Effect of Irrigation Interval, Planting Date, and Cultivar on *Aspergillus flavus* and Aflatoxin Contamination of Peanut in a Sandy Soil of Niger

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ABSTRACT

Aflatoxin contamination of peanut is a major threat to consumers in West Africa. High levels of aflatoxin have been reported in West and Central Africa, particularly in Niger. Field trials were conducted from 1991 to 1994 at ICRISAT Sahelian Center, Sadore Research Station near Niamey, Niger. Various production practices were compared to examine their effects on water stress and *Aspergillus flavus* infection and aflatoxin contamination. Different levels of water stress were achieved by varying planting date and frequency of irrigation in two resistant and two susceptible cultivars. Contamination of seed with *A. flavus* and aflatoxin was determined. The susceptible cultivars 28-206 and JL 24 had much higher levels of seed infection following 3 wk or more of water stress than did the resistant cultivars. Susceptible cultivars showed up to 81% seed infection. Cultivar 28-206 had low levels of contamination when there was low water stress but became very susceptible when the period of water stress increased (3 wk of drought). Seed infection by *A. flavus* and contamination by aflatoxin were highly correlated across years and cultivars. Although seed infection by *A. flavus* was very responsive to water stress in the field, aflatoxin contamination did not increase proportionally. This may have been influenced by high soil temperatures in Niger, which may exceed 40 C. Most reports indicate that a minimum of 20 to 30 d of drought is needed for significant aflatoxin contamination, but contamination was observed after 14 d of water stress in this study.

Key Words: Water stress, drought, groundnut.

Aflatoxins are toxic, carcinogenic, teratogenic and immunosuppressive compounds produced when toxigenic strains of the fungi *Aspergillus flavus* Link. ex Fries and *A. parasiticus* Speare grow on peanut (*Arachis hypogaea* L.), maize (*Zea mays* L.), and many other agricultural commodities. Tests of human blood have shown that a very high percentage of the populations of several countries in Asia and Africa have been exposed to aflatoxins (Wild *et al.*, 1992; Abdulrazzaq *et al.*, 2001). Maize and peanuts are important in the diet of people in Asia and Africa and are likely to be the main sources of these toxins. Aflatoxins B_1 and G_1 are the most commonly produced forms in peanut. They are highly toxic to livestock and have been implicated in human diseases (Gong *et al.*, 2002). The relatively high levels of primary hepato-cellular carcinoma in exposed populations may reflect interactions between hepatitis B and C (which are related to protein deficiency in children) and aflatoxin (Gong *et al.*, 2002). In several countries where peanut is exported to earn foreign exchange, aflatoxin is the most important quality problem (Gong *et al.*, 2002).

Aspergillus infects peanut under both pre-harvest and post-harvest conditions. Pre-harvest infection by *A. flavus* and consequent aflatoxin contamination is important in the semi-arid tropics, especially when end-of-season drought occurs (Blaney, 1985; Mehan *et al.*, 1987; Waliyar *et al.*, 1994; Holbrook *et al.*, 2000). Several sources of resistance to pre-harvest infection have been identified

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(Waliyar *et al.*, 1994; Holbrook *et al.*, 2000) but often the level of resistance is low to moderate. Some of the sources of resistance have been used in ICRISAT's breeding program (Upadhyaya *et al.*, 2003)

The duration of end-of-season drought is a major factor determining the level of aflatoxin contamination. Several papers report that seed infection by *A. flavus* and aflatoxin contamination is high when periods of drought equal or exceed 20 to 30 d (Cole *et al.*, 1982, 1984, 1985; Blankenship *et al.*, 1984, 1989; Sanders *et al.*, 1985; Mehan *et al.*, 1995; Nahdi, 1996). Much of this work was conducted in temperate or semi-arid tropic environments (Mehan *et al.*, 1991), which are different from the climate in Niger. In Niger, evapo-transpiration ratios can be very high in the post-rainy period due to high air and soil temperatures and sandy soils. The objective of this research was to compare *A. flavus* infection and aflatoxin contamination in four cultivars exposed to various intensities of drought stress in Niger.

