Aflatoxins and their relationship with sugars in peanut (*Arachis hypogaea* L.)

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Abstract

Aspergillus spp. produce aflatoxins in peanut, which poses a health risk to humans and animals, as well as affecting the marketability of peanuts. The current research found that more aflatoxin is produced under rain fed (RF) compared to irrigated (IRR) conditions, and was more predominant in juvenile (R3-5 stage) compared to older (R6-8 stage) pods. No aflatoxin was recorded in marketable pods of the Streeton cultivar in either of the growing conditions, whereas the cultivar NC-7 produced aflatoxin under RF conditions only. Sugars such as glucose, fructose and sucrose were positively correlated with total aflatoxins. It appears that *Aspergillus* utilises these simple carbohydrates as substrates in biosynthesis of aflatoxins.

Key words

Aflatoxin, sugars, irrigated, rain fed, R- stage

Introduction

Peanut is a multipurpose crop providing cooking oil and vegetable protein for humans, with haulms consisting of leaves, stems and immature pods being used as cattle feed. Aflatoxin contamination of peanut is a major hazard to human and animal health, and increasingly a major constraint to commercial trading of peanuts. The fungi, *Aspergillus parasiticus* and *A. flavus* can infect pods either pre- or post-harvest and produce a particularly lethal toxin known as aflatoxin, which is a secondary metabolite. There are 4 types of aflatoxins (Aft) G1, G2, B1 and B2 and sum of these four is referred to as the total aflatoxin. The USA Food and Drug Administration established 20 ppb as the minimum acceptable level of aflatoxin in all foods other than milk. The European union has banned the import of peanut with >2 ppb of Aft B1 content and with >4 ppb of total aflatoxins in nuts prepared for human consumption. To export tree nuts to the European market, aflatoxin levels should be <3 ppb (Schatzki, 2001). It has been found that simple carbohydrates such as sucrose, glucose and maltose, but not sorbose or lactose, can serve as substrates for the synthesis of aflatoxins (Davis and Diener, 1968; Buchanan and Stahl, 1984).

Most of the research to date to understand the influence of sugars in aflatoxin production has been done under *in vitro* conditions, however, it is very important to understand the involvement of sugars in the synthesis of aflatoxin under *in vivo* conditions. The main objectives of this research were to: a) study the effect of pod development stages (R-stages), growing conditions and cultivar on aflatoxin production in peanut and (b) to study the relationship between sugars and aflatoxins under *in vivo* conditions.

Materials and methods

During the summer of 2001-02, a field experiment was conducted at the J. Bjelke Petersen Research Station in the Kingaroy area of the South Burnett region of S.E. Queensland). Two commercial cultivars ‘Streeton’ and ‘NC-7’ were included in the experiment because Streeton is reputed to have substantial

tolerance to aflatoxin contamination compared to the more susceptible NC-7 cultivar, particularly under water stress conditions. Both cultivars were grown under irrigated (IRR) and rain fed (RF) conditions in a split plot design with IRR and RF treatments as main-plots and cultivars as sub-plots with 7 replications. In the IRR treatment, irrigation was provided to maintain non-stressed conditions, via the application of approximately 25,50,40 and 40 mm at 54, 63, 96, 145 DAS (Days After Sowing), respectively. The RF treatment relied on rainfall throughout the growing season, which received 311 mm rainfall. There were 6 dry spells exceeding more than 10 days, which coincided with flowering, pegging and pod filling stages. At maturity (150 DAS), 1.8 m^2 of crop was harvested and pods were separated into different R-stages (Boote, 1986). The pod samples were freeze-dried, and R-3 to R-5 stage pods were used as such to make meal, whereas R-6 to R-8 stage pods were shelled and kernels were ground for peanut meal. Aflatoxin analysis was performed using the extraction method of Cole and Dorner (1994), with some modification. One part of peanut meal received 2 parts of (W/V) 80% methanol in centrifuge tubes and tubes were heated in a water bath at 60 ^oC for 2 hrs to extract aflatoxin. Aflatoxins were resolved using Prevail C-18 HPLC column and UV detected at 365 nm. The mobile phase was water: acetonitrile: methanol at a ratio of 58:21:21. The flow rate was 1.25 mL min^{-1}. Aflatoxins G2, G1, B2, B1 eluted at 8, 10, 12 and 14 minutes, respectively. Prior to statistical analysis, the aflatoxin data was transformed to log form (X +1) (Tubajika and Damann, 2001). However, real values are shown in tables and figures and statistical significance is indicated by letters and asterisks, respectively. The analysis of data was undertaken using the REML package of Genstat 4.2. Sugars (glucose, fructose and sucrose) in peanut pods (R3-5) and kernels (R6-8) were analysed using the HPLC technique of Naidu (1998).

Results

Treatment, cultivar and R-stage differences for aflatoxin production

At the time of harvest total aflatoxin concentration was greater under the RF treatment compared to the IRR treatment (Table 1). There was no significant difference for total aflatoxin between the two cultivars (32.1 vs. 75.4 ppb). The highest level of total aflatoxin was associated with R-4 stage pods. There was a significant interaction between cultivar and irrigation treatment. The cultivar Streeton produced the highest total aflatoxin under RF condition, while total aflatoxin levels for NC-7 were higher under IRR, but not significantly different to Streeton under RF conditions. A similar interaction was observed for R3 to R5 pods but not for pods in the R 6 to 8 stages. In the economically important pods (R6 to R8) there was no aflatoxin in Streeton under either growing environment, whereas NC-7 produced greater levels of total aflatoxins in RF compared to the IRR treatment.

Table. 1. Concentration of total aflatoxins (ppb) in different R-stages at harvest in two cultivars grown under irrigated and rain fed conditions.

