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Field Crops Research 98 (2006) 20–29
Field Crops Research
www.elsevier.com/locate/fcr

Drought, pod yield, pre-harvest *Aspergillus* infection and aflatoxin contamination on peanut in Niger

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Received 16 December 2004; received in revised form 2 December 2005; accepted 3 December 2005

Abstract

Soil moisture and soil temperature affect pre-harvest infection with *Aspergillus flavus* and production of aflatoxin. The objectives of our field research in Niger, West Africa, were to: (i) examine the effects of sowing date and irrigation treatments on pod yield, infection with *A. flavus* and aflatoxin concentration; and (ii) to quantify relations between infection, aflatoxin concentration and soil moisture stress. Seed of an aflatoxin susceptible peanut cv. JL24 was sown at two to four different sowing dates under four irrigation treatments (rainfed and irrigation at 7, 14 and 21 days intervals) between 1991 and 1994, giving 40 different 'environments'. Average air and soil temperatures of 28–34 °C were favourable for aflatoxin contamination. CROPGRO-peanut model was used to simulate the occurrence of moisture stress. The model was able to simulate yields of peanut well over the 40 environments ($r^2 = 0.67$). In general, early sowing produced greater pod yields, as well as less infection and lower aflatoxin concentration. There were negative linear relations between infection ($r^2 = 0.62$) and the average simulated fraction of extractable soil water (FESW) between flowering and harvest, and between aflatoxin concentration ($r^2 = 0.54$) and FESW in the last 25 days of pod-filling. This field study confirms that infection and aflatoxin concentration in peanut can be related to the occurrence of soil moisture stress during pod-filling when soil temperatures are near optimal for *A. flavus*. These relations could form the basis of a decision-support system to predict the risk of aflatoxin contamination in peanuts in similar environments.

Keywords: *Aspergilus flavus*; Aflatoxin; Peanut; Groundnut; Drought; Crop simulation modelling

1. Introduction

Peanut is an important crop in Niger, even though growing seasons are short and variable, and drought and high soil temperatures are common (Ntare and Williams, 1998). Aflatoxins are toxic, carcinogenic, teratogenic and immunosuppressive substances (Turner et al., 2000; Wild and Hall, 2000; Hall and Wild, 2003) produced when toxigenic strains of the fungi *Aspergillus flavus* Link. ex Fries and *A. parasiticus* Speare grow on peanuts and many other agricultural commodities. Aflatoxin concentration is the most important quality problem in peanuts worldwide with serious health implications for humans as well as livestock (D'Mello, 2003; Bhat and Vasanthi, 2003; Gong et al., 2003; Waliyar et al., 2003a). For example, the majority of children tested in a recent study in West Africa who had detectable aflatoxin levels in their blood were stunted and under weight (Gong et al., 2003).

Infection of peanut by *Aspergillus* occurs under both pre-harvest and post-harvest conditions. Pre-harvest infection by *Aspergillus* and consequent aflatoxin concentration is more important in the semi-arid tropics, especially when drought occurs in the last 20–40 days of the season (e.g. Cole et al., 1989; Sanders et al., 1993). In a series of experiments using controlled soil temperature and soil water facilities, Cole and his co-workers (Cole et al., 1985, 1989; Dorner et al., 1989) have shown that pre-harvest contamination requires a drought period of 30–50 days and a mean soil temperature in the podding zone of 29–31 °C. Drought in the absence of

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doi:10.1016/j.fcr.2005.12.001

high soil temperature does not result in contamination. Shorter periods of drought (<20 days), and drought early or late in the season, also result in lower concentrations of aflatoxin (and see Azaizeh et al., 1989). Similarly, soil temperatures in the pod zone, not the root zone, cooler or warmer than 29–31 °C also result in less aflatoxin concentration (Blankenship et al., 1984; Cole et al., 1989), even if a drought is imposed. However, these relations have not been verified under field conditions in West Africa where aflatoxin is major problem.

The work of Cole and his co-workers does, nonetheless, provide a sound basis for predicting aflatoxin contamination, or at least the risk of aflatoxin concentration above permissible limits (e.g. >10 μg kg^{-1}), if water deficit and soil temperature data are available. Of course these data are more often than not available for experiments, and certainly not available for the larger geographical areas over which there would be a risk from aflatoxin contamination. Therefore, crop simulation models, whether on a point (experiment, field; e.g. DSSAT) or pixel (district, region; e.g. GLAM) scale will be needed to estimate water deficit and soil temperature (Boote et al., 1998; Challinor et al., 2004). DSSAT-CROPGRO-peanut is a process oriented, mechanistic crop growth simulation model that simulates daily water balance, soil temperatures, and plant water deficits in response to weather inputs, soil characteristics, plant growth characteristics and crop management practices (Boote et al., 1998). This model has been successfully used to simulate soil water balance and fraction of extractable soil water (FESW), which is strongly related to physiological activity (Sinclair et al., 1987), for sandy soils in West Africa (Naab et al., 2004) and India (Singh et al., 1994). CROPGRO-peanut is particularly suited to predicting aflatoxin as it 'grows' cohorts of pods, allowing temporal effects on individual pods to be modelled and accumulated. If observed field values for infection and contamination can be quantitatively related to simulated values of FESW or water deficit from calibrated models, then these relations could form the basis of decision-support system to predict the occurrence or risk of aflatoxin contamination in peanut.

