Association of a Burrower Bug (Heteroptera: Cydnidae) with Aflatoxin Contamination of Peanut Kernels[1]

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J. Entomol Sci. 39(1): 71-83 (January 2004)

Abstract Aflatoxin contamination of peanut kernels, *Arachis hypogaea* L., was associated with feeding by a burrower bug, *Pangaeus bilineatus* (Say). Kernel samples were divided into three grade categories: total sound mature kernels (TSMK), other kernels (OK), and damaged kernels (DK); and each of these grade categories was subdivided based on evidence of burrower bug feeding. Within TSMK, 100% of detectable aflatoxin contamination was associated with burrower bug kernel feeding, and kernels with feeding sites had a significantly higher concentration of aflatoxin than kernels without feeding sites (7.5 vs 0.0 ppb). Within the OK grade category, differences in aflatoxin contamination were not significant due to the inability to conclusively examine these kernels for feeding sites. Within the DK grade category, aflatoxin concentration was significantly higher in kernels with feeding sites than in kernels without observable feeding sites (286.5 vs 0.4 ppb), and 99.9% of contamination was associated with burrower bug feeding. Across all grade categories, aflatoxin levels were 65X higher in kernels with observable burrower bug feeding, and 98% of all aflatoxin contamination was associated with burrower bug feeding. The DK grade category had the highest concentration of aflatoxin and accounted for 45% of total contamination. Burrower bug-induced aflatoxin contamination of the TSMK grade category is particularly significant because this source would be most difficult to remove from the food supply. Contamination of the DK category is also economically significant because this grade component is specifically examined for *Aspergillus* at the buying point, and growers are severely penalized for detection.

Key Words *Pangaeus bilineatus, Arachis hypogaea, Aspergillus flavus,* groundnut, economic injury

Aflatoxin contamination is a major economic concern of the peanut, *Arachis hypogaea* L., industry. Aflatoxins are highly toxic, mutagenic, teratogenic, and carcinogenic metabolites of the molds *Aspergillus flavus* Link and *A. parasiticus* Speare (Rustom 1997). Peanut kernels become contaminated with aflatoxin only when the kernels are first colonized or invaded by *Aspergillus* and subsequent conditions favor fungal growth and toxin production (Dorner et al. 1989). When soil moisture conditions are adequate, fungal invasion of the pod elicits production of phytoalexins which inhibit fungal growth and prevent aflatoxin contamination. Drought stress favors aflatoxin production by increasing soil temperature and lowering kernel water content.

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Increased temperatures favor *Aspergillus* growth and decreased kernel water content suppresses phytoalexin production, thus promoting aflatoxin production (Dorner et al. 1989).

Damaged pods typically have higher aflatoxin levels than intact pods (Schroeder and Ashworth 1965, Rustom 1997), and a number of pod-feeding arthropods have been linked to increased aflatoxin contamination of peanut kernels. These arthropods include termites (Isoptera) (McDonald and Harkness 1963, McDonald et al. 1964, Lynch et al. 1990), white grubs (Coleoptera: Scarabaeidae) in the genus *Heteronyx* (Graham 1982), a seed web moth, *Etiella behrii* Zeller (Lepidoptera: Pyralidae) (Graham 1982), and mites (Acari, suborder Astigmata) in the genera *Caloglyphus* and *Tyrophagus* (Aucamp 1969). In the southeastern United States, the lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller) (Lepidoptera: Pyralidae), thrives under the drought stress conditions which favor aflatoxin, and this insect has been shown to enhance *Aspergillus* invasion of kernels and subsequent aflatoxin contamination (Lynch and Wilson 1991, Bowen and Mack 1993).

A burrower bug, *Pangaeus bilineatus* (Say), is known to be capable of extensive feeding on peanut kernels (Smith and Pitts 1974, Chapin et al. 2001, Chapin and Thomas 2003). Adults and nymphs of *P. bilineatus* feed directly on peanut kernels by piercing the pods with needle-like mouthparts. This feeding results in light-yellow to dark-brown lesions on the kernel as described by Smith and Pitts (1974). The highest population densities of *P. bilineatus* occur during late pod-fill and populations persist until harvest (Smith and Pitts 1974, Chapin and Thomas 2003). Increased kernel injury from *P. bilineatus* has been associated with some reduced tillage systems and drought stress (Chapin et al. 2001, Chapin and Thomas 2003).

