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**AFLATOXIN SURVEY OF MAIZE FROM THE 1978
CROP IN THE SOUTH BURNETT REGION OF
QUEENSLAND**

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SUMMARY

A rapid screening test was applied to 805 samples from the 1978 maize crop in Queensland. Of 140 samples selected as positive by the test, aflatoxins were detected in 15 samples in which average levels were 0.045 mg kg⁻¹ total aflatoxins B₁ B₂ G₁ and G₂. Of 70 samples chosen at random from the remaining 665, only two contained detectable levels, averaging 0.007 mg kg⁻¹ total aflatoxins.

I. INTRODUCTION

Aflatoxins are highly toxic substances that can be produced by the *Aspergillus* group of fungi. They constitute a large group of closely related compounds, but four members, aflatoxins B₁ B₂ G₁ and G₂, are the most commonly detected in agricultural commodities. Toxicity has been demonstrated in all animal species tested with some differences in susceptibility. At dietary levels well below those shown to produce acute effects, some aflatoxins, particularly B₁, are extremely potent hepatocarcinogens. The presence of these substances in foodstuffs therefore constitutes a danger to both man and animals (Allcroft 1969; Destroy, Lillehoj and Ciegler 1971).

Aflatoxins have been reported as contaminants of maize in many parts of the world (Alpert, Hutt, Wogan and Davidson 1971; Cambell and Stoloff 1974; Girgis, El-Sharif, Rofael and Nesheim 1977; Krishnamachari, Nagarajan, Bhat and Tilak 1975; Nwokolo and Okonkwo 1978, Shank, Wogan, Gibson and Nondasuta 1972).

Contamination can occur both on crops in the field and under improper storage. Many of the conditions leading to problems in storage have been reviewed by Hesseltine (1976), but investigations into field contamination show this problem to be complex. Factors implicated include water stress and insect damage (Anderson, Nehring and Wichser 1975. Fennell, Kwolek, Lillehoj, Adams, Zuber, Calvert, Guthrie, Bockholt, Manwiller and Jellum 1977).

During 1977, 40 samples of maize from the South Burnett region of Queensland were analysed for aflatoxin and four contained levels ranging from 0.011 to 0.100 mg kg⁻¹ total aflatoxins B₁ B₂ G₁ and G₂. These samples however, were too small in size and number to be considered in any way representative of the whole year's crop (B. J. Blaney unpublished data).

Queensland production of maize has remained fairly steady at approximately 80 000 tonnes a year over the last 20 years, which is about half the Australian production. Principal growing areas are Wide Bay-Burnett, Darling Downs and the Far North regions. Most of the maize is used in animal feeds with a small but increasing amount entering human foods. Recent amendments to the Stock Food Regulations of Queensland set a limit for maize of 0.02 mg kg^{-1} aflatoxin B_1 (Anon. 1979).

The aim of the present survey was to evaluate the extent of field and farm contamination of the 1978 maize crop in the South Burnett region.

II. METHODS

Samples were examined from approximately 6 000 tonnes of maize received at one depot of the South Burnett region. A spear sampler was used to obtain five samples from each truckload of grain which were combined to give one 5-kg sample. Of this, 2 kg were tested for aflatoxins. During the 1978 season, samples were taken from 805 truckloads representing maize from 107 growers.

The 2 kg maize samples were coarsely cracked using a maize cracker so that each kernel was reduced to five or more particles. These particles varied greatly in size and a small quantity of fines was unavoidable. The cracked sample was spread out on a stainless steel tray to a depth of 2 cm and inspected under long wave ultra violet light (365 nm) in a viewing cabinet. The samples were mixed by hand during viewing so that practically all particles were examined. This procedure occupied 5 to 10 min. per sample.

Samples were classified according to the number of bright, greenish-yellow fluorescent (BGY positive) fragments present (regardless of size). In this way, 140 samples containing five or more BGY positive particles were selected for chemical analysis. In addition, 70 samples were taken at random from the remaining 665 samples containing less than five fluorescing particles.

All selected samples were hammermilled to pass through a 1-mm screen. Subsamples of 50 g were taken by division and analysed for aflatoxin. This involved aqueous acetone extraction and ferric hydroxide gel cleanup as described by the Association of Official Analytical Chemists (1975), followed by estimation by two-dimensional thin layer chromatography on silica gel. Diethyl ether was used in the first development, followed by 10% acetone in chloroform. Quantification was achieved by visual comparison with standard aflatoxins developed in the same manner.

Confirmation of aflatoxin identity was gained by colour change from blue to yellow upon reaction with dilute sulphuric acid, and by thin layer chromatographic separation of derivatives after reaction with hydrochloric acid.

Limits of detection for the method were 0.002 mg kg^{-1} aflatoxins B_1 and G_1 ; $0.0005 \text{ mg kg}^{-1}$ aflatoxins B_2 and G_2 . This implied a limit of 0.005 mg kg^{-1} total aflatoxins $B_1 B_2 G_1$ and G_2 .

III. RESULTS

Table I shows the total number of samples received (grouped into five growing areas), the number of samples in each BGY group, the number of samples analysed and results of positive aflatoxin tests.