The overall aim of the work reported here was to examine whether measured *A. flavus* infection and aflatoxin concentration in the field in Niger, West Africa, could be predicted from simulated values of FESW, and hence form the basis of a risk model. Waliyar et al. (2003) grew four peanut cultivars at different sowing dates and levels of irrigation in each of 4 years at Sadore in Niger, West Africa and measured *A. flavus* infection, aflatoxin concentration, and pod yield. The objectives of this study were, therefore: (i) to examine the effects of year, sowing date and irrigation treatment (giving 40 different 'environments') on pod yield, *A. flavus* infection and aflatoxin concentration in a susceptible peanut cultivar, JL24 grown in the field; and (ii) to quantify relations between observed values of *A. flavus* infection and aflatoxin concentration with simulated soil moisture or drought stress (FESW).

2. Materials and methods

Experiments were carried out at the ICRISAT Sahelian Centre, Sadore, Niger (13°15′N, 2°17′E) between 1991 and 1994. The soil at Sadore is classified as Psammentic Paleustalf (West et al., 1984) comprising 93% sand. The soil moisture holding capacity in the root zone (top 60 cm) is 44 mm (West et al., 1984). Sadore has a short growing season averaging 90 days with a long-term (1931–90) annual rainfall of 545 mm (Sivakumar et al., 1993). The experiments were situated within 500 m of the research station weather station.

Four cultivars were grown in each experiment (described below and see Waliyar et al. (2003b) for full details) but only results from one cultivar, JL24, are presented here as the overall aim was establish relations between infection/contamination and drought (FESW), rather than genotypic differences in these relations; also genetic model coefficients for these genotypes were not available. JL24 is a short duration Spanish cultivar susceptible to *Aspergillus* infection and aflatoxin contamination.

2.1. Experimental design and cultural practices

In 1991, the experimental design was a randomised complete block in a split–plot arrangement with three replications. Four levels of irrigation (7, 14, and 21 days intervals and rainfed [no irrigation]) were used as main plots. At each irrigation, 20 mm of water was applied using sprinkler irrigation. There were four dates of sowing (sowing with the onset of rain, followed by sowing every 10 days thereafter) as subplots. In 1992, 1993 and 1994 the same experimental design was used except that only two dates of sowing (sowing with the onset of rain, and sowing 15 days later) were used. The plot size was 3.5 × 5.0 m (seven rows of 5 m) with spacing of 50 cm between rows and 10 cm within the row (20 plants m^{-2}). Irrigation treatments were buffered by a 5 m strip.

Before planting, fields were prepared using an animal drawn plough and broadcast with 40 kg ha^{-1} of P_2O_5. At planting seeds were treated with Thioral (25% heptachlore and 25% Thiram) at the rate of 3 g kg^{-1} of seed. Seeds were hand planted. During the cropping season one to three hand-weedings were carried out using local implements. In this environment groundnut experiments are usually treated with carbofuran to control nematodes, which cause variable growth (Sharma et al., 1992; Waliyar et al., 1992). However, carbofuran was not applied in these experiments in case it affected *A. flavus*.

Plants were hand harvested at full maturity (95–100 d). All the pods (mature and immature) were removed from plants and immediately brought to the crop-work area where they were dried in mesh trays at ambient air temperatures of 30 to 35 °C. Under these conditions pods

dry to <10% kernel moisture content in 3–4 days, avoiding further aflatoxin contamination.

2.2. *A. flavus* and aflatoxin determination

A random sample of 300 pods were hand shelled, out of which a random sub-sample of 100 seeds was tested in the laboratory to assay for percent infection by *Aspergillus* (Waliyar and Zambettakis, 1979). More than 90% of the soil fungal population was *A. flavus,* and other species (e.g. *A. niger, A. parasiticus, Penicillium* spp., *Rhizopus* spp.) were only present occasionally. In brief, seeds were surface sterilized by soaking for 3 min in a 0.1% aqueous solution of mercuric chloride, and were rinsed three times with sterile distilled water and placed on a filter paper in 10 cm diameter sterile Petri dishes at 25 °C. To maintain high humidity, 1 to

Table 1
Rainfall between sowing and harvest, irrigation applied, calculated evaporation, and calculated fraction extractable soil water (FESW) between flowering and harvest (FL to H), and in the 25 days before harvest (H-25 days) in experiments conducted between 1991 and 1994 at the Sahelian Centre, Sadore, Niger