Worldwide, six species of cydnids have been reported to feed on peanut roots or pods (Lis et al. 2000), but we are not aware of any data associating burrower bug feeding with aflatoxin contamination of peanut kernels. *Pangaeus bilineatus* has been speculated to be a potential vector of plant viruses (Sailer 1954), and *Cyrtomenus bergi* Froeschner causes fungal invasion of cassava roots, *Manihot esculenta* Crantz, in South America (CIAT 1980). Widstrom (1979) stated that the relationship between soil insects and aflatoxin in peanut has not received as much attention as might be expected based on the seriousness of the problem, growth habit of the crop, and the fact that aflatoxin was first discovered in peanut. Given our observation that *P. bilineatus* feeds most extensively on peanut pods under the late-season drought stress conditions which favor aflatoxin production, our objective was to determine whether peanut kernels with burrower bug feeding sites had increased levels of aflatoxin.

Materials and Methods

Kernel categories. A 16-ha field of 'NC-V11' peanut in Aiken Co., SC, was known to have a high level (46.6 ± 7.1%) of kernels injured by *P. bilineatus* based on pod collections taken 1 wk prior to digging. The field had also been subjected to severe late season drought stress and, thus, was considered a likely candidate for aflatoxin contamination. The crop was harvested by the grower and within 24 h of combining, was dried to 11% moisture in standard peanut wagons (3.5 to 4.5 metric ton capacity). A hand scoop was used to haphazardly collect five peanut samples of approximately 500 g each from each of two wagons. Our objective was to collect potentially aflatoxin-contaminated peanuts rather than to representatively sample the wagons or field from which the peanuts were harvested. The ten samples were shelled and then

placed in a 1.1 Kw microwave oven for 2 min at 80% power to allow later removal of the testa (seed coat) and examination of the kernels for evidence of burrower bug feeding. Microwaving is known to destroy aflatoxin. Farag et al. (1996) demonstrated substantial reductions in aflatoxin with microwave treatments as short as 3 min on the low setting of an oven rated at 1.3 Kw. However, Luter et al. (1982) reported that when the kernel sample temperature did not reach 150°C, and no browning occurred, there was no measurable reduction in aflatoxin. Although our microwave interval was shorter than in these experiments, and no kernel browning occurred, the technique we used to remove the seed coat may have caused subsequent underestimation of aflatoxin levels. We did not measure kernel temperature. A mechanical shaker screen (0.6 by 2.6 cm) was used to separate each sample into three grade categories as defined by USDA standards (USDA 1998): total sound mature kernels (TSMK), other kernels (OK), and damaged kernels (DK). The TSMK category is the sound mature kernel component large enough to ride the screen standard plus any sound split kernels which pass through the screen. The OK category refers to smaller whole kernels, usually immature, which pass through the screen standard. The DK category is the kernel component that rides the screen but is judged to be inedible due to disease, insect, or other damage sources. Very few kernels with burrower bug feeding sites would be considered damaged in the normal grading process, in part because the testa is not removed during commercial grading. Therefore, to avoid confusion, in this paper we refer to kernels with burrower bug feeding sites as fed-on kernels rather than damaged or injured kernels, unless they met the criteria for the damaged kernel grade category. The lesser cornstalk borer and several species of wireworms (Elateridae) commonly feed on peanut pods in the South Carolina coastal plain, but pod scarification was <2% in the collected samples and, therefore, this pest complex should not have had any significant effect on aflatoxin contamination.