Materials and Methods

Experiments were carried out at Sadore, Niger in the rainy seasons of 1991, 1992, 1993, and 1994. Sadore (13°15′N, 2°17′E) is situated near the town of Say, 45 km south of Niamey. Sadore has a short cropping season of about 90 d and average annual rainfall of 545 mm (mean of past 60 yr). End-of-season drought is a common occurance.

The soil at Sadore is classified as a sandy (93%) silicious isohyperthermic Psammentic Paleustalf (West *et al.*, 1984). The topsoil layer (0 to 15 cm) contains 960 g kg^{-1} sand and 30 g kg^{-1} clay with an effective cation exchange capacity of 0.9 Cmol kg^{-1}, and an organic matter content < 0.2%. The soil is acidic, with a pH (KCl) of 4.1. Phosphorus is the most soil-limiting factor for crop growth. The available P is about 2.8 mg kg^{-1}, with a total P content of 68 mg kg^{-1}. Water infiltration rate is in excess of 100 mm h^{-1}, and a rapid hydraulic conductivity assures a quick return to field capacity after a rainfall event. Available moisture ranges from 0.07 to 0.10 cm^3/cm^3 (West *et al.*, 1984; Waliyar *et al.*, 1992).

In 1991, the experimental design was a split plot with three replications. Main plots consisted of four levels of irrigation: T1 = every week, T2 = every 2 wk, T3 = every 3 wk, Control = no irrigation (rainfed). Each irrigation consisted of 20 mm water applied with a sprinkler. A combination of cultivars and dates of sowing constituted the sub plots. Two resistant (55-437 and J 11) and two susceptible (JL 24 and 28-206) cultivars were used on four dates of sowing (at the onset of rain, followed by sowing every 10^{th} d thereafter). In 1992, 1993 and 1994, the same experimental design was used except that only two dates of sowing (at the onset of rain, and sowing 15 d after) were used. The plot size was 3.5 m x 5 m (7 rows of 5 m) with a spacing of 50 cm between rows and 10 cm between plants.

Fields were prepared and 40 kg ha^{-1} of P_2O_5 was applied using an animal drawn plough. At planting seeds were hand planted and treated with thioral (25% heptachlore and 25% thiram) at the rate of 3 g kg^{-1} of seed. During the cropping seasons one to three hand-weedings were carried out using local implements. No other crop protection measures were taken.

In 1991, four plantings were performed on 27 May, 8 June, 21 July, and 2 Aug., and harvests were performed on 5 Sept., 19 Sept., 3 Oct., and 17 Oct. In 1992, plantings were performed on 25 June and 20 July and harvested on 20 Oct. and 2 Nov. In 1993, plantings were done on 5 and 22 July and harvested on 7 Oct. and 1 Nov. In 1994, plantings were done on 8 and 27 July and harvested on 13 Oct. and 1 Nov. Plants from each plot were hand harvested and pods were removed from plants. To avoid further *A. flavus* infection and aflatoxin contamination, collected pods were placed in a roofed shelter and exposed to ambient air temperatures of 30 to 35 C for rapid drying. After 3 to 4 d, 300 pods were hand shelled and a 100 seed sample was tested in the laboratory to assay for seed infection by *A. flavus*. Seeds were surface sterilized by soaking for 3 min in a 0.1% aqueous solution of mercuric chloride, rinsed three times with sterile distilled water, and placed on a filter paper in 10-cm diam. sterile petri dishes. To maintain high humidity, 1 to 2 mL of distilled water was added every day during the first 5 d. After a 7 d incubation at 25 C, seeds contaminated by *A. flavus* and other fungi were counted.

In each year, aflatoxin content was measured in a bulk sample from the three replications of each treatment by Enzyme Linked Immunosorbent Assay (ELISA) (Transia, Lyon, France). For each sample, 100 g of seed was ground and a 20 g sub-sample was used for extraction in 60 mL of an aqueous methanol solution (80% by volume). The sample was then homogenized at high speed for 3 min and filtered using a Whatman no. 1 filter. Samples were diluted with methanol solution to 1:15, 1:75 and 1:375, and 50 µL of diluted extracts were placed in duplicates into sample wells. The optical density was read at a wavelength of 450 nm with the aid of a micro-titration plate reader.