Sowing date	Irrigation treatment	Rainfall (mm)	Irrigation applied (mm)	Evaporation (mm)	FESW	
					Fl to H	H-25 days
1991						
1	7 days	500	300	563	0.86	0.86
	14 days	500	160	563	0.78	0.71
	21 days	500	80	563	0.77	0.71
	Rainfed	500	0	563	0.74	0.65
2	7 days	430	280	591	0.79	0.59
	14 days	430	140	591	0.71	0.45
	21 days	430	80	591	0.69	0.37
	Rainfed	430	0	591	0.65	0.37
3	7 days	246	220	455	0.66	0.51
	14 days	246	120	455	0.52	0.35
	21 days	246	80	455	0.49	0.28
	Rainfed	246	0	455	0.39	0.14
4	7 days	225	220	487	0.58	0.48
	14 days	225	120	487	0.46	0.41
	21 days	225	80	487	0.38	0.32
	Rainfed	225	0	487	0.27	0.23
1992						
1	7 days	456	320	705	0.79	0.43
	14 days	456	160	705	0.69	0.29
	21 days	456	100	705	0.61	0.16
	Rainfed	456	0	705	0.58	0.14
2	7 days	401	240	633	0.61	0.29
	14 days	401	100	633	0.49	0.13
	21 days	401	60	633	0.44	0.10
	Rainfed	401	0	633	0.42	0.09
1993						
1	7 days	408	280	578	0.80	0.67
	14 days	408	140	578	0.69	0.50
	21 days	408	100	578	0.66	0.41
	Rainfed	408	0	578	0.58	0.30
2	7 days	304	280	662	0.63	0.43
	14 days	304	140	662	0.51	0.28
	21 days	304	80	662	0.39	0.12
	Rainfed	304	0	662	0.37	0.10
1994						
1	7 days	608	260	591	0.81	0.64
	14 days	608	120	591	0.73	0.48
	21 days	608	80	591	0.68	0.46
	Rainfed	608	0	591	0.68	0.46
2	7 days	469	220	571	0.69	0.43
	14 days	469	100	571	0.57	0.34
	21 days	469	60	571	0.50	0.35
	Rainfed	469	0	571	0.50	0.35

2 mL of distilled water was added every day during the first 5 days. After 7 days, the number of seeds contaminated by *Aspergillus* was counted.

For each treatment in 1992 and 1993 aflatoxin content was measured using an enzyme linked immuno-sorbent assay (ELISA: Transia Society, France) technique (Waliyar et al., 1994) in a bulk sample from the three replications. HPLC was used to verify ELISA data. In 1994, aflatoxin content was measured in each replicate. Aflatoxin content was not measured in 1991. For each analysis, 100 g of seed was randomly picked and ground in a mill. A 20 g sub-sample was then extracted in an aqueous methanol solution (80% v/v). Into this sub-sample, 60 mL of methanol solution was added. The sample was then homogenized at high speed for 3 min and filtered using a Whatman No. 1 filter. To determine aflatoxin concentration from each dilution (1:15, 1:75 and 1:375), 50 μl of diluted extracts were placed in duplicates into the wells. The optical density was read at a wavelength of 450 nm with the aid of a micro-titration plate reader.

Analysis of variance was carried out for each year separately using raw data (pod yield and aflatoxin) or angular transformed values (percentage infection) using Genstat 5 (Genstat 5 Committee, 1987). Pod yield data was fairly variable, and original replicate data was re-checked to confirm values, resulting in missing values being substituted for observed values in four plots.

2.3. Prediction of soil water balance

CROPGRO-peanut model was calibrated using genetic coefficients for cv. 55–437, a short duration Spanish type similar to JL24, using the observed experimental and site (soil characteristics and weather) data to simulate total dry weight and pod yield using procedures described in Boote et al. (1998) and Hunt and Boote (1998). The drained upper and lower limits were 0.132 and 0.058 $cm^3\ cm^{-3}$, respectively, and the soil depth was 0.6 m, giving 44 mm available water. Key parameters include the cultivar genetic coefficients for phenology (timing of vegetative and reproductive stages), growth, pod addition rate and pod yield. Daily rainfall, air and soil temperature, radiation and pan evaporation were obtained from the weather station at Sadore. The model was calibrated on the whole data set to give the best prediction of phenology and pod yield. Once the model was calibrated, the average fraction of extractable soil water (FESW) in the root zone between flowering and harvest, or in the last 40–25 days of the growing season, in each of the 40 'environments' was calculated using the simulated daily soil water content and available water content. Rates of change in FESW were also calculated, but as durations from flowering to harvest were mostly similar, and irrigation was applied at 7–21 days intervals, this approach did not improve predictions.

