AFLATOXIGENIC ASPERGILLI IN FOODS AND FEEDS IN UGANDA

TERESA K. SEBUNYA

Faculty of Veterinary Medicine
Department of Veterinary Parasitology and Microbiology
Makerere University, Kampala, Uganda

and

DAVID M. YOURTEE[1]

Division of Pharmaceutical Sciences—Toxicore Laboratory
Schools of Pharmacy and Medicine
University of Missouri-Kansas City
Kansas City, MO 64108-2792

Accepted for Publication December 13, 1989

ABSTRACT

Studies conducted during the sixties and the seventies on food crops in Uganda showed that the populace was exposed to consumption of aflatoxin-contaminated foods. These studies also linked the highest incidence of liver cancer in the world to the presence of high levels of aflatoxins in the food and beverages. After a lapse of a decade, it was of interest to investigate the occurrence of aflatoxins and aflatoxigenic fungi in staple Ugandan food crops and poultry feeds derived from these foodstuffs. A simple, rapid and reproducible procedure was used. The procedure consisted of growing or culturing feed grains on a selective medium, Aspergillus flavus/parasiticus *agar (AFPA) followed by screening for aflatoxin producing fungi on a coconut agar medium (CAM) under UV light with a subsequent confirmatory screening method for aflatoxin production by the fungi in pure culture. Fifty-four samples consisting of corn and peanuts, soybean and poultry feed were analyzed for content of aflatoxigenic.* A. flavus/parasiticus *and 25 of the samples were also screened for aflatoxins B_1 and G_1, zearalenone, sterigmatocystin, ochratoxin A, citrinin, vomitoxin, and diacetoxyscirpenol (DAS). Aflatoxigenic* A. flavus/parasiticus *was detected from the majority of corn*

---

[1] Reprint Requests: David M. Yourtee, Ph.D., Associate Professor of Medicine and Pharmacy, Division of Pharmacology—Toxicore Laboratory, Schools of Pharmacy and Medicine, University of Missouri-Kansas City, 2411 Holmes Street, Kansas City, MO 64108-2792.

Journal of Food Quality **13** (1990) 97–107. *All Rights Reserved.*

(77%), peanuts (36% human food and 83.3% animal feed) and poultry feed (66.6%), but not from soybean samples. Two samples out of 25 contained detectable levels of aflatoxin B_1 *(20 ppb). For the first time other mycotoxins, zearalenone (3 samples) and vomitoxin (2 samples) were detected in corn from Uganda.*

INTRODUCTION

Numerous studies have shown that the aflatoxigenic fungi, *Aspergillus flavus* and *A. parasiticus* commonly occur in agricultural commodities including soybean, peanuts, corn, and mixed animal feeds in sub-Sahara Africa and around the world (Gbodi *et al.* 1986; El Maghraby and Maraghy 1987; Harrison *et al.* 1987). The aflatoxins and other potent environmental chemical carcinogens, originate from these fungi. The most serious effects of consumption of aflatoxin-contaminated foods and feeds are suspected to occur in Africa, south of the Sahara. It is there in countries such as Uganda, that studies have linked the highest incidence of liver cancer in the world to the presence of high levels of aflatoxins in food and beverages (Alpert *et al.* 1971; Peers and Linsell 1973; Van Rensburg *et al.* 1985). Recently, studies have demonstrated the presence of aflatoxins and their metabolites in urine samples from Zimbabwe (Nyatti and Mutiro 1987) and Nigeria (Bean 1988). The presence of aflatoxins in the urine of people on the African continent further emphasizes the reality of consuming aflatoxin-contaminated foods.

In Uganda, corn and peanuts form the basis for the staple diet of the majority of the people. Earlier studies demonstrated the occurrence of aflatoxins in significant levels in corn and peanuts at points of purchasing and consumption in Uganda (Lopez and Crawford 1967; Alpert *et al.* 1971). Lopez and Crawford (1967) also demonstrated that addition of water to peanut samples which were subsequently sealed in polyethylene bags, increased the growth of *A. flavus* and the titer of aflatoxin within seven days. Whereas the main agriculture marketing company in Uganda endeavors to control parasites in stored food crops through fumigation, neither the human food nor the poultry feed are monitored for presence of aflatoxin, so human health may be at risk from these agents.