Statistical analysis was carried out using raw data and arcsin transformed values for *A. flavus*. For combined analysis of seed infection by *A. flavus*, only two planting dates were selected for 1991 (first and third planting date). Aflatoxin data were analyzed with each year used as a replication (1992-1994). Analysis of variance was performed on all data.

Results

The ANOVA of seed infection data showed highly significant effects ($P < 0.001$) of cultivars, irrigation, planting date, and their interactions. The combined analyses also showed differences between years. Therefore, the analysis of data of these variables is presented separately for each year (Table 1).

***Seed infection by* A. flavus.** Total rainfall at Sadore was 488 mm in 1991. Seed infection by *A. flavus* was significantly affected by cultivars ($P < 0.001$). The highest rate of seed infection was found in susceptible cultivars JL 24 and 28-206 (Table 2). Differences ($P < 0.001$) in seed infection depended on the irrigation treatment. On the first sowing date, the percentage of seed infected by *A. flavus* varied from 2 to 7% as irrigation interval increased

Table 1. Results of analysis of variance of preharvest *A. flavus* (%) infection of peanut seed at Sadore during 1991-1994.

Source of variation	df.	F – probability 1991	1992	1993	1994
Replicates	2				
Irrigation (T)	3	<0.001	<0.001	<0.001	<0.001
Error (a)	6				
Date of planting (D)	1	<0.001	<0.001	<0.001	<0.001
Variety (V)	3	<0.001	<0.001	<0.001	<0.001
D x V	3	<0.001	<0.001	<0.001	<0.001
D x T	3	0.382	0.177	0.904	<0.001
V x T	9	<0.001	<0.001	<0.001	<0.001
D x V x T	9	0.008	0.027	0.996	<0.001
Error (b)	56				

in resistant 55-437 and from 1 to 33% for the susceptible cultivar 28-206. Cultivar 28-206 exhibited low infection rates with weekly irrigation, but seed infection was particularly high in 28-206 after 3 wk of water stress (Table 2). In the second planting date, seed infection in JL 24 was 34% in plots irrigated weekly (Table 2). In the third planting date, drought stress became severe even with weekly irrigation. Seed infection for the resistant cultivar 55-437 was 5% with weekly irrigation and 13% for rainfed plots. Colonization in JL 24 was 41% with weekly irrigation and 68% in rainfed plots. In the fourth planting date, rates of the seed infection were significantly higher, particularly for the susceptible cultivar JL 24, which reached 78.1% seed infection in rainfed plots (Table 2). In 1991, there was a prolonged period of drought and pod yield was greatly reduced. Therefore, the number of pods and seeds for testing was limited and aflatoxin content was not measured this year.

Table 2. Effect of planting dates of four peanut cultivars on preharvest seed infection of peanut by *A. flavus* in 1991.

Irrigation frequency (T)[a]	Planting date (D)[b]	Cultivars (C) 55-437	J 11	28-206	JL 24	Mean
		----------%----------				
T1	D1	2	3	1	26	8
	D2	4	0	4	34	10
	D3	5	2	15	41	15
	D4	5	3	26	52	21
T2	D1	5	5	8	33	13
	D2	5	5	15	43	17
	D3	7	6	18	50	21
	D4	7	7	30	59	26
T3	D1	6	17	13	39	16
	D2	6	5	19	49	20
	D3	9	9	27	54	25
	D4	8	9	38	66	30
Control	D1	7	7	33	51	25
	D2	8	8	35	59	28
	D3	13	10	41	67	33
	D4	14	12	53	78	39

SE (T) = 1.550 SE (T x D) = 2.460 SE (T x C x D) = 4.527
SE (D) = 1.103 SE (T x C) = 2.430
SE (C) = 1.080 SE (D x C) = 2.194

[a]Irrigation (20 mm water) intervals were T1 = 1 wk; T2 = 2 wk; T3 = 3 wk; Control = rainfed (no irrigation).

