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Comparative Susceptibility of Four Experimental Peanut Lines and the Cultivar Florunner to Preharvest Aflatoxin Contamination

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ABSTRACT

Four peanut genotypes, selected as resistant to invasion by *Aspergillus flavus* in laboratory screening with rehydrated, stored seed and Florunner cultivar were subjected to preharvest drought and temperature conditions conducive to *A. flavus* invasion and aflatoxin contamination. Preharvest aflatoxin contamination of peanuts has been previously correlated with geocarposphere temperature and moisture conditions during drought. All genotypes tested were highly contaminated with aflatoxin. This study indicates that a critical assessment should be made of the value of using the current laboratory method to select germplasm for resistance to *A. flavus* invasion and assuming resistance to aflatoxin contamination under field conditions.

Key Words: Peanuts, drought resistance, resistant varieties.

Economic losses experienced by the US peanut industry, which are attributable to aflatoxin contamination, have occurred during years with peanut growing seasons characterized by late season drought. Studies over the past four years, in environmental control plots at the USDA, ARS, National Peanut Research Laboratory, have elucidated the geocarposphere conditions during drought that result in preharvest aflatoxin contamination of the peanut cultivar Florunner (1,2,3,4,5,7,14,15). Peanuts subjected to the described drought stress conditions during the last 30-50 days of the growing season were contaminated with aflatoxin at harvest, though not always visibly moldy (5,14). Peanut kernel invasion by *Aspergillus flavus* Link and *A. parasiticus* Speare prior to harvest occurred under conditions both conducive and non-conducive for development of aflatoxin (15). Higher percentages of kernels were invaded when peanuts were subjected to late season drought as compared to peanuts not subjected to preharvest drought stress (5,14). No aflatoxins were detected in peanuts that were grown without stress, although these peanuts were invaded by the toxigenic fungus to a considerable extent (5,14).

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The possible use of genetic resistance to kernel invasion by *A. flavus* and subsequent aflatoxin production has been investigated (8,9,10,11,12,13). Recent research has demonstrated significant postharvest varietal resistance to *A. flavus* invasion of rehydrated mature peanut kernels (8,10,11) and the data have been interpreted to indicate a possible resistance, or tolerance, to preharvest invasion under field conditions. Wilson *et al.* (17) subjected unshelled and shelled peanuts from germplasm accessions previously reported to be resistant to colonization by *A. flavus* to high humidity conditions. Aflatoxin levels that occurred were compared with those of an easily colonized genotype and a commercially grown cultivar, Florunner. All tested genotypes had appreciable levels of aflatoxin after 9-10 days storage in high humidity. They concluded that peanut genotypes with postharvest laboratory resistance to seed colonization by *A. flavus* did not show any advantage over genotypes that exhibited moderate laboratory resistance to colonization when both were subjected to high humidity environments.

The purpose of the present study was to evaluate and compare the resistance of four genotypes selected with a postharvest laboratory screening method and Florunner, a current commercially grown genotype, to preharvest kernel invasion by *A. flavus* and subsequent aflatoxin contamination. All five genotypes were challenged in rainfall control plots with drought conditions and elevated soil temperatures previously found to be optimum for preharvest aflatoxin contamination of the cultivar, Florunner (2,4,5,7).

Materials and Methods

Peanut genotypes tested were A7404, A72118, UF77316, UF791041, and Florunner. On May 1, 1984, 6 rows of peanuts were planted in each of 2 rainfall control plots (3) containing Tifton sandy loam soil in a 91-cm row pattern. Two rows of each of two of the resistant genotypes along with 2 rows of Florunner were planted in each plot. The plots were 5.5 x 12.3 m and had been constructed to prevent lateral moisture movement into the plots from surrounding soil. Both plots were maintained the same throughout the growing season and were irrigated until ninety-two days after planting when precise soil environmental