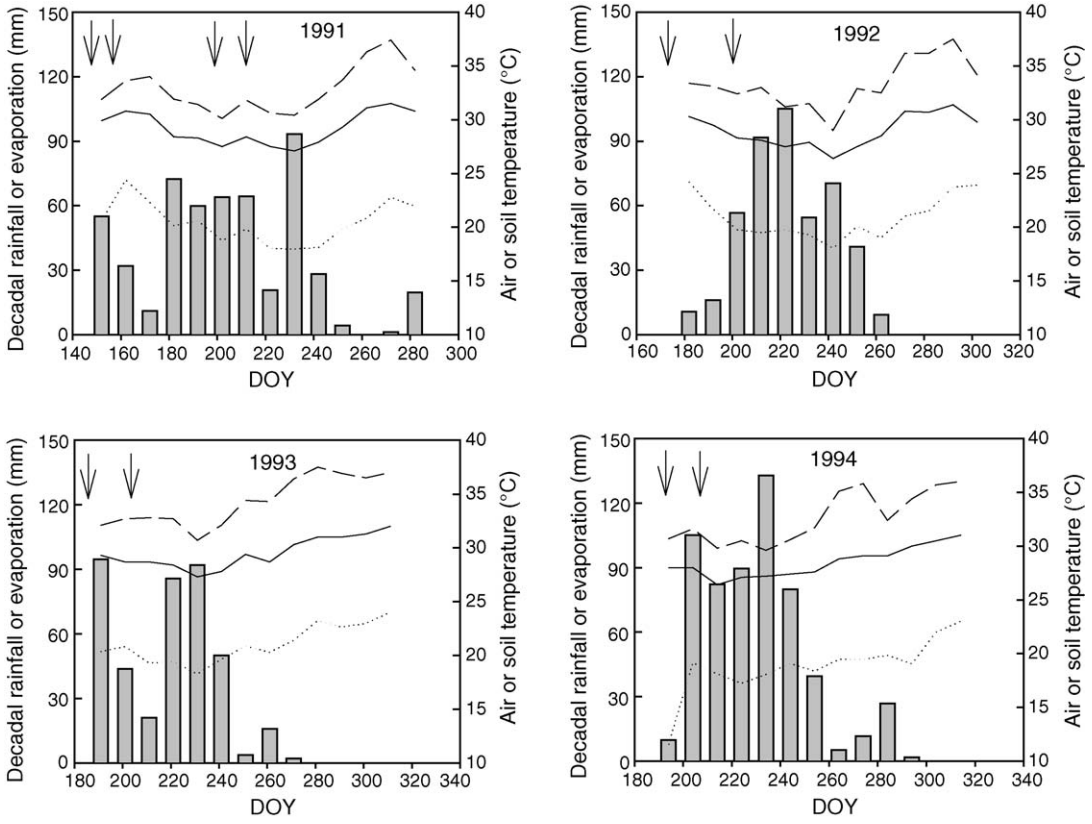

Fig. 1. Decadal values of total rainfall (bars) and evaporation (long-dashed line), and average air (solid line) and soil (dotted line) temperature at Sadore, Niger between 1991 and 1994. Arrows show planting dates in each year.

Table 2
Effect of sowing date and irrigation treatment on observed days to 50% flowering, number of plants at harvest, pod yield, *A. flavus* infection (transformed values in parenthesis) and aflatoxin concentration in cv. JL24 during 1991–1994

Sowing date	Irrigation treatment	Days to 50% flowering	No. plants at harvest (m^2)	Pod yield (kg ha^{-1})	%*A. flavus* (arcsine%)	Aflatoxin (μg kg^{-1})
1991						
1	7 days	30	17.4	1061	26 (30)	[a]
	14 days	30	16.5	722	33 (35)	[a]
	21 days	27	17.8	945	39 (39)	[a]
	Rainfed	29	16.3	1315	51 (46)	[a]
2	7 days	34	6.3	332	34 (36)	[a]
	14 days	38	4.1	211	43 (41)	[a]
	21 days	32	5.8	242	49 (44)	[a]
	Rainfed	37	6.7	491	59 (51)	[a]
3	7 days	32	7.1	295	41 (40)	[a]
	14 days	32	8.2	147	50 (45)	[a]
	21 days	31	6.4	171	54 (47)	[a]
	Rainfed	34	6.7	194	67 (55)	[a]
4	7 days	37	8.4	316	52 (46)	[a]
	14 days	27	7.6	153	59 (50)	[a]
	21 days	28	9.6	170	66 (54)	[a]
	Rainfed	27	8.7	247	78 (62)	[a]
	SED (9, 24 d.f.)[b]	2.2	1.13	101.1	5.3 (3.3)	–
1992						
1	7 days	31	2.5	581	19 (26)	73
	14 days	33	3.1	579	23 (29)	91
	21 days	29	4.4	750	33 (35)	111
	Rainfed	33	4.4	1231	47 (43)	152
2	7 days	28	3.3	238	30 (33)	78
	14 days	26	2.4	56	44 (42)	125
	21 days	28	4.1	112	61 (51)	149
	Rainfed	28	2.9	142	81 (64)	197
	SED (3, 8 d.f.)	1.8	0.54	216.4	2.2 (1.5)	[c]
1993						
1	7 days	25	8.7	129	22 (28)	34
	14 days	25	9.2	118	28 (32)	45
	21 days	24	10.1	142	34 (35)	97
	Rainfed	24	10.2	130	41 (40)	120
2	7 days	24	8.5	90	39 (38)	34
	14 days	24	7.3	57	41 (40)	82
	21 days	24	9.6	86	51 (46)	107
	Rainfed	24	7.3	102	60 (50)	132
	SED (3, 8 d.f.)	0.4	0.57	21.4	1.4 (0.8)	[c]
1994						
1	7 days	28	9.9	119	21 (27)	38
	14 days	28	9.4	110	20 (27)	38
	21 days	28	10.5	115	21 (28)	38
	Rainfed	28	9.1	107	23 (28)	38
2	7 days	28	7.4	75	27 (32)	39
	14 days	29	7.9	74	33 (35)	41
	21 days	29	9	69	42 (40)	59
	Rainfed	28	8.6	53	57 (49)	208
	SED (3, 8 d.f.)	0.3	0.8	8.8	1.0 (0.7)	5.8

[a] Not analysed.
[b] SED: standard error of the difference.
[c] Unreplicated bulk sample.