[b]Planting dates were D1 = 27 May (onset of rain); D2 = 8 June; D3 = 21 July; D4 = 2 Aug.

In view of large percentage of seeds colonized by *A. flavus* in the third and fourth planting dates, only a normal planting date and a late planting date (15 d after first planting) were included in subsequent years. Total rainfall was 530 mm in 1992. In the first planting date, seed infection increased as the irrigation interval increased in both resistant and susceptible cultivars. Cultivar 28-206 had 10% seed infection compared to 19% for JL 24 in plots irrigated weekly. At longer irrigation intervals, these lines showed similar levels of seed infection (Table 3).

In the second planting date, colonization was 11% in the resistant cultivar 55-437 with weekly irrigation and 15% in the rainfed plots. Both susceptible cultivars had higher levels of seed infection in the second planting than the first. Infection was somewhat lower in 28-206 compared to JL 24, which showed the highest levels (Table 3).

Total rainfall was 534 mm in 1993. Seed infection was lower in the first planting date than in the previous years. The susceptible cultivar (JL 24) had 41% and the resistant cultivar (55-437) had 3% seed infection in the rainfed plots (Table 4). Seed infection was greater in the second planting date than the first, although differences were small in the resistant cultivars (Table 4).

Total rainfall in 1994 was 768 mm, which was much

Table 3. Effect of planting dates of four peanut cultivars on preharvest seed infection by *A. flavus* in Niger, 1992.

Irrigation frequency (T)[a]	Planting date (D)[b]	Cultivars (C) 55-437	J 11	28-206	JL 24	Mean
		----------%----------				
T1	D1	6	7	10	19	11
	D2	11	8	18	30	17
T2	D1	8	9	22	23	16
	D2	13	9	30	44	24
T3	D1	12	10	32	33	22
	D2	13	9	40	61	31
Control	D1	13	12	46	47	30
	D2	15	16	53	81	41
Mean	D1	10	9	28	31	19
	D2	13	10	35	54	28

SE (T) = 1.674 SE (T x D) = 1.98 SE (T x C x D) = 3.688
SE (D) = 0.747 SE (T x C) = 2.997
SE (C) = 1.435 SE (D x C) = 1.782

[a]Irrigation (20 mm water) intervals were T1 = 1 wk; T2 = 2 wk; T3 = 3 wk; Control = rainfed (no irrigation).

[b]Planting dates were D1 = 25 June (onset of rain); D2 = 20 July.

higher than in the previous years. The first planting date showed the lowest percentage of seed infection by *A. flavus* for all cultivars across irrigation treatments. J 11 showed seed infection of 1% with one irrigation every week and reached 3% seed infection in rainfed plots. Similarly, the seed infection was 11% for 28-206 compared to 23% for JL 24 in rainfed plots (Table 4). The second planting date had higher levels of seed infection, particularly in JL 24, which had 57% of seeds colonized (Table 5).

Table 4. Effect of planting dates of four peanut cultivars on preharvest seed infection by *A. flavus* in Niger, 1993.

Irrigation frequency (T)[a]	Planting date (D)[b]	Cultivars (C) 55-437	J 11	28-206	JL 24	Mean
		----------%----------				
T1	D1	0	1	9	22	8
	D2	2	2	14	29	12
T2	D1	1	1	10	28	10
	D2	4	3	16	31	13
T3	D1	2	2	11	34	12
	D2	5	4	17	41	17
Control	D1	3	2	12	41	15
	D2	6	5	18	50	20
Mean	D1	2	1	11	31	11
	D2	4	3	16	38	15

SE (T) = 0.405 SE (T D) = 0.537 SE (T x C x D) = 1.077
SE (D) = 0.249 SE (T x C) = 0.814
SE (C) = 0.408 SE (D x C) = 0.539

[a]Irrigation (20 mm water) intervals were T1 = 1 wk; T2 = 2 wk; T3 = 3 wk; Control = rainfed (no irrigation).
[b]Planting dates were D1 = 5 July (onset of rain); D2 = 22 July.