The presence of aflatoxigenic fungi in foods or commodities might signify the potential for aflatoxin production within a substrate under conditions of optimum temperature and moisture. Hence, studies continue to be directed towards surveys of the occurrence of *A. flavus/parasiticus* in feeds and foods in different parts of the world (Romano and Fernandez 1986; Harrison *et al.* 1987). However, studies on aflatoxin in food crops in Uganda in the past 10 years have not been reported, nor have extensive studies ever been conducted on the occurrence of aflatoxigenic *A. flavus/parasiticus* in Ugandan foodstuffs. The objective of this study, then, was to investigate the occurrence of aflatoxigenic fungi and aflatoxins in the staple food crops of corn, peanuts, soybeans, and some of the poultry feeds derived from these Ugandan foodstuffs.

Recently, a number of reliable mycological methods of detection and identification of aflatoxigenic fungi in food crops have become available. For laboratories with minimum equipment and a need for results in a minimum time, the aflatoxigenic fungi detection and identification may be divided into 3 categories:

(1) Rapid presumptive tests on differential culture media, i.e., *Aspergillus flavus* and *parasiticus* agar (AFPA) identify samples that may contain the suspected aflatoxigenic fungi (Pitt *et al.* 1983).
(2) Rapid screening procedures on coconut agar medium (CAM) (Davis *et al.* 1987) measure the ability of the suspected aflatoxigenic fungi to produce aflatoxin.
(3) Qualitative methods to determine the types of aflatoxins produced by the aflatoxigenic aspergilli growing in pure culture (Filtenborg and Frisvad 1980).

It was desirable to employ such procedures to detect aflatoxigenic fungi and aflatoxin in the selected feedstuffs and compare them to chemical methods. An advantage of the mycological methods is that they could easily be employed under conditions in a developing country.

MATERIALS AND METHODS

Samples

Corn kernels, soybean seed, peanut seed and poultry feed (breeder's mash) were from food and feed stores of the Produce Marketing Board (PMB), Kampala, and those of the Animal Feed Mill at Jinja, Uganda. The samples were collected over a period of four weeks during July–August, 1987 to comprise 50 Kg bulk lots of each type of food or feed. The peanut samples were differentiated into two categories, i.e., those that the PMB had earmarked for human consumption which were in apparent good condition, and those that had been rejected for human consumption because of being apparently moldy. The two types had been stored separately at PMB stores. From each bulk lot twenty 1 Kg sample lots were obtained at random. Each of the 1 Kg samples was packed into a brown paper bag. After all the samples had been collected, they were packed into polyethylene sheet-lined boxes for shipment to the United States of America for analysis. On arrival in the United States, after the holding period for agricultural clearance, the samples were divided into two storage groups. A portion of the sample (about 100 g) was removed and placed into plastic containers and stored at 22–25°C. The remainder of the samples were stored at −20°C. For purposes of general comparison in aflatoxigenic fungi and aflatoxin content two samples of American peanuts were obtained one from a vendor in a public recreation facility (salted peanuts) and the other sample was raw peanuts from a Kansas City store.

Mycological Studies

Fifty-four sample lots were studied for external/internal content of aflatoxigenic *A. flavus/parasiticus*. Before culture, about 50 g of the sample to be cultured was held at −20°C for 72 h to kill mites and other insects that might interfere with analysis. Preliminary experiments revealed that common fungi such as *Mucor* and *Rhizopus* tended to overgrow the plates rapidly and obscure growth of the desired *A. flavus/parasiticus*. Therefore, the samples were surface sterilized briefly (1–3 min) with 1% sodium hypochlorite solution and washed 3 times with sterile distilled water before culture. Although this procedure may underestimate the external contribution of aflatoxigenic fungi, it was found to suppress the growth of the common fungi sufficiently while allowing surface entrapped/internal *A. flavus/parasiticus* to be expressed during culturing and to reduce complications in chemical analysis for mycotoxins. In the direct plating technique, 5 items (grains, etc.) were cultured per plate for 10 plates to make a total of 50 items per sample.

Samples of poultry feed (Breeder's mash) of 10 g were put into a sterile wide-mouth bottle. Ninety mL of sterile 1% peptone water was added to each sample and each was handshaken for 2 min after which the sample was serially diluted and plated (King *et al.* 1984).

