Peanut Science (1983) 10, 43-47

Field Performance of Two Peanut Cultivars Relative to Aflatoxin Contamination

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ABSTRACT

Two runner-type peanut cultivars, "Sunbelt Runner" and "Florunner," were compared under differing field conditions for contamination of the seed by *Aspergillus flavus* Link and aflatoxin. Laboratory tests had shown marked differences in resistance between the two cultivars. Peanuts were grown on three nonirrigated farms during 1980 using two planting dates and three harvest dates for each cultivar. Peanuts grown on two farms experienced moderate to severe drought stress and both cultivars contained high levels of aflatoxin. Peanuts on the third farm received adequate rainfall and contained very low levels of aflatoxin. Microflora, grade and aflatoxin data showed that Sunbelt Runner (reported to be resistant to *A. flavus* infection) had no advantage over Florunner (reported to have moderate resistance to *A. flavus*) in reducing levels of *A. flavus* and subsequent aflatoxin contamination under field conditions. Levels of infection and contamination were related primarily to environmental conditions, (especially drought stress), during pod maturation. These and prior results show that the current laboratory assay method for selecting resistant lines should be carefully reassessed.

Key Words: aflatoxin resistant, *A. flavus* resistant, peanut varieties, drought stress, mycotoxin

Aflatoxin produced by *Aspergillus flavus* Link in agricultural commodities is a primary concern of the agricultural industry and the consumer. The U. S. peanut industry has been a leader in prevention, detection, and removal of aflatoxin (11). Research scientists (2,3,4,10) have found that most contamination occurs in the field under drought related environments or in farmers stock storage. Mixon (8) developed a laboratory screening method and found that seed of different varieties had a wide range of resistance to invasion by *A. flavus*. Using this screening method he selected several peanut lines that showed a high degree of resistance (6). Wilson et al. (12) reported that such lines or cultivars may have advantages in the field but not during storage.

The purpose of this paper is to present information from field scale studies on the relative seed contamination by *A. flavus* and aflatoxin of two peanut cultivars that have been reported to differ in resistance to *A. flavus* in laboratory tests (6).

Materials and Methods

"Sunbelt Runner" (A7109) and "Florunner" which have been reported to have high and intermediate resistance to *A. flavus*, respectively, were compared under commercial management at three locations. The plantings were made by each of three grower cooperators who planted both cultivars at each of two dates. The early planted peanuts of each cultivar were harvested on two different dates (first and second). The first harvest (digging) was conducted to allow a preliminary evaluation prior to optimum maturity and this harvest date was approximately the same for both cultivars and all three farms. However, the 2nd (early planting) and 3rd (the late planting) harvests were selected by a University of Georgia Extension Agronomist (Frank McGill). These later harvests were selected for optimum maturity and maximum yield and thus these har-

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vest dates were usually different for each cultivar. The experiment consisted of 2 cultivars x 2 planting dates x 3 farms (12 plots) with an additional harvest for the early plantings making a total of 18 seed lots (2 cultivars x 3 farms x 3 harvests). The three farms were located in Terrell County, Georgia, several miles apart to increase the probability that the farms would be exposed to different environmental conditions. Each plot was at least 1 ha. The soil preparation, cultural practices, harvesting, and drying were according to the latest recommendations of the Cooperative Extension Service. Soil and seed microflora, rainfall, and plant conditions were monitored throughout the growing and harvest season. The peanuts were marketed at four commercial buying stations and minilots (136 kg minimum) were removed with the official grade pneumatic sampler (1). Comparative lots of Sunbelt Runner and Florunner were marketed at the same commercial facility. The peanuts of each minilot were cleaned and shelled, and the shelled peanuts including LSK (loose shelled kernels) were prepared for aflatoxin analyses as shown in Fig. 1. Two 26-kg subsamples (A and B) were used in the analyses. Two additional 26-kg subsamples (C and D) were placed in storage (-18 C) in case additional data were needed in the evaluation of the cultivars.