Aflatoxin contamination. In analyses using years as replicates, aflatoxin levels were significantly ($P < 0.001$) different under different irrigation schemes (Table 6). There were also significant ($P < 0.006$) differences in planting dates; the second planting date always had more aflatoxin contamination than the first planting. Significant differences ($P < 0.001$) between genotypes were observed (Table 6).

Aflatoxin contamination was high in susceptible cultivars and varied from 11 μg kg^{-1} with weekly irrigation in the first planting, to 179 μg kg^{-1} with no irrigation (control) and late planting (Table 7). Resistant cultivars had much less aflatoxin contamination, but there was always an increased level of toxin in the second planting date (Table 7). The only highly significant interaction was genotype x irrigation. This was attributed to increases in aflatoxin levels in the susceptible cultivar with increasing irrigation intervals. The aflatoxin content increased when the irrigation intervals increased. The susceptible cultivar JL 24 showed 48, 58 and 82 μg kg^{-1} in first planting date when irrigated every 1, 2 or 3 wk, respectively (Table 7).

Table 5. Effect of planting dates of four peanut cultivars on preharvest seed infection by *A. flavus* in Niger, 1994.

Irrigation frequency (T)[a]	Planting date (D)[b]	Cultivars (C) 55-437	J 11	28-206	JL 24	Mean
		----------%----------				
T1	D1	1	1	8	21	8
	D2	5	4	13	27	12
T2	D1	1	1	9	20	8
	D2	6	5	14	33	15
T3	D1	3	2	11	21	9
	D2	7	6	16	42	18
Control	D1	4	3	11	23	10
	D2	8	7	18	57	23
Mean	D1	2	2	10	21	9
	D2	7	6	15	40	17

SE (T) = 0.483 SE (T x D) = 0.713 SE (T x C x D) = 1.429
SE (D) = 0.371 SE (T x C) = 0.971
SE (C) = 0.486 SE (D x C) = 0.715

[a]Irrigation (20 mm water) intervals were T1 = 1 wk; T2 = 2 wk; T3 = 3 wk; Control = rainfed (no irrigation).
[b]Planting dates were D1 = 8 July (onset of rain); D2 = 27 July.

Relationship between A. flavus and aflatoxin contamination. There was a high positive correlation between seed infection by *A. flavus* and aflatoxin contamination across all cultivars and years of the study ($r = 0.87$; $P < 0.0001$; $n = 96$). With increased levels of seed infection by *A. flavus*, aflatoxin concentration increased in all genotypes. The correlation between seed infection and aflatoxin concentration was higher ($r = 0.81$; $P < 0.0001$; $n = 24$) in susceptible JL 24 and lowest ($r = 0.64$; $P = 0.0004$; $n = 24$) in resistant 55-437 (Fig. 1). However, high levels of seed infection by *A. flavus* did not always lead to extremely high aflatoxin concentration.

Table 6. Analysis of variance of aflatoxin concentration (μg kg^{-1}) in infected peanut seed at Sadore, Niger.

Source of variation	d.f.	s.s.	m.s.	F ratio	F prob.
Rep (Years)	2	7111	3555.5	20.39	0.002
Irrigation (T)	3	15662.8	5220.9	29.94	<0.001
Error (a)	6	1046.3	174.4		
Date of sowing (D)	1	3144	3144	13.39	0.006
T x D	3	1777.8	592.6	2.52	0.131
Error (b)	8	1878.2	234.8		
Variety (V)	3	120163.8	40054.6	84.3	<0.001
T x V	9	18967.4	2107.5	4.44	<0.001
D x V	3	2594.7	864.9	1.82	0.156
T x D x V	9	1855.4	206.2	0.43	0.91
Error (c)	48	22807.3	475.2		
Total	95	197008.7			

Table 7. Effect of planting dates and irrigation on aflatoxin concentration in four groundnut cultivars at Sadore, Niger.