The material for culture was plated on *Aspergillus flavus/parasiticus* agar (AFPA) (Pitt *et al.* 1983). All plates were incubated at 28–30°C for an initial period of 48 to 72 h before examination. The grain samples (corn, peanuts, soybean) were examined for the characteristic orange reverse coloration growth of *A. flavus/parasiticus*. AFPA plates were used with a spread technique to differentiate and select for the characteristic *A. flavus/parasiticus* conidia in the breeder's mash.

Aspergillus flavus/parasiticus agar positive samples were cultured on Czapek's agar (Raper and Fennell 1965) in duplicate, one plate at 25°C and the other at 37°C for a minimum of 7 days before examination; and on malt extract agar (powdered malt extract 20 g, peptone 1 g, glucose 20 g/L) incubated at 25°C. These media were for morphological identification and confirmation of *A. flavus/parasiticus*.

Suspected *A. flavus/parasiticus* cultures were subsequently put on coconut agar medium (CAM) (Davis *et al.* 1987) and incubated at 28–30°C for a minimum of 2 days after which they were inspected daily for a blue fluorescent zone around the colonies on exposure to longwave UV light.

Thin-Layer Chromatography (TLC) and High Performance Thin-Layer Chromatography (HPTLC) of Fungal Growth on CAM

Isolated fungi that tested positive on CAM and a number of CAM negative Aspergilli were tested for ability to elaborate aflatoxin in CAM medium using TLC and HPTLC. The aflatoxin studies employed 7 to 10 day cultures. Plugs of CAM medium with the fungal growth were picked up with short capillary tubes, 8 mm or 2 mm diameter, and deposited on silica gel plates (Analtech) 250 m and 150 m, respectively (Filtenborg and Frisvad 1980). The solvent employed was 90:9:1 chloroform:acetone:methanol. Identification of the aflatoxins liberated by a given fungal strain was made in comparison to controls of aflatoxin B_1, B_2, G_1, and G_2 (Sigma).

Analysis of Mycotoxins in Food/Feeds

A number of aflatoxigenic fungi positive and negative samples were analyzed for content of aflatoxin B_1 and G_1. They were also screened for zearalenone, sterigmatocystin, ochratoxin A, citrinin, vomitoxin and diacetoxyscirpenol (DAS). This screening was according to the method of Rottinghaus, *et al.* (1982). The detection limit for the various mycotoxins were: aflatoxin B_1, 10 ppb; aflatoxin G_1, 20 ppb; zearalenone, 50 ppb; citrinin, 400 ppb; vomitoxin, 500 ppb; sterigmatocystin, 800 ppb; T_2 and DAS, 2 ppb.

RESULTS AND DISCUSSION

Aflatoxigenic fungi were present in a majority of the samples analyzed (Table 1). Aflatoxigenic *A. flavus/parasiticus* was recovered from corn, peanuts, chicken feed (breeder's mash) and American peanut, but not from soybeans. Among the peanuts, more samples from the rejected type were positive for aflatoxigenic fungi than those earmarked for human consumption. Hence, among peanut samples, visible presence of fungal contamination may indicate possible presence of aflatoxigenic fungi, although other fungi may be present as well. In this case, other fungi, e.g., *Aspergillus niger, Rhizopus spp* and other nonaflatoxin producing Aspergilli, e.g., *Aspergillus ochraceus* and *Aspergillus tamarri* were also present. The rejected peanuts most likely would end up used as animal feed with possible resultant health risks and economic loss to the animal industry, particularly the poultry industry, where so much processed feed is consumed.

The number of *A. flavus/parasiticus* positives varied widely in the various samples. Table 2 shows the concentration of *A. flavus/parasiticus* in breeder's

TABLE 1.
OCCURRENCE OF AFLATOXIGENIC *A. FLAVUS/PARASITICUS*
IN FEEDS AND FOODS FROM UGANDA

Type of Sample	Lots Studied	Number with Aflatoxigenic fungi and (%)[1]
Corn	13	10 (77%)
Peanuts (human consumption)	11	4 (36%)
Peanuts (rejects)	12	10 (83.3%)
Breeder's mash	9	6 (66.6%)
Soybean	7	0 (0%)
American peanut	2	1
Total	54	31

1. A majority of plates per lot with one or more positive grains were required for a sample lot to be considered positive

mash from a Jinja animal feed processing facility. The concentration of suspected *A. flavus/parasiticus* varied from 100 to 7500 organisms per gram. Of the 45 suspected isolates chosen and tested on HPTLC, 11 samples or 24.4% were positive for aflatoxin production. The ability of the suspected *A. flavus/parasiticus* isolates from any one sample in the total sampled to produce aflatoxins varied from 0 to 60% of the isolates. The CAM screening procedure for aflatoxin production generally tied well with the HPTLC results in that only 2 positive samples were missed by the CAM procedure.