The testa was completely removed from all TSMK by hand as each kernel was examined for the discolorations or pits characteristic of burrower bug feeding (Smith and Pitts 1974). The TSMK category was subdivided into three subcategories according to the degree of burrower bug feeding: not detectable, minor feeding (1 to 10 feeding sites per kernel), and major feeding (>10 feeding sites per kernel). Split kernels were included in the not detectable, minor feeding, or major feeding categories based on 0, 1 to 5, and >5 feeding sites per kernel half, respectively. Split kernels comprised only 8% of all TSMK weight. Kernels in the OK category were only subdivided into two feeding categories, no detectable feeding or fed-on. The testa could not be totally removed from most (≈80%) of the OK category, and it was impossible to determine the number of feeding sites or to state with certainty that these kernels had not been fed-on. The DK category was also subdivided into two categories, no detectable feeding or fed-on, because some of these kernels were too deteriorated to enumerate feeding sites or to determine conclusively that they had not been fed on. Each of the seven kernel categories was weighed, then placed in a sealed plastic bag and refrigerated at ≈3.5°C for up to 1 wk prior to shipment to the National Peanut Research Laboratory for aflatoxin assay.

Aflatoxin assay. The aflatoxin concentration in each sample component was determined by blending all kernels with methanol-water (80:20, v/v; 2 mL/g) and subjecting extracts to the high performance liquid chromatography (HPLC) method of Dorner and Cole (1988) with certain modifications. The HPLC system consisted of a Waters 3.9 × 150 mm Nova-PAK C_{18} column with a mobile phase of water-methanol-butanol (700 + 355 + 12; v/v/v). Instead of using postcolumn iodination to enhance

fluorescence of aflatoxins B_1 and G_1, postcolumn derivatization was achieved with a photochemical reactor (Joshua 1993) placed between the column and a Shimadzu Model RF551 fluorescence detector with excitation and emission wavelengths of 365 and 440 nm, respectively. Injection solvent consisted of methanol-water (62 + 38, v/v) with 0.1% acetic acid. Aflatoxin standards were prepared from crystals according to AOAC method 970.44 (AOAC 1995), and aflatoxin determinations were not corrected for recovery. Sample aflatoxin concentrations were calculated by dividing the total aflatoxin mass (ng) by the total kernel mass (g) for all kernel components of each sample.

Data analysis. Analysis of variance (PROC GLM, SAS Institute 1985) was used to detect differences in aflatoxin concentration among grade categories, among feeding categories, among feeding categories within each of the three grade categories, and between fed-on and no-detectable-feeding kernel categories across all grades. Aflatoxin concentration data were transformed to log(x + 1) prior to analysis to homogenize variance, but means and standard errors are reported in the original scale. The significance level for all statistical tests was set at $\alpha = 0.05$. A protected LSD test was used to make mean separation decisions where significant F ratios were detected and more than two means were compared. The same statistical procedures were used to compare the percentage of sample aflatoxin contributed by: each grade category, each feeding category, feeding categories within each grade category, and feeding categories pooled into fed-on or not observably fed-on across all grades. Percentage data were transformed to arcsine (x) prior to analysis to homogenize variance and normalize the distribution, but means and standard errors are presented in the original scale.

Results

Percentage of kernels with burrower bug feeding. Of total sample kernel weight, 83.75, 10.51, and 5.76% were in the TSMK, OK, and DK categories, respectively (Table 1). Within the TSMK kernel category, 49.38% of the sample, by weight, had no detectable feeding; 30.19% had minor feeding; and 20.41% had major feeding. Within the OK kernel category, only 5.72% of kernel weight had detectable feeding sites. Within the DK kernel category, 11.65% of the sample had no detectable feeding; and 88.35% of kernel weight had observable burrower bug feeding. Across all grade categories, 48.07% of sample weight had observable burrower bug feeding sites.