R-stage	IRR growing condition		RF growing condition		Mean
	NC-7	Streeton	NC-7	Streeton	
R3 pods	49.8	0.0	3.1	183.7	59.2 b
R4 pods	146.8	179.2	107.0	419.7	213.2 a
R5 pods	39.6	0.0	10.0	121.6	42.8 bc
R6 kernels	0.0.	0.0	12.7	0.0	3.2 d

R7 kernels	0.0	0.0	5.5	0.0	1.4 d
R8 kernels	0.0	0.0	10.6	0.0	2.7 d
Mean	39.4 b	29.9 b	24.8 b	120.8 a	
Mean of Growing conditions	34.6 b (IRR)		72.8 a (RF)		

Note: Values followed by the same letter are not significantly different (P=0.05)
Differences between the cultivars for components of aflatoxin production

When averaged over all treatments, the major component of total aflatoxin was Aft G2 in Streeton and Aft G1 in NC-7 (Figure 1). Aft G1 was completely absent in Streeton. The contribution of B2 and B1 was not significant to the total aflatoxin in both Streeton and NC-7.

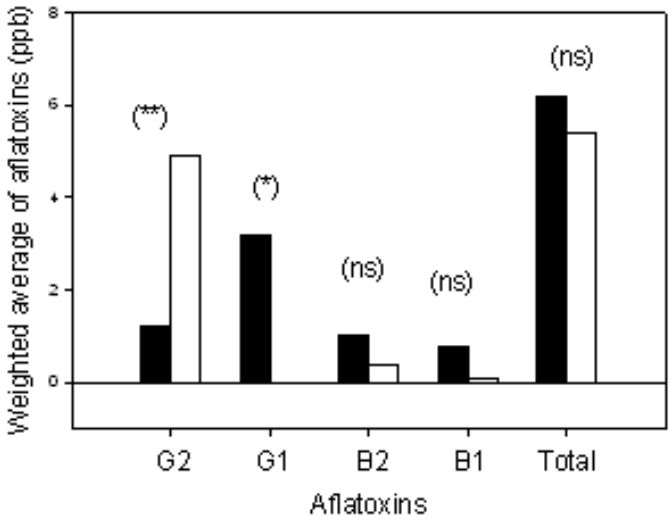

Figure 1. Weighted average of aflatoxin components (ppb) in NC-7 (■) and Streeton (□) at harvest. (ns) non significant; (*) P<0.05; (**) P<0.01

Relationships between sugars and total aflatoxin

Total aflatoxin was significantly and positively correlated with glucose, fructose, and sucrose at harvest time (Table 2). All three sugars were also positively correlated with Aft G2. Aft G1 was positively correlated with fructose only. There was no relationship between Aft B2 and sugars. A significant positive correlation between Aft B1 and sucrose was also found.

Table 2. Correlation coefficients (r) between sugars and aflatoxin components at harvest.

Aflatoxin	Glucose	Fructose	Sucrose

Aft G2	0.63**	0.43*	0.70**
Aft G1	ns	0.38*	ns
Aft B2	ns	ns	ns
Aft B1	ns	ns	0.48*
Total	0.62**	0.46*	0.67**

Note: ** - P < 0.01; * - P < 0.05; ns – non significant

Discussion

In the present experiment aflatoxins were found more under RF conditions compared to the IRR treatment. Under rain fed conditions, higher infection by *Aspergillus spp*. may have occurred due to poor functioning of the plant immune response and associated reduction in phytoalexin production (Wotton and Strange (1987). Reduced kernel moisture content and water content of the entire plant has been shown to increase the susceptibility of the plant for fungal invasion and hence aflatoxin production (Holbrook *et al*., 1994b; Cruikshank *et al*., 2000) Aflatoxin was mostly found in juvenile and immature pods, which also contained high sugar concentrations. This indicates that higher levels of these sugars either enhance fungal infection and/or they serve as important substrates in the biosynthesis of aflatoxin (Buchanan and Stahl, 1984). Cultivar differences in the components of aflatoxin were observed. The fungus *A. parasiticus* produces all 4 toxin types i.e. afltoxin G1, G2, B2 and B1 whereas *A.flavus* produces only aflatoxin B2 and B1 (Pitt, 1993). Higher concentrations of all three sugars lead to higher Aft G2 (Table 2). These results indicate that the type of toxin produced by *Aspergillus spp* may depend on the host cultivar, or alternatively cultivars are preferentially infected by a particular fungal species/strain, which then led to differences in the composition of aflatoxin.

The cultivar NC-7 produced more aflatoxins in mature pods compared to Streeton, which is consistent with previous reports indicating that Streeton possesses a substantial level of tolerance to aflatoxin contamination (Cruickshank *et al*., 2000). The current research suggests that these cultivar variations may be associated with differences in sugar profiles of maturing pods and their interaction with the aflatoxin producing *Aspergillus* fungus. Further work is in progress to identify the mechanisms responsible for the genotypic differences for aflatoxin production, particularly in mature pods.

Conclusion

The results of this study demonstrated that aflatoxin production was greater under water limiting conditions compared to irrigated conditions. NC-7, a known aflatoxin susceptible cultivar produced more aflatoxins in the marketable pods than Streeton, which is a renowned drought resistant cultivar. The results suggest that choosing a drought tolerant variety for cultivation under rain fed conditions may significantly minimise the risk of aflatoxin contamination. Aflatoxin is mostly found in the juvenile pods with higher sugar content than mature pods, which contains lower levels of sugar. In future the project is aiming on peanut cultivar differences for sugar composition and aflatoxin production under water stress conditions.

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