conditions previously determined to be optimum for aflatoxin contamination (1,2,3,4,5,7,14,15) were initiated. Peanuts in the plots received no water after 92 days and the soil temperature was elevated throughout the remainder of the growing season until harvest (September 20, 1984; 142 days after planting). To accomplish soil temperature elevation, nine General Electic 73.2 m long, 240 V, 1600 W heating cables were installed back and forth across the plot with adjacent runs approximately 10 cm apart and 12.7 cm deep. The entire cross-sectional area of the plot was covered with the cable pattern. An on-off heating thermostat was used to regulate the operation of the cables and maintain the temperature of the soil in the plot above a minimum prescribed limit. Soil temperature and moisture measurements, 5 cm below the soil surface, were obtained automatically every two hours throughout the growing season (1,3). At harvest, peanuts were dug by hand, dried in inverted windrows protected from rainfall, picked with a plot combine, and shelled with a sample sheller. All kernels from each cultivar were then separated by vibratory screens into commercial grade categories based on kernel diameter (jumbo, medium, Number one (No. 1), split, other edible, oil stock, and loose shelled kernels (LSK)). Visibly damaged kernels were removed from each category and combined to form a separate category (damaged). The undamaged kernels of each category were then analyzed for aflatoxin except for a small portion separated for an evaluation of *A. flavus* invasion. Previous to the aflatoxin analysis, categories with kernels weighing more than 200 g were divided into 1-5 subsamples of 100-200 g to facilitate analysis of all kernels. Aflatoxin analyses were accomplished with a high pressure liquid chromatography method (2).

Results and Discussion

Geocarposphere temperatures and moisture levels for the treament period at the 5 cm deep level for the plots are shown in Table 1. Mean soil temperatures of the plots were not significantly different. Mean soil moistures were different for the 2 plots but the tensions in both were greater than 3 bars and both plots were in an extreme drought condition during the treatment period.

Table 1. Mean geocarposphere temperatures and moisture levels at the 5 cm deep level during the treatment period (97-142 days after planting).

Plot	Treatment	Mean Soil Temperature (C)	Mean Soil Moisture (bars tension)
1	Drought	29.1 a[1/]	3.75 a
2	Drought	29.1 a	4.53 b

[1/] Means in each column followed by the same letter are not significantly different at the 5 percent level as evaluated by the Waller-Duncan K-Ratio T-Test (16).

Results of aflatoxin analyses of the various commercial grade categories of the 5 genotypes are presented in Table 2. Peanuts from all genotypes and size categories were contaminated with aflatoxin except the Florunner and UF791041 jumbo kernels and the A72118 LSK. No significant differences in aflatoxin levels between genotypes were indicated in any one of the edible size categories. Some differences in aflatoxin levels occurred between genotypes in the inedible categories; however, none of the experimental genotypes had statistically lower levels in these categories than the commercial cultivar Florunner except the A72118 damaged kernels. The damaged kernels contained the highest levels of aflatoxin for each genotype tested. Also, toxin levels were generally inversely proportional to kernel size by genotype excluding the damaged kernels.

Table 2. Aflatoxin contamination[1] (ppb) by commercial size category for the peanut genotypes evaluated.

Size Category	Genotypes[2/]				
Edible Kernels	Florunner	UF77316	A72118	UF791041	A7404
Jumbo	ND[3/] a A	174 a A	82 a A	ND a A	6 a A
Medium	283 a A	704 a A	424 a A	616 a A	1144 a A
No. 1	578 a A	3290 a AB	10 a A	834 a A	1420 a A
Split	174 a A	395 a A	201 a A	22 a A	192 a A
Other edible	1922 a AB	924 a A	258 a A	1036 a A	1302 a A
Inedible Kernels					
Oil Stock	7040 bc B	11370 ab B	1070 c A	17700 a B	7880 bc A
LSK	549 a A	20 a A	ND a A	1266 a A	4115 a A
Damaged	57150 d C	335000 a C	29600 e B	77400 c C	293500 b B

[1/] Determined by high pressure liquid chromatography.

[2/] Means in each row followed by the same lowercase letter are not significantly different at the 5 percent level as evaluated by the Waller-Duncan K-Ratio T-Test (16). Similarly, means in each column followed by the same capital letter are not significantly different at the 5 percent level.

[3/] Not detected.

Davidson *et al.* (6) reported that the cultivar Sunbelt Runner, also reported to have resistance to seed colonization by *A. flavus* (12), showed much higher levels of *A. flavus* invasion and subsequent aflatoxin contamination when grown under field conditions ideal for aflatoxin contamination (1980 crop year). In that study, neither Sunbelt Runner nor Florunner exhibited any indication of "field" resistance to aflatoxin contamination.

Mehan and McDonald (8) have selected cultivars resistant to seed invasion and colonization by toxigenic *A. flavus* isolates and/or to aflatoxin production following invasion by the fungus. They have also screened several peanut cultivars for seed resistance in the field, both under natural conditions and with fungal inoculum added in the pod zone. Some cultivars with resistance to seed colonization also showed resistance to seed invasion by *A. flavus*. None of the cultivars tested have shown complete resistance to aflatoxin production; however, significant cultivar differences occurred. Some of the cultivars with good resistance to colonization by *A. flavus* proved good substrates for aflatoxin production, while others that were highly susceptible to fungal colonization were not good substrates for toxin production.