Aflatoxin contamination. Sample aflatoxin concentrations ranged from 0.3 to 141.5 ppb with a mean of 30.0 ± 13.7 ppb (Table 2). Within the TSMK category, aflatoxin concentrations were 0.0 ± 0.0, 4.2 ± 3.9, and 12.7 ± 10.5 ppb for kernels with no detectable feeding, minor feeding, and major feeding, respectively ($F = 2.90$; df = 2,18; $P = 0.08$). When the minor and major feeding categories were combined in the TSMK category, the mean aflatoxin concentration of 7.5 ± 4.4 ppb for fed-on kernels was significantly greater than the zero level found in kernels without detectable feeding ($F = 6.35$; df = 1,9; $P = 0.033$). Within the OK category, aflatoxin contamination levels were 5.7 ± 4.5 ppb in kernels without detectable feeding, and 2455.8 ± 2339.1 ppb in kernels with observable feeding sites ($F = 1.04$; df = 1,9; $P = 0.34$). Within the DK category, aflatoxin contamination levels were 0.4 ± 0.3 ppb in kernels without detectable feeding, and 286.5 ± 138.1 ppb in fed-on kernels ($F = 19.6$; df = 1,9; $P = 0.0017$). When the three grade categories were combined, aflatoxin contamination

Table 1. Weight (g) of peanut kernel categories based on grade and burrower bug feeding criteria; Aiken Co., SC, 2001

	TSMK*			OK		DK		
Sample	Not detectable**	Minor	Major	Not detectable	Fed-on	Not detectable	Fed-on	Total sample
1	132.38	74.72	47.70	36.23	0.73	2.73	19.91	314.40
2	143.73	71.12	43.31	28.44	1.54	3.49	13.67	305.30
3	120.50	84.97	50.37	32.33	2.15	0.96	14.00	305.28
4	124.59	82.55	50.02	33.54	0.23	0.91	9.88	301.72
5	113.61	76.94	63.37	25.42	2.14	1.30	21.50	304.28
6	118.55	75.58	46.06	26.67	2.51	3.44	13.17	286.98
7	122.26	73.25	55.08	25.84	3.36	4.49	16.55	300.83
8	119.15	77.52	54.41	29.87	2.63	0.65	16.38	300.61
9	128.49	70.84	57.98	29.32	2.02	1.51	13.33	303.49
10	126.84	76.84	48.44	31.04	0.85	0.77	15.50	300.28
Mean ± SE	125.01 ± 2.70	76.43 ± 1.43	51.67 ± 1.91	29.97 ± 1.08	1.82 ± 0.31	2.03 ± 0.44	15.39 ± 1.08	302.32 ± 2.14
% of total sample wt.	41.35	25.28	17.09	9.91	0.60	0.67	5.09	

* Kernel grade categories (TSMK = total sound mature kernels, OK = other kernels, DK = damaged kernels) as defined by USDA grade criteria (USDA 1998).

** Burrower bug kernel-feeding categories: not detectable = no observable burrower bug feeding, minor = 1-10 feeding sites per kernel, major = >10 feeding sites per kernel, fed-on = burrower bug feeding evident but feeding sites could not be enumerated.

Table 2. Aflatoxin concentration (ppb) in peanut kernels categorized by grade and burrower bug feeding criteria; Aiken Co., SC, 2001

		TSMK*			OK		DK		Total sample	
Sample	Aflatoxin ppb	Not detectable**	Minor	Major	Not detectable	Fed-on	Not detectable	Fed-on	Not detectable	Fed-on
1	10.1	0.00	39.00	0.80	0.00	2.20	0.00	10.80	0.00	22.15
2	141.5	0.00	0.00	3.60	2.10	23486.00	0.00	498.40	0.34	332.75
3	0.3	0.00	0.00	0.90	0.00	2.50	0.00	3.10	0.00	0.62
4	18.1	0.00	1.20	106.20	1.30	0.00	0.40	0.00	0.28	37.93
5	11.1	0.00	0.70	12.30	1.50	1059.60	0.00	11.20	0.27	20.38
6	0.3	0.00	0.00	0.00	0.00	0.00	0.00	6.90	0.00	0.66
7	0.8	0.00	0.00	0.00	5.00	0.00	0.00	6.60	0.85	0.74
8	37.7	0.00	0.00	0.00	45.60	0.00	3.40	608.50	9.12	66.03
9	59.4	0.00	1.10	3.30	1.40	0.00	0.00	1328.60	0.26	124.71
10	20.2	0.00	0.00	0.00	0.10	8.10	0.00	390.90	0.02	42.83
Mean ± SE	30.0 ± 13.7	0.0 ± 0.0cA	4.2 ± 3.9bcA	12.7 ± 10.5bcA	5.7 ± 4.5bcA	2455.8† ± 2339.1abA	0.4 ± 0.3cB	286.5† ± 138.1aA	1.1 ± 0.9B	64.9 ± 32.1A
Grade category means			3.8 ± 2.2b		134.2 ± 119.7ab		258.2 ± 124.6a		29.9 ± 13.7	

