ($P = 0.036$), transformed aflatoxin B1 ($P = 0.026$), and transformed total aflatoxin content ($P = 0.026$). Effects

of background genotype were detected for growth score ($P = 0.049$), and for the transformed values of aflatoxins B1 ($P = 0.036$), B2 ($P = 0.023$), and their total ($P = 0.034$). Oleate level had no effect on growth, color, or development of fluffy colonies, but did influence ($P < 0.05$) contents of aflatoxins B1 and B2 and total aflatoxin whether transformed or not. No interaction between background genotype and oleate level was detected for any trait.

Examining the back-transformed aflatoxin contents (Table 3), the accumulation of toxins in the five background genotypes occurred in the following order: NC 7, VA-C 92R, NC-V 11, and NC 9 and NC 10C, which were very close in value to each other. Mean content of each aflatoxin form was greater in the high-oleic variants (Table 4). Averaged across all background

Table 2. Mean squares from analysis of variance of five near-isogenic pairs of normal- and high-oleic peanut lines

Source	df	Growth score ^x	Color score ^x	Fluffy score ^x	Aflatoxin B1 ^y	ln (B1+0.5)	Aflatoxin B2 ^y	ln (B2+0.5)	Aflatoxin B1+B2 ^y	ln(B1+B2+0.5)
Bag	9	4.28** z	6.12	20.08	35,660,347	2.5245**	48,397	2.6384*	38,302,627	2.5307**
Position	9	26.72***	80.16***	20.16	165,518,877***	7.4354***	227,378***	10.8353***	177,994,607***	7.5163***
Lines	9	3.28	3.16	14.40	56,662,170**	2.6707**	82,013*	3.5317**	61,017,065**	2.6864**
Genotype	4	5.04**	5.88	25.72	36,591,233	3.0334**	50,025	4.6373**	39,268,211	3.0638**
Oleate level	1	0.04	0.64	1.44	226,656,199***	5.7815**	336,864***	6.1234**	244,469,017***	5.7881**
Genotype × oleate	4	2.32	1.12	6.36	34,234,600	1.5302	50,289	1.7783	36,902,931	1.5336
Error	72	2.00	4.08	9.96	27,105,186	1.1029	41,070	1.5261	29,236,146	1.1123
Mean		8.4	5.4	2.0	5,349	7.81	184	4.24	5,533	7.84
CV (%)		16.7	37.4	157.7	97.3	13.4	110.0	29.2	97.7	13.5

^x 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.

^y Parts per billion.

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

Table 3. Main effect means for background genotypes averaged across two oleate levels (normal and high)

Source	Growth score ^x	Color score ^x	Fluffy score ^x	Aflatoxin B1 ^y	ln (B1+0.5)	Back-transformed B1 ^y	Aflatoxin B2 ^y	ln (B2+0.5)	Back-transformed B2 ^y	Aflatoxin B1+B2 ^y	ln(B1+B2+0.5)	Back-transformed B1+B2 ^y
NC 7	8.90 a ^z	5.86	3.60	6,368	8.35 a	4,243 a	219	4.91 a	135 a	6,588	8.38 a	4,380 a
NC 9	8.30 ab	5.20	2.60	5,205	7.43 b	1,687 b	198	3.84 b	46 b	5,403	7.46 b	1,737 b
NC 10C	8.20 ab	4.66	1.00	4,016	7.44 b	1,708 b	138	3.74 b	42 b	4,154	7.47 b	1,751 b
NC-V 11	7.86 b	5.30	0.90	4,099	7.83 ab	2,522 ab	126	4.19 ab	66 ab	4,225	7.86 ab	2,589 ab
VA-C 92R	9.06 a	6.00	1.90	7,057	7.99 ab	2,955 ab	239	4.50 ab	89 ab	7,297	8.02 ab	3,045 ab
LSD _{0.05}	0.90	ns	ns	ns	0.66		ns	0.78		ns	0.66	

^x 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.

^y Parts per billion.

^z Means followed by the same letter are not significantly different ($P < 0.05$) by *t* test. ns = not significant.