In corn, the average percentage of aflatoxin producing *A. flavus/parasiticus* in the suspected population (22.9%) was similar to that found in breeder's mash. Within isolates (Table 3) from individual samples aflatoxigenicity varied from 7.6 to 75%. However, the percentage of aflatoxigenicity in individual corn samples averaged about 3.5%.

The types of aflatoxins elaborated in pure culture varied between the source of the isolate (Table 4). Peanuts were the most extensive producers of aflatoxigenic fungi with varied capacity to produce aflatoxigenic types. Some of the isolates from peanuts produced the four aflatoxins (B_1, B_2, G_1, G_2) in apparently large quantities as suggested from TLC spot intensity. Aflatoxigenic isolates from the various sample types seemed to produce qualitatively similar patterns of mycotoxins, e.g., isolates from peanuts often produced the B_1, G_1 pattern; while fungal isolates from corn and animal feed produced B_1 only.

TABLE 2.
CONCENTRATION OF *A. FLAVUS/PARASITICUS* IN BREEDER'S MASH FROM A JINJA ANIMAL FEED PROCESSING FACILITY

Sample No.	Concentration of AFPA[a] Positive Fungi per Grain	Fluorescense in CAM[b] Medium No. Positive/No. Tested x/5	Aflatoxins Produced on HPTLC[c] No. Positive/No. Tested x/5	Type of Aflatoxin Produced
Y120	7.5×10^3	0/5	0/5	NA*
Y121	3.5×10^3	2/5	2/5	B_1
Y122	2×10^3	0/5	0/5	NA
Y123	2.4×10^3	0/5	0/5	NA
Y124	2×10^3	0/5	1/5	B_1
Y125	2.1×10^3	1/5	1/5	B_1
Y126	8×10^2	2/5	3/5	B_1
Y127	1×10^3	3/5	3/5	B_1
Y129	1×10^2	1/5	1/5	B_1, G_1

[a] AFPA = *Aspergillus flavus/parasiticus* agar
[b] CAM = Coconut agar medium
[c] HPTLC = High Performance Thin Layer Chromatography
* NA = Not applicable

Table 5 is a comparison of analyses for aflatoxigenic fungi and aflatoxins. Twenty-five samples were analyzed for aflatoxin B_1 and G_1 and other mycotoxins. Aflatoxigenic *A. flavus/parasiticus* was recovered from 18 out of the 25 samples, but aflatoxin B_1 was detected at 20 ppb in only 2 samples, i.e., in one sample of corn (Y208) and in a poultry sample (Y121). Sample Y208 was one of the few corn samples that was rejected by the Produce Marketing Board as food for human consumption, based on unsightly appearance. In the laboratory, this sample appeared discolored and moldy and aflatoxigenic. *Aspergillus/parasiticus* was isolated from it. No other potential mycotoxin producing fungi was recovered from sample Y208. Of interest is the detection of the other mycotoxins, besides aflatoxins, zearalenone and vomitoxin in corn. This is the first report of the occurrence of these mycotoxins in foodstuffs from Uganda.

Although *A. flavus/parasiticus* and aflatoxins were detected, findings on the samples from Uganda are somewhat unexpected. This area of the world is one where high temperatures and humidity would be optimum for *A. flavus/parasiticus* growth. The serial dilution and sample treatment techniques for isolating

TABLE 3.
AFLATOXIGENIC A. *FLAVUS/PARASITICUS* IN CORN FROM UGANDA

AFPA* Positive Fungi Recovered x/50 (%)	Percent Aflatoxigenic Samples x/50 x 100	Percent Aflatoxigenic Fungi from a Suspect Population
13/50 (26)	1/50 (2)	1/13 (7.6)
19/50 (38)	3/50 (6)	3/19 (15.8)
4/50 (8)	2/50 (4)	2/4 (50)
14/50 (28)	2/50 (4)	2/14 (14.4)
7/50 (14)	2/50 (4)	2/7 (28.5)
5/50 (10)	2/50 (4)	2/5 (40)
4/50 (8)	3/50 (6)	3/4 (75)
12/50 (24)	2/50 (4)	2/12 (16.6)
5/50 (10)	2/50 (4)	2/5 (40)