Means within rows followed by the same letter are not significantly different; Fisher's protected LSD ($P < 0.05$). Uppercase letters compare means within the same grade category. Lowercase letters compare means across all grade categories. Mean separations based on analysis of log(x+1), but means ±SE reported in original scale.

* Kernel grade categories (TSMK = total sound mature kernels, OK = other kernels, DK = damaged kernels) as defined by USDA grade criteria (USDA 1998).

** Burrower bug kernel-feeding categories: not detectable = no observable burrower bug feeding, minor = 1-10 feeding sites per kernel, major = >10 feeding sites per kernel, fed-on = burrower bug feeding evident but feeding sites could not be enumerated.

† Note that although the mean of the OK, fed-on category is greater than that of the DK, injured category; log transformation accounts for the reverse ranking in the lowercase mean separation notation.

levels were 1.1 ± 0.9 ppb in kernels without detectable feeding, and 64.9 ± 32.1 ppb in kernels with observable burrower bug feeding (F = 19.55; df = 1,9; P = 0.0017). Aflatoxin concentrations averaged 3.8 ± 2.2, 134.2 ± 119.7, and 258.2 ± 124.6 ppb for TSMK, OK, and DK grade categories, respectively; and the DK aflatoxin level was significantly greater than that of the TSMK category (F = 4.26; df = 2,18; P = 0.03). Kernels with burrower bug feeding in OK and DK grade categories had higher aflatoxin levels than the other five feeding categories (F = 4.83; df = 6,54; P = 0.0005).

Percentage of aflatoxin contamination. Within the TSMK category, 77.3 ± 15.9, 22.7 ± 15.9, and 0.0 ± 0.0% of aflatoxin contamination was attributable to kernels with major, minor, and no detectable burrower bug kernel feeding, respectively. The percentage of contamination attributable to kernels with major feeding was significantly greater than that of the other two categories (F = 5.75; df = 2,10; P = 0.017) (Table 3). When the minor and major feeding categories were combined in the TSMK category, 100% of aflatoxin contamination was attributable to kernels with burrower bug feeding ($F = \infty$; df = 1,5; $P < 0.0001$). When all TSMK samples were pooled; 0.0, 32.6, and 67.4% of the total aflatoxin contamination in the composite sample were attributable to kernels with no feeding, minor feeding, and major feeding, respectively. This unreplicated composite is not subject to statistical analysis but serves as an additional indicator of the association between burrower bug feeding and aflatoxin contamination. Within the OK category, 51.9 ± 16.7 and 48.1 ± 16.7% of aflatoxin contamination was attributed to kernels without and with observable burrower bug feeding sites, respectively (F = 0.01; df = 1,9; P = 0.97). When all OK samples were pooled, 95.8% of the aflatoxin contamination in the composite sample was found in kernels with observable feeding sites. Within the DK category, 10.0 ± 10.0 and 90 ± 10.0% of aflatoxin contamination was attributed to kernels without and with observable burrower bug feeding sites, respectively (F = 15.98; df = 1,9; P = 0.003). When all DK samples were pooled, 99.9% of the aflatoxin contamination in the composite sample was found in kernels with observable feeding sites. When the three grade categories were combined, 6.9 ± 5.4 and 93.1 ± 5.4% of aflatoxin contamination was attributed to kernels without and with observable burrower bug feeding sites, respectively (F = 61.52; df = 1,9; P = 0.0001). When all samples were pooled across all grade categories, 98.2% of aflatoxin contamination in the composite sample was found in kernels with observable burrower bug feeding sites.