Table 4. Main effect means for oleate levels averaged across five background genotypes

Source	Growth score ^x	Color score ^x	Fluffy score ^x	Aflatoxin B1 ^y	ln (B1+0.5)	Back-transformed B1 ^y	Aflatoxin B2 ^y	ln (B2+0.5)	Back-transformed B2 ^y	Aflatoxin B1+B2 ^y	ln(B1+B2+0.5)	Back-transformed B1+B2 ^y
High	8.44	5.32	2.12	6,855 a ^z	8.05 a	3,136 a	242 a	4.48 a	88 a	7,097 a	8.08 a	3,226 a
Normal	8.48	5.48	1.88	3,844 b	7.57 b	1,938 b	126 b	3.99 b	53 b	3,970 b	7.60 b	1,994 b
LSD _{0.05}	ns	ns	ns	2,076	0.42		81	0.49		2,156	0.42	

^x 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.

^y Parts per billion.

^z Means followed by the same letter are not significantly different ($P < 0.05$) by *t* test. ns = not significant.

genotypes, there was nearly a twofold increase in aflatoxin B1, B2, and total aflatoxin in the high-oleic lines compared with the normal lines. The increase was apparent in both untransformed and back-transformed data.

Although the interaction between background genotype and oleate level was not statistically significant (Table 2), the variance among high-oleic lines was substantially greater than that among normal lines for all three aflatoxin components (6,240,727 versus 841,856 ppb² for aflatoxin B1, 9350 versus 682 ppb² for aflatoxin B2, and 6,730,233 versus 886,881 ppb² for total aflatoxin). Two-tailed *F* tests of the variances were not significant for any component in spite of the up to eightfold difference in magnitude. Differences

between the high- and normal-oleic members of individual pairs were not consistent across background genotypes. The difference was greatest in the VA-C 92R background, followed by NC 9. In the NC-V 11 background, the normal line actually produced slightly more aflatoxins B1 and B2 than the high-oleic line (data not shown).

Test 2. The use of trays separated by plastic spacers to relieve any down-pressure on petri dishes at lower levels resulted in better growth, more uniform color development, fewer fluffy colonies, and higher levels of aflatoxin production than did use of stacked petri dishes (Table 5). The coefficient of variation was reduced for all traits. Because of the increase in precision achieved with this technique, more effects were detected in test 2. The

effects of background genotype, oleate level, and their interaction were significant ($P < 0.0415$) for growth and color scores and for all components of aflatoxin, untransformed or transformed, except the effect of interaction between background genotype and oleate level on untransformed aflatoxin B2. Fluffy colony scores were affected only by tray.

The order of background genotypes in accumulating aflatoxins was dependent on whether or not the data were log transformed (Table 6). The ordering was not consistent with that found in test 1, although seeds of NC 7 were again the most contaminated. In general, NC 7 and NC 12C had high levels, VA-C 92R and VA 93B had the lowest levels, and the other genotypes were intermediate.

Table 5. Mean squares from analysis of variance of five near-isogenic pairs of normal- and high-oleic peanut lines

Source	df	Growth score ^x	Color score ^x	Fluffy score ^x	Aflatoxin B1 ^y	ln (B1+0.5)	Aflatoxin B2 ^y	ln (B2+0.5)	Aflatoxin B1+B2 ^y	ln(B1+B2+0.5)
Tray	4	0.89	0.20	1.30 ^z	3,714,506,972**	4.0897**	3,864,621**	4.1481**	3,954,951,476**	4.0909**
Entry	15	3.69**	3.17**	0.27	918,115,370**	1.0394**	1,654,824**	1.8581**	995,579,308**	1.0582**
Genotype	7	2.77**	2.80**	0.21	509,283,101**	0.5091**	1,165,251**	0.9786**	557,751,716**	0.5200**
Oleate level	1	21.01**	15.31**	0.31	8,509,983,647**	9.3954**	14,702,776**	17.8960**	9,232,133,290**	9.5949**
Genotype × oleate	7	2.13*	1.80**	0.31	242,395,029*	0.3759**	280,404	0.4466**	256,756,332*	0.3769**
Error	60	0.79	0.55	0.39	105,610,306	0.1102	179,045	0.1442	113,681,277	0.1107
Mean		9.2	9.3	1.1	36,907	10.3137	1141	6.7622	38,048	10.3424
CV (%)		9.6	8.0	57.2	27.8	3.2	37.1	5.6	28.0	3.2

^x 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.