3. Results

3.1. Weather

Total rainfall during the experimental period between 1991 and 1994 ranged from 500 to 608 mm (Table 1, Fig. 1), typical values for Sadore in Niger (Sivakumar et al., 1993). Within individual sowing date × year combinations, rainfall varied from 225 to 608 mm and pan evaporation from 455 to 705 mm. Under rainfed conditions pan evaporation exceeded rainfall by >100% in some years and only in the first sowing date (S1) in 1994 did rainfall exceed evaporation. Therefore, in some sowing date × year combinations frequent irrigation meant that excess water may have been applied. Mean daily air and soil temperature during pod-filling averaged 28–30 and 31–35 °C, respectively, generally increasing towards the end of the season as rainfall declined (Fig. 1). Environmental conditions were therefore well within the range likely to favour aflatoxin concentration.

3.2. Flowering time, stand counts and pod yield

JL24 flowered 25–30 days after sowing in most experiments and, with the exception of 1991, irrigation treatments had little effect on flowering time (Table 2). In 1991, flowering times were as late as 38 days, but these delays were not systematically related to irrigation treatment or sowing date. All experiments were harvested around 95–100 days after sowing (mean 97 ± 2.1 days) except for the third and fourth sowing date in 1991 (75 days) and the first sowing in 1992 (117 days). Therefore, durations from flowering to harvest were similar, between 65 and 70 days.

Experiments were established at 20 plants m^{-2}, but only at the first sowing date in 1991 was final plant sand close to this value (Table 2). Plant stands were generally <10 m^2 and in some cases as low as 2.4 plants m^{-2}. Clearly drought, and probably nematodes (Sharma et al., 1992; Waliyar et al., 1992) had a substantial effect on stand establishment. However, there was no relation ($r^2 = 0.13$) between pod yield and stand count.

There were significant effects of sowing date on pod yield in all years and significant effects of irrigation frequency in 1991 only (Table 3). There were no significant sowing date × irrigation interactions. Pod yields were quite variable, with rainfed plot yields often exceeding that of plots receiving supplementary irrigation. Pod yield varied with year and sowing date, from 1315 kg ha^{-1} at the first sowing date (S1) in 1991 down to 53 kg ha^{-1} at S2 in 1994 (Table 3). Delayed sowing always reduced pod yield but there was no consistent effect of irrigation treatment on pod yield.

The model predicted phenology and yields of the whole data set reasonably well (Fig. 2; $r^2 = 0.67$, $P < 0.001$, $n = 40$), with a root mean square deviation (RMSD) of 244 kg ha^{-1}. The rainfed pod yield at S1 1992 (circled in Fig. 2) was much higher than predicted and without this point $r^2 = 0.78$. Predicted yields for three irrigation treatments at S1 and S2 in 1991 were noticeably higher than observed yields.

3.3. Aspergillus infection and aflatoxin concentration

There were significant effects of irrigation and sowing date on *Aspergillus* infection in all years (Table 3). There were also significant sowing date × irrigation interactions in 1992 and 1994. The percent infection with *Aspergillus* (untransformed) ranged from 19 to 81% (Table 2). Generally, infection was higher in 1991 and lower in 1994, and it increased with late sowing. For example, in 1991 mean percent infection was 37, 46, 53 and 63 in S1 to S4, respectively. Likewise, infection increased as the irrigation frequency declined from 7-day intervals to none (rainfed). For example, in S1 in 1991, infection was 26, 33, 39 and 51% at 7, 14 and 21 days intervals, and rainfed, respectively.

Aflatoxin concentration was not measured in 1991 but between 1992 and 1994 values ranged from 34 to 208 μg kg^{-1}. In 1994, the only year in which replicate values were analysed, there were significant effects of sowing date, irrigation and their interaction (Table 3). Aflatoxin concentration was affected by year, sowing date and irrigation treatment in a similar manner to *Aspergillus* and there was a good relation ($r^2 = 0.71$, $P < 0.001$, $n = 24$)

Table 3
Mean sums of squares for replicate (Rep.), irrigation and sowing date (Date) effects on pod yield, percentage infection with *Aspergillus* (%*A. flavus*, angular transformed) and aflatoxin (1994 only) in cv. JL24 in 1991–1994

	1991			1992			1993		1994		
	d.f.	Pod yield	%*A. flavus*	d.f.	Pod yield	%*A. flavus*	Pod yield	%*A. flavus*	Pod yield	%*A. flavus*	Aflatoxin
Rep.	2	263608	82	2	308410	7	421	5	60	14	225
Irrigation	3	1780135***	516**	3	149828	666***	1038	169***	301	106***	9966***
Residual	6	13590	35	6	121179	6	1919	2	367	<1	86
Date	3	1780135***	526***	1	2522827**	1228***	12604**	596***	12178***	807***	14123***
Date × irrigation	9	34209	<1	3	151660	50*	327	3	78	72***	9808***
Residual	24	36345	33	8	159813	9	842	2	96	3	117
Total	47			23							

* Significance at $P < 0.05$.
** Significance at $P < 0.01$.
*** Significance at $P < 0.001$.