When grade categories were compared, 26.7 ± 12.6% of aflatoxin contamination was attributed to TSMK, 22.5 ± 10.4% to OK, and 50.8 ± 13.4% to DK (F = 1.22; df = 2,18; P = 0.318) (Table 4). When all samples were pooled, 10.6% of aflatoxin contamination in the unreplicated composite sample was attributed to TSMK, 44.1% to OK, and 45.3% to the DK category. Kernels with observable feeding sites in the DK grade category contributed more to aflatoxin contamination (50.8 ± 13.4%) than any other kernel category (F = 3.7; df = 6,54; P = 0.0037). Among the remaining kernel categories, 17.1 ± 10.2% of sample aflatoxin contamination was attributable to TSMK major feeding, 15.7 ± 10.1% to OK fed-on, 9.6 ± 9.2% to TSMK minor feeding, 6.9 ± 5.4% to OK no feeding, 0.003 ± 0.002% to DK no feeding, and 0.0 ± 0.0% to the TSMK no feeding category. None of these means were significantly different (Table 4).

Discussion

Percentage of kernels with burrower bug feeding. The 48% of total kernel weight and 49% of TSMK category weight exhibiting burrower bug feeding in the

Table 3. Percentage of peanut sample aflatoxin contamination based on burrower bug feeding criteria within grade categories and within the total sample; Aiken Co., SC, 2001

	TSMK*			OK		DK		Total	
Sample	Not detectable**	Minor	Major	Not detectable	Fed-on	Not detectable	Fed-on	Not detectable	Fed-on
1	0.0	98.7	1.3	0.0	100.0	0.0	100.00	0.00	100.00
2	0.0	0.0	100.0	0.2	99.8	0.0	100.00	0.20	99.80
3	0.0	0.0	100.0	0.0	100.0	0.0	100.00	0.00	100.00
4	0.0	1.8	98.2	100.0	0.0	100.0	0.00	0.80	99.20
5	0.0	6.5	93.5	1.7	98.3	0.0	100.00	1.10	98.90
6	—	—	—	—	—	0.0	100.00	0.00	100.00
7	—	—	—	100.0	0.0	0.0	100.00	54.20	45.80
8	—	—	—	100.0	0.0	0.0	99.98	12.00	88.00
9	0.0	28.9	71.1	100.0	0.0	0.0	100.00	0.20	99.80
10	—	—	—	31.1	68.9	0.0	100.00	0.10	99.90
Mean ± SE	0.0 ± 0.0B	22.7 ± 15.9B	77.3 ± 15.9A	48.1 ± 16.7A	51.9 ± 16.7A	10.0 ± 10.0B	90.0 ± 10.0A	6.9 ± 5.4B	93.1 ± 5.4A
Pooled samples	0.0	32.6	67.4	4.2	95.8	0.1	99.9	1.8	98.2

Means within the same grade category, or total sample category, followed by the same letter are not significantly different; Fisher's protected LSD ($P < 0.05$). Mean separations based on analysis of arcsine(×), but means ±SE reported in original scale.

* Kernel grade categories (TSMK = total sound mature kernels, OK = other kernels, DK = damaged kernels) as defined by USDA grade criteria (USDA 1998).

** Burrower bug kernel-feeding categories: *not detetable* = no observable burrower bug feeding, *minor* = 1-10 feeding sites per kernel, *major* = >10 feeding sites per kernel, fed-on = burrower bug feeding evident but feeding sites could not be enumerated.