Irrigation frequency (T)[a]	Planting date (D)[b]	Cultivars			
		55-437	J 11	28-206	JL 24
		mg/kg			
T1	D1	0.70	0.60	11.30	48.40
	D2	3.30	1.10	16.80	50.30
T2	D1	1.50	1.40	20.30	58.20
	D2	5.00	2.70	26.60	83.00
T3	D1	2.70	1.90	23.30	82.40
	D2	5.80	4.50	28.70	105.20
Control	D1	3.20	3.20	33.00	113.70
	D2	9.50	8.80	58.80	178.80
Mean	D1	2.00	1.80	22.00	75.70
	D2	5.90	4.30	32.70	104.30

SE (T) = 3.81 SE (T x D) = 5.84 SE (D x V) = 8.32
SE (T x V x D) = 16.48 SE (D) = 3.13
SE (T x V) = 11.55 SE (V) = 6.29

[a]Irrigation (20mm water) intervals were T1 = 1 wk; T2 = 2 wk; T3 = 3 wk; Control = rainfed (no irrigation).

[b]D1 = planting at onset of rain; D2 = planting ca. 15 d after PD 1.

Discussion

The percentage of seed infected by *A. flavus* and the concentration of aflatoxin in the seeds were high even when irrigation was applied weekly (Tables 2 to 5). This is attributed to the low and erratic rainfall and high evapotranspiration found at the Niger test site. Given the high soil temperatures in Niger, the plants may have been under heat or water stress even when irrigation was provided. Sanders *et al.* (1985) conducted studies to determine the duration of end-of-season drought stress necessary for pre-harvest *A. flavus* infection and aflatoxin production in a temperate climate. They reported that the threshold stress period for pre-harvest infection by *A. flavus* and subsequent aflatoxin contamination was between 20 and 30 d. In this study, much shorter periods (7 to 14 d) between irrigations led to seed infection and aflatoxin production. This difference may be due to differences in soil types and other environmental differences between the two study locations. Mehan *et al.* (1991) reported that *A. flavus* infection and aflatoxin contamination levels were much lower in seed of all genotypes from Vertisols than in seed from Alfisols across locations and seasons. There were no marked differences between light sandy soils and red sandy loam soils (Alfisols) in respect of seed infection by *A. flavus* and aflatoxin contamination. Graham (1982) reported greater infection by *A. flavus* and more aflatoxin on peanuts that were grown in sandy and sandy loam soils. This observation was confirmed by our