^y Parts per billion.

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

Table 6. Mean for eight background genotypes averaged across oleic acid levels

Genotype	Growth score ^x	Color score ^x	Fluffy score ^x	Aflatoxin B1 ^y	ln (B1+0.5)	Back-transformed B1 ^y	Aflatoxin B2 ^y	ln (B2+0.5)	Back-transformed B2 ^y	Aflatoxin B1+B2 ^y	ln(B1+B2+0.5)	Back-transformed B1+B2 ^y
NC 7	9.7 ab ^z	9.7 ab	0.9	45,572 a	10.6698 a	43,035 a	1,416 b	7.1724 a	1,302 a	46,988 a	10.6999 a	44,353 a
NC 9	9.7 ab	9.6 abc	1.1	39,641 ab	10.4412 abc	34,240 abc	1,247 bc	6.9575 ab	1,051 ab	40,888 ab	10.4716 abc	35,298 abc
NC 10C	8.9 cd	9.0 cde	1.3	35,268 bc	10.3072 bcd	29,947 bcd	1,034 cd	6.7454 bc	850 bc	36,302 bc	10.3355 bcd	30,807 bcd
NC-V 11	9.0 bcd	9.1 bcd	1.1	35,500 bc	10.1421 d	25,389 d	1,018 cd	6.4794 c	651 c	36,517 bc	10.1678 d	26,051 d
NC 12C	9.8 a	10.0 a	1.0	48,200 a	10.5797 ab	39,330 ab	1,816 a	7.2078 a	1,349 a	50,015 a	10.6140 ab	40,702 ab
Gregory	9.6 abc	9.5 abc	1.2	32,892 bc	10.1571 cd	25,774 cd	962 cd	6.5713 c	714 c	33,854 bc	10.1850 cd	26,502 cd
VA-C 92R	8.4 d	8.4 e	1.2	27,781 c	10.0716 d	23,662 d	764 d	6.4266 c	618 c	28,545 c	10.0976 d	24,285 d
VA 93B	8.8 d	8.8 de	0.9	30,399 c	10.1406 d	25,350 d	872 cd	6.5370 c	690 c	31,271 c	10.1678 d	26,051 d
LSD _{0.05}	0.8	0.7	ns	9,193	0.2969		379	0.3397		9,538	0.2977	

^x 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.

^y Parts per billion.

^z Means followed by the same letter are not significantly different ($P < 0.05$) by *t* test. ns = not significant.

Table 7. Means for oleate levels averaged across background genotypes

Oleate	Growth score ^x	Color score ^x	Fluffy score ^x	Aflatoxin B1 ^y	ln (B1+0.5)	Back-transformed B1 ^y	Aflatoxin B2 ^y	ln (B2+0.5)	Back-transformed B2 ^y	Aflatoxin B1+B2 ^y	ln(B1+B2+0.5)	Back-transformed B1+B2 ^y
High	9.8 ^z	9.7 ^z	1.2	47,220**	10.6564**	42,461**	1,570**	7.2351**	1,387**	48,790**	10.6887**	43,859**
Normal	8.7	8.8	1.0	26,593	9.9710	21,396	712	6.2892	538	27,305	9.9961	21,940
LSD _{0.05}	0.4	0.3	ns	4,597	0.1485		189	0.1699		4,769	0.1488	

^x 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.

^y Parts per billion.

^z ** Denotes means for high-oleic lines that are significantly different ($P < 0.01$) from the mean for normal-oleic lines by *t* test. ns = not significant.