Table 4. Percentage of peanut sample aflatoxin contamination based on grade categories and burrower bug feeding criteria across all grade categories; Aiken Co., SC, 2001

	TSMK*				OK			DK		
Sample	Not detectable**	Minor	Major	Total	Not detectable	Fed-on	Total	Not detectable	Fed-on	Total
1	0.0	92.0	1.2	93.2	0.0	0.1	0.1	0.00	6.80	6.80
2	0.0	0.0	0.4	0.4	0.1	83.7	83.9	0.00	15.80	15.80
3	0.0	0.0	48.2	48.2	0.0	5.7	5.7	0.00	46.10	46.10
4	0.0	1.8	97.4	99.2	0.8	0.0	0.8	0.01	0.00	0.01
5	0.0	1.6	23.1	24.7	1.1	67.1	68.2	0.00	7.10	7.10
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	100.00	100.00
7	0.0	0.0	0.0	0.0	54.2	0.0	54.2	0.00	45.80	45.80
8	0.0	0.0	0.0	0.0	12.0	0.0	12.0	0.02	88.00	
9	0.0	0.4	1.1	1.5	0.2	0.0	0.2	0.00	98.30	98.30
10	0.0	0.0	0.0	0.0	0.1	0.1	0.2	0.00	99.80	99.80
Mean ± SE	0.0 ± 0.0b	9.6 ± 9.2b	17.1 ± 10.2b	26.7 ± 12.6A	6.9 ± 5.4b	15.7 ± 10.1b	22.5 ± 10.4A	0.003 ± 0.002b	50.8 ± 13.4a	50.8 ± 13.4A
Pooled samples	0.0	3.5	7.2	10.6	1.8	42.2	44.1	0.003	45.3	45.31

Means followed by the same letter are not significantly different; Fisher's protected LSD ($P < 0.05$). Uppercase letters compare means of grade category totals. Lowercase letters compare feeding category means across all grade categories. Mean separations based on analysis of arcsine(x), but means ±SE reported in original scale.

* Kernel grade categories (TSMK = total sound mature kernels, OK = other kernels, DK = damaged kernels) as defined by USDA grade criteria (USDA 1998).

** Burrower bug kernel-feeding categories: not detectable = no observable burrower bug feeding, minor = 1-10 feeding sites per kernel, major = >10 feeding sites per kernel, fed-on = burrower bug feeding evident but feeding sites could not be enumerated.

analyzed samples is similar to the 47% incidence of kernel feeding observed in representative field sampling prior to harvest. The fact that only 6% of OK weight had observable burrower bug feeding is a reflection of our inability to thoroughly remove the testa from these immature kernels. Therefore, it is likely that we significantly underestimated the true level of feeding on this kernel category. Our results may also be partially due to burrower bugs feeding preferentially on more mature kernels (Smith and Pitts 1974). Although 88% of the DK category had observable burrower bug feeding, some kernels were too deteriorated to conclusively determine if they had been fed on. Therefore, the actual level of burrower bug feeding may have been greater. In any case, burrower bug feeding was primarily responsible for the high level of damaged kernel weight in our samples (6%). Even in the absence of aflatoxin, grade penalties are imposed for each percent of damaged kernels above 2.5% (USDA 1998). Commercial grade reports from the study field ranged up to 4% damaged kernels, which is similar to the 6% level we found; because hull weight is included in the total weight for commercial grading.

Aflatoxin contamination. Our data demonstrate an association between burrower bug feeding and aflatoxin contamination in that, across all three grade categories, aflatoxin concentration was 65X greater in kernels with burrower bug feeding. Our results also indicate that burrower bug feeding contaminated TSMK peanuts with aflatoxin when no detectable level of aflatoxin was found in the absence of burrower bug feeding on this kernel category. Aflatoxin contamination of TSMK peanuts is particularly significant because contaminated kernels without obvious damage are difficult to detect and remove and, therefore, of primary concern (Hill et al. 1983). The human consumption tolerance for aflatoxin is set at 20 ppb by the FDA and at 10 ppb by FAO/WHO (Rustom 1997). Although the mean aflatoxin concentration of 3.8 ppb in the TSMK category was below these tolerance levels, the concentrations of 11.6 ppb in sample 1 and 21.0 ppb in sample 4 are noteworthy. In the case of whole-kernel products, the risk of an individual consumer ingesting significant levels of aflatoxin increases because the effects of a few highly contaminated kernels are not diluted (Chiou and Tsao 1997).