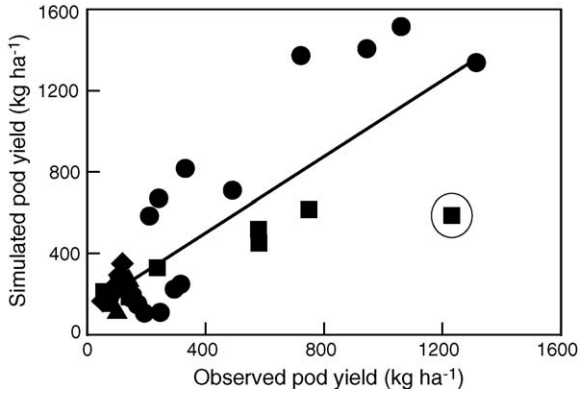

Fig. 2. Relation between simulated and observed pod yields of cv. JL 24 with CROPGRO-peanut model across all sowing dates and irrigation treatments during 4 years (1991 through 1994): $Y = 125.8 + 0.94X$ ($r^2 = 0.67$; $P < 0.001$; $n = 40$). Key to symbols: ●, 1991; ▲, 1992; ■, 1993; ◆, 1994.

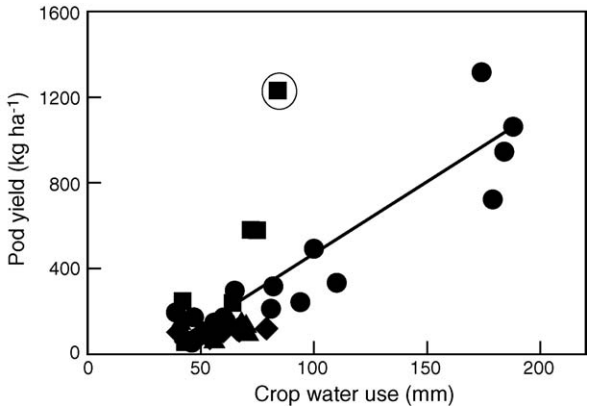

Fig. 4. Relation between observed pod yield and simulated crop water use across all sowing dates and irrigation treatments during 4 years (1991 through 1994): $Y = -203.1 + 6.7X$ ($r^2 = 0.62$; $P < 0.001$; $n = 40$). Key to symbols: ●, 1991; ▲, 1992; ■, 1993; ◆, 1994.

between aflatoxin concentration and percent infection (Fig. 3).

3.4. Pod yield, crop water use and FESW

Simulated crop water use varied from 193 mm at S1 in 1991 to only 44 mm at S4 in 1991 and S2 in 1992. As a proportion of total water use (plant and soil evaporation, deep drainage and runoff), these values represented between 34 and <10% of total water use. Crop water use was noticeably higher at S1 and S2 in 1991 (>100 mm) than in other years and sowing dates (all <100 mm). Rainfall was well distributed in 1991 (Fig. 1) and early sowings suffered no periods of prolonged drought nor periods of excessive rainfall which would have checked plant growth and hence limited crop water use. Across years, sowing dates and irrigation treatments there was a good relation ($r^2 = 0.62$, $P < 0.001$, $n = 40$) between pod yield and simulated crop water use ($r^2 = 0.75$ without rainfed S2 in 1992, circled point in Fig. 4), though as noted previously pod yields were lower than predicted in some treatments (e.g. S1 in 1991).

The simulated fraction of extractable soil water (FESW) between flowering and harvest ranged from 0.86 to 0.27 (Table 1). In the last 25 days of the season values of FESW ranged from 0.86 to 0.09. Across all sowing dates, FESW was greater and soil moisture conditions therefore more favourable with more frequent irrigation. However, in all years FESW was lower with later sowing. If stress is defined as FESW < 0.50, then most treatments experienced some degree of drought stress, particularly towards the end of the season, despite irrigation. For example, at S1 in 1991, where decadal rainfall totals were between 20 and 70 mm in all

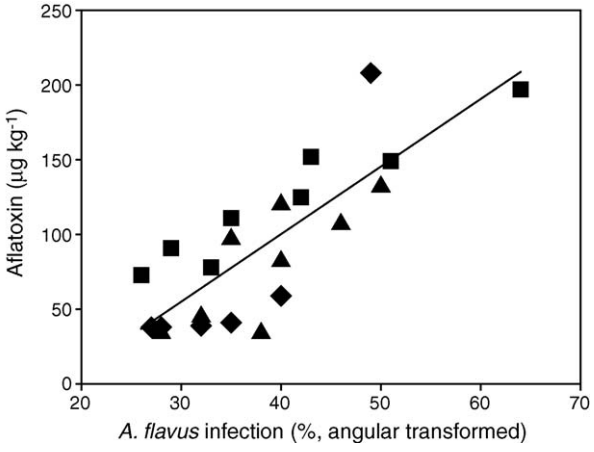

Fig. 3. Relation between observed aflatoxin concentration and *Aspergillus* infection across all sowing dates and irrigation treatments during 3 years (1992 through 1994): = −90.3 + 4.73*X* ($r^2 = 0.71$; $P < 0.001$; $n = 24$). Key to symbols: ▲, 1992; ■, 1993; ◆, 1994.