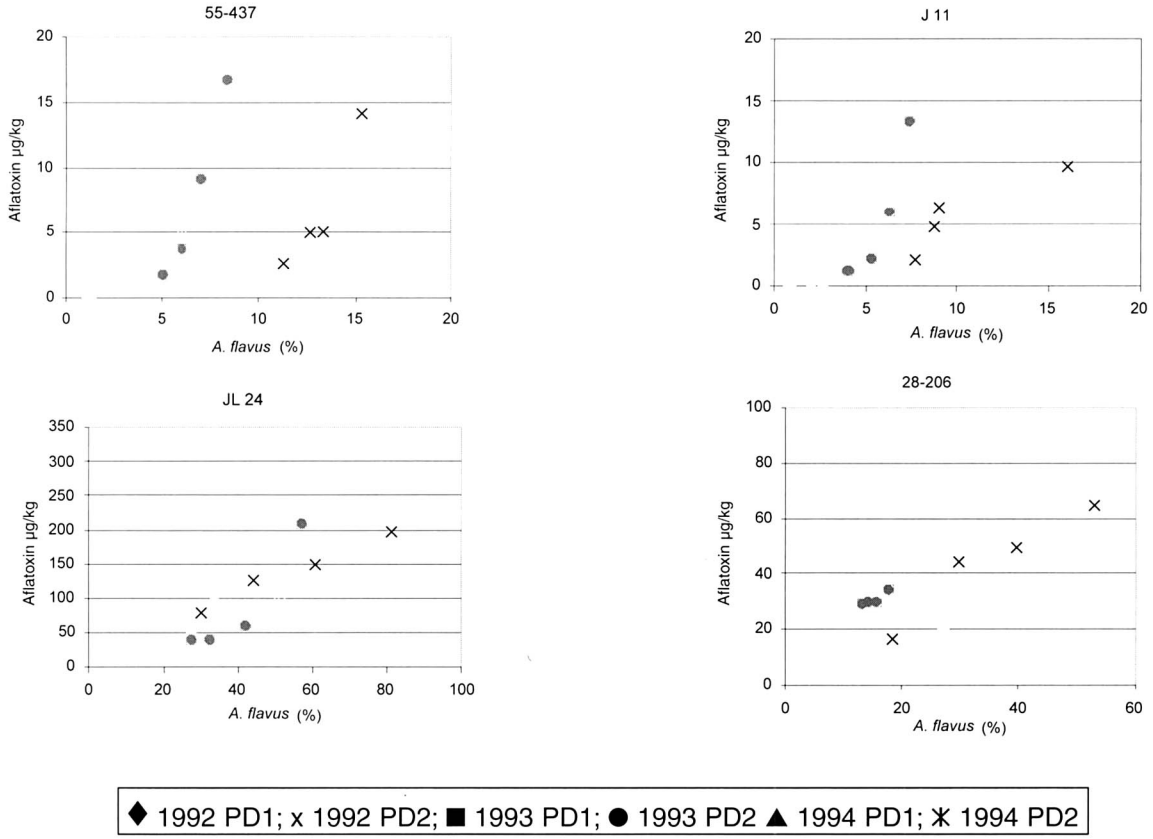

Fig. 1. Relationship between *A. flavus* infection and aflatoxin concentration at two planting dates for four cultivars during 1992 to 1994. (PD1 = first planting date; PD2 = second planting date).

data.

It is well known that the seed infection by *A. flavus* is greater with prolonged periods of drought (Blankenship *et al.*, 1980, 1983, 1984, 1989; Cole *et al.*, 1982, 1984, 1985; Hill *et al*, 1983; Sanders *et al.*, 1985; Mehan *et al.*, 1995; Nahdi, 1996). This is consistent with the results obtained in our study, which showed that a drought period of 3 wk or more leads to very high levels of seed infection. As many as 81% of the kernels of susceptible cultivars were infected by *A. flavus* (Table 3). This high percentage of *A. flavus*-infected seed did not, however, always result in the extremely high concentrations of aflatoxin found in other reports such as Holbrook *et al.* (2000) or Sanders *et al.* (1984, 1985). Severe water stress and high temperatures, particularly with late plantings, are believed to have limited toxin production. Temperature has been shown to influence toxin production (Sanders *et al.*, 1984). Cole *et al.* (1985) determined that the optimum mean pod-zone (5 to 10 cm) soil temperature for aflatoxin production ranged from 28 to 30.5 C. At very high soil temperatures (> 35 C), toxin production may be suppressed (Cole *et al.*, 1985). The temperature at 5 to 10 cm soil depth at Sadore is high during the last months of crop growth. Soil temperatures ranged from 26 (early morning) to 47 C (early afternoon). The mean soil temperature varied from 29 to 37 C. Sanders *et al.* (1985) indicated that more than 20 d, but probably less than 30 d, of drought stress at soil temperatures of 28 to 30.5 C are required for pre-harvest aflatoxin contamination. Increased duration of drought and temperature stress generally resulted in increased percentages of kernels infected by *A. flavus* (Sanders *et al.*, 1985). This is also confirmed by our data.

Under severe drought stress, the levels of seed infection and aflatoxin contamination were still low for the resistant cultivars 55-437 and J11. Cultivar 28-208 showed high levels of colonization only under prolonged water stress. Under the normal water regime the level of seed infection by *A. flavus* and aflatoxin production remained low. JL 24 was the most susceptible cultivar, showing consistent and high levels of seed infection and aflatoxin contamination.

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