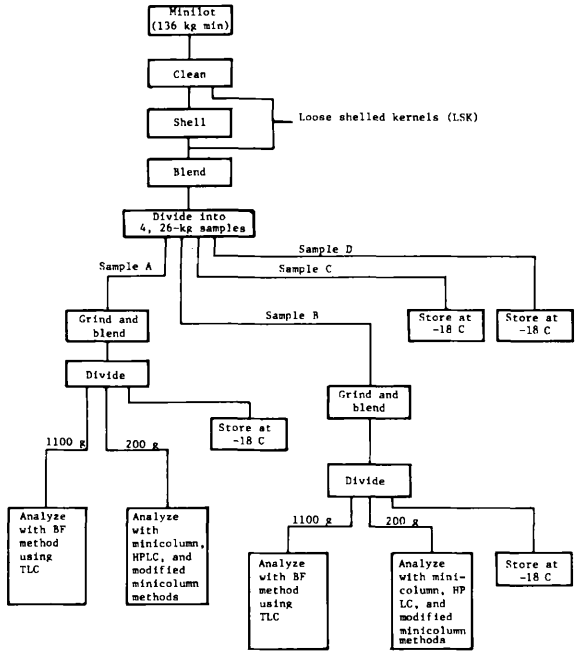

Fig. 1. General outline of sample preparation and analyses for estimating the mean level of aflatoxin of each minilot.

Four analytical methods were used to provide more precision in estimating the aflatoxin concentration in each ground subsample. The BF method using thin layer chromotography (TLC) (9) and a modified minicolumn using TLC were performed by the Fruit and Vegetable Processed Products Division, AMS, USDA, in Albany, Georgia. The Holaday minicolumn (5) and high-pressure liquid chromotograph (HPLC) analyses were conducted at the National Peanut Research Laboratory. The modified minicolumn method consisted of adding 60 mL of water to 100 mL of the minicolumn filtrate and blending for 30 sec. Hexane (70 mL) was added to the aqueous filtrate and blended for an additional 30 sec. The entire 230 mL was then centrifuged and 50 mL of the methanol-water extract was analyzed as with the standard BF procedure. High pressure liquid chromotograph analyses were performed using Waters Associates instrumentation including M-6000A pumps, a WISP 710B auto injector, and a radial compression module equipped with normal phase absorbent. The aflatoxins were detected with a Varian Fluorichrom detector and quantitated with a Waters Associates data module. The toluene extracts of peanut samples used in millicolumn analyses were also used in HPLC analyses for the aflatoxins. The mobile phase was water saturated chloroform supplemented with 0.6% methanol. Aflatoxin values obtained from all four methods were averaged for each subsample. If there was wide disagreement between values for a particular subsample, another subsample of meal was analyzed and all values averaged.

Peanuts were sampled on August 8, 13, 20, 25, September 3 and at harvest (in mid-September). Approximately 2000 kernels, handshelled from peanuts dug at random were plated out from each location at each sampling date. Numbers and kinds of fungi on and within kernels were estimated by plating both untreated and surface-sterilized (0.5% sodium hypochlorite solution [clorox], 5 min.) material on 2% malt extract agar and 10% malt salt agar and incubating at 25 C and 37 C for a week.

Most of the data was analyzed by averaging the data for each of the 18 seed lots. An analysis of variance was conducted on the aflatoxin data by considering all aflatoxin determinations for each individual seed lot. Differences were assumed to be totally related to differences in resistance of the two cultivars.

Results

Cultural, agronomic and harvesting information for each of the three locations are presented in Table 1. The cultural and agronomic practices were similar for all three farms. There were slight differences in soil type, prior crops and seeding rate. These differences did not appear to affect the overall results of this study.

Table 1. Cultural, agronomic and harvesting data for Sunbelt Runner and Florunner peanuts grown on three farms in Terrell County, GA during CY 1980.