*Two samples were negative

TABLE 4.
ABILITY OF 79 ISOLATES OF *A. FLAVUS* FROM PEANUT, CORN, AND POULTRY FEED TO PRODUCE AFLATOXINS

Source of Fungi	Aflatoxin Produced	Number of Isolates
Peanut	B_1	6
	B_1, G_1	21
	B_1, B_2	1
	B_1, G_1, B_2, G_2	5
	B_1, B_2, G_1	2
Corn	B_1	19
	B_1, G_1, B_2, G_2	2
Animal feed	B_1	10
	B_1, G_1	1
American peanuts (unsalted)	B_1	12

TABLE 5.
OCCURRENCE OF *ASPERGILLUS FLAVUS/PARASITICUS* AND AFLATOXINS IN UGANDAN FOODS/FEEDS

		Mycological and Chromatographic Analysis		Chemcial Analysis Mycotoxins in Food/Feed
Sample Number	Type of Food/Feed	*A. flavus/ Parasiticus*	Type of Aflatoxins	
Y120	Chicken feed	+	not aflatoxigenic	ND*
Y121	" "	+	B_1	Detected[a]
Y124	" "	+	B_1	ND
Y126	" "	+	B_1	"
Y129	" "	+	B_1, G_1	"
Y162	Peanuts	+	B_1, B_2, G_1	"
Y166	"	+	B_1, G_1	"
Y169	"	+	B_1	"
Y172	"	+	B_1, G_1	"
Y173	"	+	B_1, B_2, G_1, G_2	"
Y182	"	+	B_1, G_1	"
Y183	"	-Negative-		"
Y185	"	+	B_1, B_2	"
Y186	"	-Negative-		"
Y188	"	-Negative-		"
Y193	Corn	-Negative-		"
Y194	"	+	B_1, B_2, G_1, G_2	"
Y195	"	+	B_1	"
Y206	"	-Negative-		"
Y208	"	+	B_1	Detected[b]
Y214	"	+	B_1	Detected[c]
Y215	"	+	B_1	Detected[d]
Y216	Soybean	-Negative-		ND
Y220	" "	-Negative-		"
AMP2-2	Peanuts	+	B_1	"

[a] Sample #Y121 contained about 20 ppb aflatoxin B_1

[b] " #Y208 contained about 20 ppb aflatoxin B_1

[c] " #Y214 contained about 0.5 - 1 ppm zearalenone and < 0.5 ppm vomitoxin

[d] " #Y215 contained < 0.5 ppm zearalenone

* ND means not detected

colonies may have led to a lower percentage of samples containing *A. flavus/ parasiticus*. In samples from the Southern United States, Harrison *et al.* (1987) found a high percentage (32%) of *A. flavus/parasiticus* isolated from corn to be aflatoxigenic. The percentage of aflatoxigenic *A. flavus* from poultry feed in Spain was about 39% (Romano and Fernandez 1986). From a field perspective, the occurrence of lower levels of aflatoxigenic *Aspergillus flavus/parasiticus* and aflatoxins in the samples from Uganda could be attributed to a number of factors among which are the practice of fumigation in the storage facility to control parasites at the Produce Marketing Board. Control of parasites in grain by use of insecticides appears to reduce aflatoxin contamination which may increase in insect damaged crops following increased *A. flavus* growth (Diener 1983). Interference competition is known to occur between *A. flavus* and *A. niger* when cultured together in corn with resultant interference with aflatoxin production but not *A. flavus* growth in the laboratory (Wicklow *et al.* 1980). The long-term effect of this interference competition among fungal species is not known under field and storage conditions in Uganda.

The findings here, however, do suggest a potential health hazard of aflatoxigenic fungi prevailing in staple foods of corn and peanuts as well as in the poultry feeds in Uganda. Because aflatoxins could accumulate quickly in these foods, given the right conditions of humidity and temperature in storage and rainfall where the crops are grown, continued monitoring and surveillance for aflatoxins and other mycotoxins in foods/feeds under different conditions would be recommended.