As was found in test 1, the high-oleic trait increased aflatoxin contamination across genotypes. On average, the increase was again nearly twofold (Table 7), but there were detectable differences in the amount of increase depending on the background genotype. This variation in difference produced the observed interaction (Table 5). The magnitude of the difference between high- and normal-oleic lines (Table 8) ranged from 21 to 244% for untransformed aflatoxin B1 (13 to 330% for back-transformed values), 48 to 367% for untransformed aflatoxin B2 (47 to 502% for back-transformed values), and 22 to 247% for untransformed aflatoxin B1 (14 to 333% for back-transformed values). The increases were not significant for several aflatoxin components in NC 7, the background genotype with the greatest average levels of contamination, and in VA 93B, a background genotype with relatively low average contamination in this study. There were two cases of inconsistency in the results using transformed versus untransformed data. In the NC 7 background, untransformed aflatoxin B2 was higher (1,792 versus 1,098 ppb, $P = 0.021$) in the high-oleic line than in the normal, while the difference was not significant in the transformed data. In the VA 93B background, the converse was observed: the difference was significant ($P = 0.007$), when using transformed data but not when using untransformed data. These are minor inconsistencies when viewed in the overall context of the study. In no case in test 2 was aflatoxin contamination even numerically lower in the high-oleic line than in the corresponding normal-oleic line.

The high-oleic lines had increased ability to support production of aflatoxin. It should be reiterated that the increase in

oleate level associated with the so-called high-oleic trait of peanut is achieved by a corresponding reduction in the level of linoleate, the fatty acid reported to affect aflatoxin production. The extent to which high-oleate peanuts may be predisposed to aflatoxin contamination may be modulated by other genotypic factors. These differences could be caused by epistatic interaction between the genes conditioning the high-oleic trait and other genes in the background genotype, or they could be the result of linkage between the high-oleate gene and the gene or genes influencing aflatoxin production. It is not possible to differentiate between the two possibilities using the data collected in this study. It should be possible to differentiate them by studying high-oleic lines developed by mutagenesis rather than by backcrossing.

Under field conditions, Holbrook et al. (7) found that low linoleic acid level had no measurable effect on preharvest aflatoxin contamination, and proposed that reducing linoleic fatty acid composition does not appear to be a viable approach toward reducing preharvest aflatoxin contamination in peanut. The results of our study may appear to be inconsistent with those of Holbrook et al., but there were two fundamental differences in the methods employed in the two studies. First, Holbrook et al. (7) monitored preharvest aflatoxin contamination in seeds from normal- and high-oleic plants inoculated in the field and deliberately subjected to drought stress while we examined the effect of the high-oleic trait on postharvest contamination in seeds under laboratory conditions chosen to favor fungal growth and aflatoxin synthesis. The results of the two studies are not directly comparable. Results of our investigation are applicable

to seeds in storage. Second, Holbrook et al. (7) compared an array of high-oleic lines with two checks: the standard cultivar Florunner and the multiply disease-resistant germ plasm line Tifton 8. Their contrast between genotypes confounded the high-oleic trait with numerous other genetic differences. We compared pairs of lines with nearly the same background genotypes, one member of the pair with normal oleate level and the other with the high-oleate trait. This pairing helps separate the effect of background genotype from that of contribution of the fatty acid profile.

Results from these studies have important implications for postharvest handling and storage in peanuts. Because these data suggest increased ability of high-oleic lines to support aflatoxin production during storage, it is critical for growers, shellers, and processors to take all precautions to prevent growth of *Aspergillus* spp. in seed of cultivars of this type. These data also support the importance of developing high-oleic lines with background genotypes that have reduced ability to support aflatoxin production to offset the increased susceptibility conditioned by the high-oleic trait.