Item	Farm A	Farm B	Farm C
Soil type	Sandy loam (heavy)	Sandy loam (light)	Sandy loam (medium)
Prior crop	Corn	Bahia grass (7 years)	Peanuts
Soil pH	5.7	6.3	6.5
Winter cover crop	None	None	Rye
Lime application - kg/ha	1122	4487	2243
Preplant fertilizer application - kg/ha	673 - 5-10-15	673 - 5-10-10	561 - 7-14-21
Preplant land preparation	Mow, disc harrow (3)[1], turn, bed	Mow, disc harrow (3)[1], subsoil (2)[1], turn, bed	Mow, turn, bed
Preplant and post-emerge herbicides	Balan and Vernam Dyanap, Amiben (1)[1]	Balan and Vernam Dyanap, Lasso, Dinitro (1)[1]	Balan, Vernam, Klean Krop, Lasso (1)[1]
First planting	4/23/80	4/23/80	4/23/80
Second planting	5/10/80	5/1/80	5/18/80
Seeding rate (kg/ha)			
Sunbelt Runner	123	84	135
Florunner	123	112	135
Postplant fertilizer - kg/ha	0	785 - 5-10-10 plus Zinc	0
Insecticide application	Nudrin (2)[1]	Toxaphene (1)[1], Lanate (4)[1], Azodrin (3)[1]	Furadan (1)[1], Lanate (4)[1]
Fungicide applications	Bravo (5)[1], Sulphur (1)[1]	Bravo (10)[1], Sulphur (8)[1]	Bravo (7)[1]
Late herbicide applications	Butoxone (1)[1]	Butoxone (1)[1]	None
Cultivations	2	2	1
Landplaster - kg/ha	0	561	561
Rainfall - cm	44.4	47.5	69.1
First harvest Sunbelt Runner and Florunner	9/3/80	9/2/80	9/3/80
Second harvest			
Sunbelt Runner	9/9/80[2]	9/6/80	9/11/80
Florunner	9/9/80	9/15/80	9/17/80
Third harvest			
Sunbelt Runner	9/9/80[2]	9/13/80	9/18/80
Florunner	10/13/80	9/22/80	10/14/80
Planting conditions	Good	Good	Good
Growing conditions	Hot, dry, moderate plant stress	Hot, dry severe plant stress	Good
Harvest conditions	Good	Good	Good
Approximate yield[3] kg/ha			
Sunbelt Runner	1414	561	3365
Florunner	1273	729	4936

[1]Numbers in parenthesis indicate the number of cultivations or applications.
[2]Optimum harvest dates for Sunbelt Runner on Farm A was the same for the early and late planting because the severe late season drought had completely stopped maturation of the Sunbelt Runner, and vine and peg conditions were deteriorating very rapidly.
[3]Yields were averaged over the 2nd and 3rd harvests.

Farm A and farm B had insufficient rainfall and the peanuts were stressed while the rainfall for farm C was considered adequate (Fig. 2). Plant stress was most severe for farm B because of its light sandy soil. On farm B the peanut plants wilted and the seed became loose in the hull just prior to harvest. Soil temperatures during pod development were extremely high and yields were extremely low. Even though farms A and B had about the same total rainfall, farm A had more rainfall than farm B

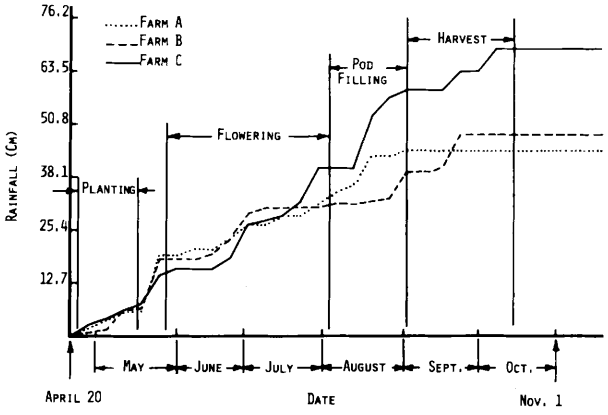

Fig. 2. Measured rainfall during CY 1980 peanut growing season for three farms in Terrell County, GA.

during the early part of the growing season and the heavier soils of farm A tended to hold moisture longer and reduce plant stress when compared to the lighter soils of farm B. Sunbelt Runner and Florunner had approximately the same yields (averaged over last 2 harvests) from farms A and B, but Florunner yielded higher than Sunbelt Runner on farm C. Sunbelt Runner matured 4 to 27 days earlier than Florunner on farms A and B.