ACKNOWLEDGMENTS

The authors wish to thank Dr. G. Rottinghaus of the Veterinary Diagnostic Laboratory, University of Missouri, Columbia, Missouri, for assistance in mycotoxin analysis; Dr. S. W. Peterson of USDA Midwest Area Northern Regional Research Center, Peoria, Illinois, for assistance in identification of fungi; and Dr. M. A. Klich of USDA Southern Regional Research Center, New Orleans, Louisiana, for her helpful advice. This work was conducted when the first author was on a Fulbright Research Fellowship and was partially supported by the University of Missouri, Kansas City, Missouri. We also thank Makerere University, Uganda, for allowing the first senior leave of absence to carry out this tudy and for permission to publish this paper.

REFERENCES

ALPERT, M. E., HUTT, M.S.R., WOGAN, G. N. and DAVIDSON, C. S. 1971. Association between aflatoxin content of food and hepatoma frequency in Uganda. Cancer *28*, 253–260.

DEAN, T. A. 1988. Development of a high performance liquid chromatography method for aflatoxins in human urine and analysis of aflatoxin metabolite excretion in Nigerians. Ph.D. Thesis, University of Missouri, Kansas City.

DAVIS, N. D., IYER, S. K. and DIENER, U. L. 1987. Improved method of screening for aflatoxin with a coconut agar medium. Appl. Environ. Microbiol. *53*, 1593–1595.

DIENER, L. U. 1983. Aflatoxin and *Aspergillus flavus* in corn. Craftmaster printers, Opelika, Alabama.

EL MAGHRABY, O. M. and EL MARAGHY, S. S. 1987. Mycoflora and mycotoxins of peanut (Arachis hypogoea L.) seeds in Egypt. 1. Sugar fungi and natural occurrence of mycotoxins. Mycopathologia *98*, 165–170.

FILTENBORG, O. and FRISVAD, I. C. 1980. A simple screening method for toxigenic moulds in pure culture. Lebensin-Wiss-U Technol. *13*, 128–130.

GBODI, T. A., NWUDE, N., ALIU, Y. O. and IKEDIOBI, C. O. 1986. The mycoflora and some mycotoxins found in corn (Zea mays) in the plateau state of Nigeria. Vet. Hum. Toxicol. *28*, 1–5.

HARRISON, M. A., CLAYTON, S. J. and CARPENTER, J. A. 1987. Incidence of aflatoxigenic isolates of *Aspergillus flavus/parasiticus* obtained from Georgia corn processing plants. J. Food Qual. *10*, 101–105.

KING, A. D., PITT, J. I., BEUCHAT, L. R. and CORRY, J.E.L. 1984. *Methods for the Mycological Examination of Food*, Plenum Press, New York.

LOPEZ, A. and CRAWFORD, M. A. 1967. Aflatoxin content of peanuts sold for human consumption in Uganda. Lancet *2*, 1351–1354.

NYATTI, C. B. and MUTIRO, C. F. 1987. A survey of urinary aflatoxin in Zimbabwe. Int. J. Epidemiol. *16*, 516–519.

PEERS, F. G. and LINSELL, T. 1973. Dietary aflatoxins and liver cancer. A population based study in Kenya. Br. J. Cancer *27*, 473–484.

PITT, J. I., HOCKING, A. D. and GLEN, D. R. 1983. An improved medium for the detection of *Aspergillus flavus* and *A. parasiticus*. J. Appl. Bact. *54*, 109–114.

RAPER, K. B. and FENNELL, D. I. 1965. The genus *Aspergillus*. The William and Wilkins Co.

ROMANO, M.A.M. and FERNANDEZ, S. G. 1986. Mycoflora of commercial poultry mixed feeds. Poultry Science *65*, 284–287.

ROTTINGHAUS, G. E., OLESEN, B. and OSWIELER, G. D. 1982. Rapid screening method for aflatoxin B_1, zearalenone, ochratoxin A, T-2 toxin, diacetoxyscirpenol and vomitoxin. Am. Assoc. Vet. Lab. Diag., 25th Annual Proceedings, pp. 477–484.

VAN RENSBURG, S. J., COOK-MOZAFFANI, P., VAN SCHALKWYK, D. D. and VAN DER, J. J. 1985. Hepatocellular carcinoma and dietary aflatoxin in Mozambique and Transkei. J. Canc. *51*, 713–26.

WICKLOW, D. T., HESSELTINE, C. W., SHORTWELL, O. L. and ADAMAS, G. L. 1980. Interference competition and aflatoxin levels in corn. Phytopathology *70*, 761–764.