Our failure to demonstrate a significant association between burrower bug feeding and aflatoxin contamination of the OK category is not surprising given our inability to determine with any certainty whether feeding occurred on most kernels in this category. It is probable that most of the contamination detected in the OK samples without observable feeding sites was actually related to burrower bug feeding.

The association of burrower bug feeding with consistently high levels of aflatoxin contamination in the DK category is significant because when grower lots are graded, the DK grade component is specifically examined for the presence of *A. flavus*. Although electronic screening can be used to remove much of the severely discolored DK component and, hence, eliminate most of this aflatoxin source from the food supply, the grower is likely to have already been economically penalized based on *A. flavus* detection. In fact, two grower lots marketed from the study field were consigned to the oil market due to detection of *A. flavus* even after the lots were re-cleaned and re-sampled at the buying point. The fact that the DK grade category had the highest level of aflatoxin contamination is also consistent with previous aflatoxin studies (Whitaker et al. 1998).

Percentage of aflatoxin contamination. Analysis of the percentage of aflatoxin contamination contributed by kernel categories reinforces the degree of association with burrower bug feeding in that, on average, 93% of aflatoxin (98% of pooled

sample aflatoxin) was attributable to kernels with feeding sites. It is probable that much, if not all of the remaining aflatoxin was also due to undetectable burrower bug feeding in the OK and DK categories. On average, 27% of all aflatoxin (11% of pooled sample aflatoxin) was found in the TSMK category where it is most likely to contaminate food, and all of this TSMK aflatoxin was associated with burrower bug feeding. In a survey of aflatoxin contaminated grower lots, Whitaker et al. (1998) found that 7% of total aflatoxin mass was in the TSMK grade component. Within the TSMK category, although the kernel component with major feeding weighed 32% less than the minor feeding category, the major feeding component accounted for about 3.5X more aflatoxin due to the higher aflatoxin concentration in kernels with major feeding.

Within the OK category, the comparison of aflatoxin percentage found for fed-on kernel samples and kernels without detectable feeding was meaningless due to the previously mentioned inability to adequately examine the OK component for evidence of feeding, and the fact that low concentrations in samples with no detectable feeding had equal weight on a percentage basis with the high levels of aflatoxin found in the fed-on component of samples 2 and 5. This is apparent from the fact that 96% of aflatoxin contamination in the pooled OK sample was attributable to kernels with feeding sites.

Within the DK category, fed-on kernels would have accounted for even more of aflatoxin contamination than the measured 90% if not for sample 4, where results were skewed by an absence of aflatoxin in the fed-on component and a low level in the component without observable feeding. This is reflected in the pooled DK total sample where 99.9% of aflatoxin was associated with fed-on kernels.

Management implications. Our results implicate *P. bilineatus* as a previously unrecognized, but potentially significant risk factor for aflatoxin contamination of peanut. Unlike any other arthropod associated with aflatoxin contamination of peanut, *P. bilineatus* feeds on a high percentage of otherwise intact pods while leaving no external evidence of injury. We have observed a greater incidence of kernel feeding by this pest under severe late season drought stress, thus *A. flavus* kernel contamination may increase under the very conditions which favor subsequent development of mycotoxins. The potential for this pest to contaminate sound mature kernels (TSMK) is particularly noteworthy because this aflatoxin source is most likely to enter the food supply. The combination of an increased percentage of DK in grower grade samples and higher levels of *Aspergillus* or aflatoxin within the DK grade component are also likely to trigger significant grade penalties even if much of the DK aflatoxin can be removed later with electronic screening. Given the previously documented ability of *P. bilineatus* to cause economic injury through yield and grade reduction, and the potential for aflatoxin contamination demonstrated herein, it is important to develop a greater understanding of the biology of this pest such that appropriate avoidance strategies can be implemented.

Acknowledgments

We appreciate the support of South Carolina peanut growers and the National Peanut Board. We also thank William Bridges for assistance with statistical analysis. This is Contribution No. 4846 of the S. C. Agricultural Experiment Station, and is based on work supported by the CSREES/USDA, under project number SC1700191.

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