Table 2 shows the proportion of kernels colonized by the *A. flavus* group at each sampling time and the ratio of *A. flavus:A. niger* at harvest. The *A. flavus* group refers to *A. flavus* Link (87% of isolates), *A. parasiticus* Speare (10% of isolates) and *A. tamarii* Kita (3% of isolates). Colonization of peanuts by the *A. flavus* group increased from none to few kernels colonized, on August 8, to as many as 30% of the SMK colonized from location B (drought-stressed) at harvest. The damaged and loose shelled kernels showed a much greater incidence of the *A. flavus* group than did the sound kernels irrespective of cultivar, sampling time or location. The incidence of the *A. flavus* group in the SMK's was greater in general on August 20 or 25 than at the other sampling dates. The reason for this is not known. For each location colonization of peanut kernels by the *A. flavus* group reflected differences in field conditions, rather than differences between cultivars in susceptibility to the fungi. However, data from farm B, which had the greatest drought-stress, showed that Sunbelt Runner was colonized more than was Florunner at the time of highest infection. In addition, the ratio of kernels colonized by *A. flavus:A niger* was much greater for Sunbelt Runner at farm A (moderate drought) than for Florunner. Hill et al. (4) reported that serious aflatoxin contamination of peanuts is probable when the *A. flavus:A niger* ratio >19:1 but unlikely if this ratio is <9:1. Our data support that observation (Table 2 and 3).

Average grade data are presented in Table 4. Generally the outturn data for Sunbelt Runner and Florunner were similar to that reported by Mixon et al. (7). Florunner tended to have a higher seed content and lower hull content than did Sunbelt Runner. The Florunner peanuts from farms A and C were extremely immature at the first harvest. Two of three loads of Sunbelt Runner peanuts and one of three loads of Florunner peanuts from farm A were graded segregation 3 (visible *A. flavus* present). Visible *A. flavus* was found in every grade sample (6 total) from farm B (3 loads per variety) for both Sunbelt Runner and Florunner varieties. No visible *A. flavus* was found in the grade samples (8 total) from farm C.

Aflatoxin determinations are presented in Table 3. There was general agreement between analytical methods within samples and subsamples indicating that subsampling methods were acceptable and analytical errors had been minimized. Analyses of additional subsamples of meal were not required. Shelled peanut samples A and B provided sufficient data for estimating the aflatoxin levels for each lot and for determining significant differences between varieties. Thus only Samples A and B were evaluated. Sunbelt Runner peanuts from the second and third harvests from farm A and the first harvest of farm B had significantly higher aflatoxin levels than for the Florunner peanuts. However, Sunbelt Runner peanuts from the second harvest of farm B had significantly lower aflatoxin levels than for Florunner. As expected aflatoxin levels for all peanuts grown on farm C were very low. Therefore, aflatoxin contamination was evidently affected by environmental factors. Different harvest dates and different number of days to maturity for the two varieties

Table 2. Percent of surface sterilized peanut kernels colonized by the *Aspergillus flavus* group during the 1980 growing season, from three locations in Terrell County, GA and the ratio of *A. flavus:A niger* at harvest.

Variety	Farm A				Farm B				Farm C			
	Florunner		Sunbelt Runner		Florunner		Sunbelt Runner		Florunner		Sunbelt Runner	
	SMK*	Other	SMK	Other	SMK	Other	SMK	Other	SMK	Other	SMK	Other
Sample date**												
Aug. 8	-	-	-	-	0	-	11.5	-	2	-	1.5	-
Aug. 13	5	-	6.7	-	15.5	-	13.5	-	4	-	10.5	-
Aug. 20	46.1	46.5	64.3	73.5	36.4	66.5	97.8	97.0	30.3	-	37.8	-
Aug. 25	25.3	-	25.2	-	10.7	-	24.3	-	44.4	-	15.0	-
Sep. 3	2.0	-	0.8	-	20.5	-	19.2	72.5	1.5	-	16.5	-
HARVEST	5.6	79.0	6.8	68.2	17.6	82.4	29.5	80.7	2.9	23.5	5.4	78.1
	Ratio kernels colonized by A. flavus:A. niger at harvest											
	4:1	1:1	17:1	23:1	20:1	28:1	42:1	23:1	2:1	1:1	3:1	2:1

*SMK = Sound mature kernels.
**Samples were obtained from the first planting.