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Table 8. Means of eight near-isogenic pairs of normal- and high-oleic peanut lines

Genotype	Oleate	Growth score ^x	Color score ^x	Fluffy score ^x	Aflatoxin B1 ^y	ln (B1+0.5)	Back-transformed B1 ^y	Aflatoxin B2 ^y	ln (B2+0.5)	Back-transformed B2 ^y	Aflatoxin B1+B2 ^y	ln(B1+B2+0.5)	Back-transformed B1+B2 ^y
NC 7	High	9.8 ^{ns} z	9.8 ^{ns}	1.2 ^{ns}	49,935 ^{ns}	10.7318 ^{ns}	45,786 ^{ns}	1,734 ^{**}	7.3652 ^{ns}	1,580 ^{ns}	51,669 ^{ns}	10.7658 ^{ns}	47,371 ^{ns}
	Normal	9.6	9.6	0.6	41,209	10.6078	40,448	1,098	6.9796	1,074	42,307	10.6341	41,527
NC 9	High	10.0 ^{ns}	10.0 [*]	0.8 ^{ns}	48,470 ^{***}	10.7432 ^{***}	46,315 ^{***}	1,635 ^{***}	7.3351 ^{***}	1,533 ^{***}	50,105 ^{***}	10.7759 ^{***}	47,854 ^{***}
	Normal	9.4	9.2	1.4	30,812	10.1391	25,314	859	6.5799	720	31,671	10.1673	26,036
NC 10C	High	10.0 ^{***}	9.8 ^{***}	1.4 ^{ns}	46,986 ^{***}	10.6330 ^{***}	41,483 ^{***}	1,435 ^{***}	7.1702 ^{***}	1,300 ^{***}	48,421 ^{***}	10.6641 ^{***}	42,790 ^{***}
	Normal	7.8	8.2	1.2	23,550	9.9813	21,619	634	6.3205	555	24,184	10.0070	22,180
NC-V 11	High	9.8 ^{***}	9.8 ^{***}	1.2 ^{ns}	55,022 ^{***}	10.8714 ^{***}	52,648 ^{***}	1,676 ^{***}	7.3758 ^{***}	1,596 ^{***}	56,698 ^{***}	10.9013 ^{***}	54,248 ^{***}
	Normal	8.2	8.4	1.0	15,977	9.4128	12,243	359	5.5830	265	16,336	9.4343	12,510
NC 12C	High	10.0 ^{ns}	10.0 ^{ns}	1.0 ^{ns}	60,625 ^{***}	10.8740 ^{***}	52,783 ^{***}	2,486 ^{***}	7.6350 ^{***}	2,069 ^{***}	63,111 ^{***}	10.9126 ^{***}	54,863 ^{***}
	Normal	9.6	10.0	1.0	35,774	10.2855	29,305	1,145	6.7805	880	36,919	10.3155	30,196
Gregory	High	10.0 ^{ns}	9.6 ^{ns}	1.2 ^{ns}	43,645 ^{***}	10.5883 ^{***}	39,667 ^{***}	1,410 ^{***}	7.1859 ^{***}	1,320 ^{***}	45,055 ^{***}	10.6212 ^{***}	40,995 ^{***}
	Normal	9.2	9.4	1.2	22,138	9.7260	16,747	515	5.9567	386	22,653	9.7488	17,132
VA-C 92R	High	9.6 ^{***}	9.6 ^{***}	1.4 ^{ns}	38,710 ^{***}	10.4673 ^{***}	35,147 ^{***}	1,141 ^{***}	6.9430 ^{***}	1,035 ^{***}	39,851 ^{***}	10.4964 ^{***}	36,185 ^{***}
	Normal	7.2	7.2	1.0	16,852	9.6759	15,929	386	5.9101	368	17,238	9.6988	16,298
VA 93B	High	8.8 ^{ns}	9.0 ^{ns}	1.0 ^{ns}	34,369 ^{ns}	10.3419 [*]	31,005 [*]	1,041 ^{ns}	6.8708 ^{***}	963 ^{***}	35,410 ^{ns}	10.3726 [*]	31,971 [*]
	Normal	8.8	8.6	0.8	26,430	9.9392	20,727	702	6.2032	494	27,132	9.9631	21,228
LSD _{0.05}		1.1	0.9	0.8	13,001	0.4199		535	0.4805		13,489	0.4210	

^x 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.

^y Parts per billion.

^z *, **, *** Denote means for high-oleic lines that are significantly different ($P < 0.10$, $P < 0.05$, or $P < 0.01$) from the mean for the corresponding normal-oleic lines by t test. ns = not significant.

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