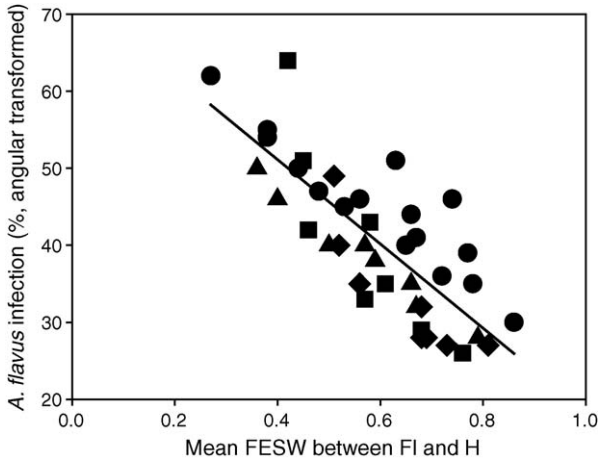

Fig. 5. Relation between observed *Aspergillus* infection and average simulated fraction of extractable soil water (FESW) between flowering (FL) and harvest (H) across all sowing dates and irrigation treatments during 4 years (1991 through 1994): $Y = 72.5–53.0X$ ($r^2 = 0.62$; $P < 0.001$; $n = 40$). Key to symbols: ●, 1991; ▲, 1992; ■, 1993; ◆, 1994.

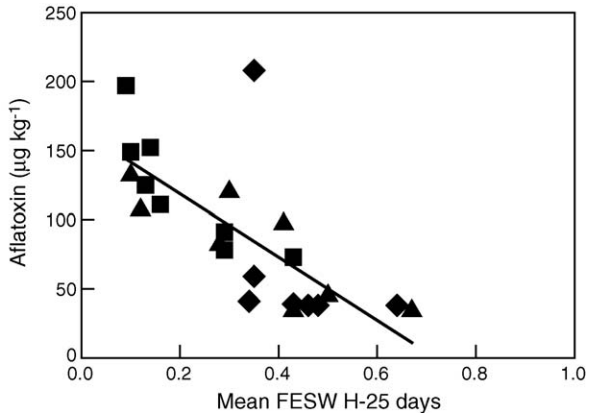

Fig. 6. Relation between observed aflatoxin concentration and average simulated fraction of extractable soil water (FESW) in the last 25 days of pod-filling (H-25 days) across all sowing dates and irrigation treatments during 3 years (1992 through 1994): $Y = 164.4–228.9X$ ($r^2 = 0.54$; $P < 0.001$; $n = 24$). Key to symbols: ▲, 1992; ■, 1993; ◆, 1994.

periods with no dry spells, FESW remained >0.6 and there were only 5 days where FSEW < 0.5. Therefore, at most only a mild and transient drought occurred at S1. In contrast, at S4 in 1991 decadal rainfall totals were between 0 and 30 mm, there were 22 days where FESW < 0.5 and drought was therefore severe.

3.5. *Aspergillus* infection, aflatoxin concentration and FESW

The year, sowing date and irrigation treatments created a wide range of water stress conditions (Table 1), which were reflected in the low yields and low simulated crop water use. Values of percentage infection with *A. flavus* (angular transformed) were most strongly negatively related to average FESW between flowering and harvest (Fig. 5; $r^2 = 0.62$, $P < 0.001$, $n = 40$); relations with FESW in the last 40 or 25 days of pod-filling accounted for half the variation of that between flowering and harvest. Aflatoxin concentration (in 1992–94 only) was also negatively related to FESW (Fig. 6; $r^2 = 0.54$, $P < 0.001$, $n = 24$), though in contrast to *A. flavus* the greatest proportion of variance was accounted for by FESW in the last 25 days of pod-filling. Thus, when water stress during pod-filling was severe, infection and aflatoxin concentration were high.

4. Discussion

The range in rainfed pod yields, from 53 to 1315 kg ha^{-1}, observed across this 4-year field study is typical for peanuts in semi-arid to arid climates (e.g. Ntare and Williams, 1998), and illustrates the variable, and harsh conditions under which small-scale subsistence farmers labour in countries such as Niger. A small delay in planting after the first rains in any given year substantially increased the risk of drought and hence reduced pod yield. Later sowings may also have established less well because of de-nitrification after the first rains. Indeed, in 1993 and 1994 pod yields hardly exceeded the amount of seed planted, typically 100 kg ha^{-1}. Yield was not related to rainfall totals, with the lowest pod yields occurring in the wettest year (1994), showing that rainfall distribution and evaporative demand is far more important (Dancette and Forest, 1986).