Table 3. Aflatoxin contamination for Sunbelt Runner and Florunner peanuts.

Variety	Total aflatoxin (ppb)[1]								
	No drought stress (Farm C)			Moderate drought stress (Farm A)			Severe drought stress (Farm B)		
	First planting		Second planting	First planting		Second planting	First planting		Second planting
	First harvest	Second harvest	(Third harvest)	First harvest	Second harvest	(Third harvest)	First harvest	Second harvest	(Third harvest)
Sunbelt Runner	1(0-4)a	5(0-35)a	14(3-75)a	10(0-75)a	283(125-500)a	246(18-761)a	527(125-761)a	257(125-500)a	484(125-779)a
Florunner	5(0-35)a	2(0-6)a	5(0-35)a	25(1-50)a	5(0-25)b	10(0-75)b	341(125-634)b	465(125-723)b	591(125-924)a

[1]Aflatoxin values are the average of eight values for Samples A and B (see Fig. 1). Values in parenthesis represent the range of the eight values. Values within a column bordered by different letters are significantly different at the 0.05 level of significance.

Table 4. Average grade data for Sunbelt Runner and Florunner peanuts.

Variety	Planting time	Harvest time	SMK[1] Farms			SS[1] Farms			OK[1] Farms			D[1] Farms			TK[1] Farms			H[1] Farms			FM[1] Farms			LSK[1] Farms			M.C.[1] Farms			Seg.[1] Farms		
			A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Sunbelt Runner	First	First	63	69	61	1	2	1	9	4	9	1	1	1	74	76	72	26	24	28	10	5	1	4	3	2	8	8	10	1	3	1
Florunner	First	First	58	69	55	1	2	1	16	6	17	0	1	0	75	78	73	25	22	27	4	5	4	3	1	5	7	7	9	1	3	1
Sunbelt Runner	First	Second	64	69	63	3	2	2	8	4	7	1	2	0	76	77	72	25	23	21	5	7	5	4	4	4	7	8	10	3	3	1
Florunner	First	Second	60	66	68	3	3	6	13	8	5	0	2	0	76	79	78	24	21	22	9	7	4	5	8	2	7	9	8	1	3	1
Sunbelt Runner	Second	Third	63	57	71	3	3	2	6	6	2	1	2	0	73	68	77	27	31	23	5	4	3	5	3	5	7	9	9	3	3	1
Florunner	Second	Third	64	58	73	11	3	2	4	6	2	1	2	0	80	69	78	21	30	20	8	15	4	14	7	4	6	10	8	3	3	1

[1]Grade factors are defined as follows:
SMK = % Sound mature seed.
SS = % Sound split seed.
OK = % Small immature seed.
D = % Damaged seed.
TK = % Total seed.
H = % Hulls.
FM = % Foreign material.
LSK = % Loose shelled seed.
M.C. = % Moisture content of seed.
Seg. = Segregation. 1 = Edible quality, 3 = Nonedible quality (A. flavus mold present).

could possibly explain some of the differences. However, over the wide range of conditions investigated, there was no real evidence that Sunbelt Runner had the potential of reducing levels of aflatoxin below that normally experienced for Florunner.

Discussion

Both Sunbelt Runner and Florunner were challenged by *Aspergillus* spp. under 3 different field environments. Two of these environments were very conducive to *A. flavus* invasion and subsequent aflatoxin production. Microflora, grading and aflatoxin data showed no advantage of Sunbelt Runner over Florunner in relation to seed resistance to invasion by *A. flavus* and subsequent aflatoxin production under field conditions. Invasion of the seed by *A. flavus* and subsequent aflatoxin production in both cultivars were influenced primarily by drought stress during pod development and not by varietal differences. These results and those reported by Wilson et al. (12) in commercial-type peanut storage tests show that certain lines and cultivars selected in the laboratory to have a low percentage of seed colonized with *Aspergillus* sp. were not resistant in commercial field and storage environments. Thus the current laboratory assay to select for genetic resistance of lines and cultivars should be carefully reassessed.

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Accepted March 23, 1983