The results of the present study, in conjunction with those of Wilson *et al.* (17), Davidson *et al.* (6), and Mehan and McDonald (8), show conclusively that certain lines or cultivars selected by a laboratory screening method to be resistant to seed invasion and colonization by *A. flavus* were highly susceptible to preharvest aflatoxin contamination when challenged with environmental conditions conducive to aflatoxin contamination. These studies indicated the need for further research to develop an accurate screening method to identify genetic resistance to preharvest aflatoxin contamination in peanut germplasm if it exists.

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Literature Cited

1. Blankenship, P. D., R. J. Cole, and T. H. Sanders. 1980. Rainfall control plot facility at National Peanut Research Laboratory. Proc. Amer. Peanut Res. Educ. Soc. 12:46 (Abstr.).
2. Blankenship, P. D., R. J. Cole, T. H. Sanders, and R. A. Hill. 1984. Effect of geocarposphere temperature on preharvest colonization of drought-stressed peanuts by *Aspergillus flavus* and subsequent aflatoxin contamination. Mycopathologia 85:69-74.
3. Blankenship, P. D., R. J. Cole, T. H. Sanders, and R. A. Hill. 1983. Environmental control plot facility with manipulable soil temperature. Oleagineaux 38:615-620.
4. Cole, R. J., P. D. Blankenship, T. H. Sanders, and R. A. Hill. 1984. Relation of preharvest aflatoxin contamination to duration of environmental stress. Proc. Amer. Peanut Res. Educ. Soc. 16:45 (Abstr.).
5. Cole, R. J., T. H. Sanders, P. D. Blankenship, and R. A. Hill. Environmental conditions required to induce preharvest aflatoxin contamination of peanuts. Biodeterioration 6. In Press.
6. Davidson, J. I., R. A. Hill, R. J. Cole, A. C. Mixon, and R. J. Henning. 1983. Field performance of two peanut cultivars relative to aflatoxin contamination. Peanut Sci. 10:43-47.
7. Hill, R. A., P. D. Blankenship, R. J. Cole, and T. H. Sanders. 1983. Effects of soil moisture and temperature on preharvest invasion of peanuts by the *Aspergillus flavus* group and subsequent aflatoxin development. Appl. Environ. Microbiol. 45:628-633.
8. Mehan, V. K. and D. McDonald. 1980. Screening for resistance to *Aspergillus flavus* invasion and aflatoxin production in groundnuts. ICRISAT Groundnut Improvement Program Occasional Paper 2:1-15.
9. Mehan, V. K. and D. McDonald. 1984. Research on the aflatoxin problem in groundnut at ICRISAT. Plant Soil 79:255-260.
10. Mixon, A. C. 1979. Developing groundnut lines with resistance to seed colonization by toxin-producing strains of *Aspergillus* species. Pest. Artic. News Summ. 25:394-400.
11. Mixon, A. C. 1981. Reducing aflatoxon contamination in peanut genotypes by selection and breeding. J. Amer. Oil Chem Soc. 58:961A-966A.
12. Mixon, A. C. and K. M. Rogers. 1973. Peanut accessions resistant to seed infection by *Aspergillus flavus*. Agron. J. 64:560-562.
13. Mixon, A. C. and K. M. Rogers. 1975. Factors affecting *Aspergillus flavus* Lk. ex Fr. colonization of resistant and susceptible genotypes of *Arachis hypogaea* L. Peanut Sci. 2:18-22.
14. Sanders, T. H., R. J. Cole, P. D. Blankenship, and R. A. Hill. 1985. Relation of Environmental Stress Duration to *Aspergillus flavus* Invasion and Aflatoxin Production in Preharvest Peanuts. Peanut Sci. 12:90-93.
15. Sanders, T. H., R. A. Hill, R. J. Cole, and P. D. Blankenship. 1981. Effect of drought on occurrence of *Aspergillus flavus* in maturing peanuts. J. Amer. Oil Chem. Soc. 58:966A-970A.
16. Waller, R. A. and D. B. Duncan. 1969. A Bayes rule for the symmetric multiple comparison problem. Jour. Amer. Stat. Assoc. 64: 1484-1499.
17. Wilson, D. M., A. C. Mixon, and J. M. Troeger. 1977. Aflatoxin contamination of peanuts resistant to seed invasion by *Aspergillus flavus*. Phytopathology 67:922-924.

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