Pod yields across all treatments in this study were variable (mean 306 kg ha^{-1}, RMSD 244 kg ha^{-1}) and in a number of years pod yields were higher in rainfed than irrigated plots, with pod yield at S1 in 1992 anomalously high. Simulations suggested that irrigated pod yields should have been higher than those observed, particularly in 1991. Although stand counts were low in most experiments, variation in pod yield was not strongly associated with stand count within or across years, and using stand count as a covariate did not reduce variability in pod yield. Previous studies have shown that nematodes cause considerable variation in groundnut growth at this site (Sharma et al., 1992; Waliyar et al., 1992). Peanuts are also sensitive to excess soil moisture and frequent irrigation may have contributed to water logging and lower yields as well (Ibrahim et al., 2002) in some treatments/years. As nematodes were not controlled in these experiments, the usual practice at this site, it is probably that the combination of drought, high soil temperature, water logging and nematodes caused variable stand counts and growth.

CROPGRO-peanut simulated pod yields reasonably well, excluding rainfed S2 in 1991. Across all years and sowings observed pod yields were broadly related to simulated crop water use (transpiration) and the amounts of water used were typical of peanuts growing in semi-arid environments (Azam-Ali et al., 1989; Dancette and Forest, 1986). Ideally it would have been useful to have some soil moisture data to verify these simulations, but CROPGRO models have been widely tested and verified, including in semi-arid, northern Ghana (Naab et al., 2004). Within sowing dates simulated crop water use often did not vary much and FESW would appear to be a much better discriminator of stress. Many crop simulation models use FESW (or fraction of transpirable soil water, fraction of available soil water) as there is a consistency in plant responses across a wide of conditions (Sinclair et al., 1987) and as such FESW is a useful physiological (stress) index.

Drought and high soil temperature were common occurrences, especially when sowing was delayed, giving rise to ideal conditions for infection and aflatoxin contamination (Cole et al., 1989; Sanders et al., 1993; Parmar et al., 1997; Marin et al., 1998). In the experiments reported here infection was widespread and aflatoxin was found at concentrations >30 µg kg^{-1} at all treatments in 1992–1994 when average FESW in the last 25 days of pod-filling was between 0.09 and 0.67 and average soil temperatures (at the nearly meteorological station) were between 31 and 35 °C. Unfortunately aflatoxin was not measured in 1991 when values of FESW were generally

higher and there should therefore have been less contamination. Previous studies have shown that a combination of >20 days stress at the end of the season with soil temperatures of 29 to 31 °C results in aflatoxin contamination. This is associated with stress reducing kernel water activity (K_{aw}) or internal RH to values between 0.85 and 0.95 (Dorner et al., 1989); when $K_{aw} > 0.95$ phytoalexins prevent contamination and when $K_{aw} < 0.85$ *A. flavus* does not grow or produce toxin.

In the experiments reported here stress durations (FESW < 0.5) were sometimes as short as 8 days. Nonetheless contamination occurred. Given adequate inoculum, infection with *Aspergillus* and contamination with aflatoxin can occur within a short period of time. For example, Diener and Davis (1967) reported the minimum time for aflatoxin production under optimal conditions is about 60 h. The *A. flavus* strain in Niger is also highly toxigenic (Waliyar, unpublished). Thus >20 days drought and high soil temperature stress are probably not necessary for contamination as long as the physiological conditions determining K_{aw} are met in some pods — principally immature or damaged pods. So even <10 days stress was enough to cause significant aflatoxin contamination. Nonetheless, it is clear that the longer and more severe the stress, the greater are levels of infection and contamination.

CROPGRO-peanut model successfully estimated pod yields over the 40 environments used in this experiment, and simulated crop water use and FESW account for a high proportion of the observed variation in pod yield, infection and contamination. Assuming that soil temperatures are favourable for *A. flavus* and aflatoxin production, i.e. around 30 °C, then mean FESW from podding to harvest or the last 25 days of pod-filling could be used to predict infection and contamination, respectively, given basic crop data (flowering, podding and maturity dates), agronomic data (sowing date, soil water holding capacity and soil type) and weather data. Where soil temperatures are outside the optimal range then soil temperature will probably be needed as a model input as well. This is the first time that we are aware of where field observations have been successfully modelled in West Africa. Nageswara Rao et al. (2004) have used a similar approach to model the risk of contamination in Queensland, using the crop simulation model APSIM to 'count' the number of stress days, which is then related to risk. These simple empirical relations provide the basis for a decision-support system (DSS) that can be used by pathologists and other crop scientists to predict infection and contamination in the field in environments where aflatoxin is a serious problem. A good example of a DSS is the DON-forecasting system in Canada (Hooker et al., 2002). These support systems will allow areas historically at high risk, as well as areas potentially at risk in the coming season, to be targeted for technology transfer, e.g. of possible ameliorating practices such as earlier harvesting or even supplementary irrigation if available, as well as the promotion of greater awareness of the health risks of aflatoxin. Nageswara Rao et al. (2004) have shown how farmers in Queensland can manage aflatoxin given a DSS.

In conclusion, this study has shown that infection and contamination occurred in the field in Niger even when stress periods were short. Infection and contamination can be predicted in peanuts using the fraction of extractable soil water when soil temperatures are not limiting aflatoxin contamination. These relations can form the basis of a decision-support system for aflatoxin risk prediction.

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