The incidence of BGY positive samples was similar in all growing areas, ranging from 44% for Wooroolin to 58% for Kingaroy-Kumbia.

Aflatoxins were detected in 15 of 140 samples containing 5 to 20 BGY fluorescent particles. Total aflatoxin levels ranged from 0.005 mg kg⁻¹ to 0.34 mg kg⁻¹ while aflatoxin B₁ ranged from 0.002 mg kg⁻¹ to 0.15 mg kg⁻¹. In 70 samples selected at random from samples containing < 5 BGY fluorescent particles, two samples contained 0.005 and 0.008 mg kg⁻¹ total aflatoxins respectively.

The 17 samples in which aflatoxins were detected were obtained from 15 growers representing all five growing areas.

TABLE 1

RESULTS OF SCREENING TEST AND AFLATOXIN ANALYSES

	Growing Area																Total				
	Wooroolin				Wondai-Proston				Gayndah-Central-Burnett				Cloyna-Murgon-Tansey-Goomeri					Kingaroy-Kumbia			
No. Growers	37				31				15				9				15				107
No. Samples	331				201				151				44				78				805
Samples per grower Mean ± S.D.	9 ± 7.3				6 ± 5.1				10 ± 6.0				5 ± 3.4				5 ± 3.0				
NIL BGY PARTICLES																					
TOTAL	185				90				69				20				33				397
No. analysed	19				12				9				3				8				51
No. positive	1				0				1				0				0				2
Aflatoxin levels (mg x 10 ⁻³) kg ⁻¹	B ₁	B ₂	G ₁	G ₂	B ₁	B ₂	G ₁	G ₂	B ₁	B ₂	G ₁	G ₂	B ₁	B ₂	G ₁	G ₂	B ₁	B ₂	G ₁	G ₂	
	2.5	—	2.5	—					4	—	4	—									
1-4 BGY PARTICLES																					
Total	108				67				48				15				30				268
No. analysed	8				4				2				2				3				19
No. positive	0				0				0				0				0				0
5-10 BGY PARTICLES																					
Total	34				36				32				4				12				118
No. analysed	34				36				32				4				11				117
No. positive	4				3				5				1				0				13
Aflatoxin levels (mg x 10 ⁻³) kg ⁻¹	B ₁	B ₂	G ₁	G ₂	B ₁	B ₂	G ₁	G ₂	B ₁	B ₂	G ₁	G ₂	B ₁	B ₂	G ₁	G ₂	B ₁	B ₂	G ₁	G ₂	
	5	—	—	—	10	1	—	—	10	1	10	1	8	1	8	1					
	2	—	4	—	25	1	—	—	4	1	8	1									
	15	1	20	—	20	1	—	—	50	3	30	2									
	2	—	4	—					10	2	1	—									
									10	1	10	1									
11-20 BGY PARTICLES																					
Total	4				8				2				5				3				22
No. analysed	4				8				2				4				3				21
No. positive	0				0				0				1				1				2
Aflatoxin levels (mg x 10 ⁻³) kg ⁻¹	B ₁	B ₂	G ₁	G ₂	B ₁	B ₂	G ₁	G ₂	B ₁	B ₂	G ₁	G ₂	B ₁	B ₂	G ₁	G ₂	B ₁	B ₂	G ₁	G ₂	
	150	20	150	20	20	1	25	1													

IV. DISCUSSION

(a) CORRELATION BETWEEN BGY FLUORESCENCE AND PRESENCE OF AFLATOXIN. Since chemical analysis of large numbers of samples is expensive and time consuming, a rapid screening technique was used in which the maize was cracked and examined under long wave ultra-violet light. This disclosed the presence or absence of a bright, greenish-yellow (BGY) fluorescence in some particles of the kernels. This fluorescence is not due to aflatoxin but is thought to be formed in the field by an interaction between kojic acid produced by *Aspergillus* spp. and a peroxidase of the viable maize kernel.

Several studies (Fennell, Bothast, Lillehoj and Peterson 1973; Shotwell, Goulden, Jepson, Kwolek, and Hesseltine 1975), have shown that the incidence of aflatoxin contamination is higher in those samples exhibiting BGY fluorescence. The technique had not previously been evaluated in this country.

While the results given in table I suggest some quantitative correlation between incidence and levels of aflatoxin with number of BGY particles, the relationship is inconclusive and this is in accordance with the findings of Shotwell *et al.* (1975). The practice in the U.S.A. in some instances has been to analyse all samples containing any BGY positive fragments. While this may provide more sensitive detection of contamination, it was not practicable in this survey where slightly more than half of the samples examined contained one or more fluorescing particle.

However, the results do indicate that samples containing the highest number of fluorescent fragments are more likely to contain aflatoxin and that use of a criterion of BGY 'positive' sample to refer to one containing five or more fluorescing particles would be satisfactory for the purposes of identifying samples most likely to contain aflatoxins in excess of regulatory levels.

Pursuant to this argument, it can be stated with confidence that those samples most likely to contain aflatoxin were analysed in the present survey.

(b) AFLATOXIN LEVELS AND SIGNIFICANCE. Of 140 samples selected by the screening test to be most likely to contain aflatoxins, positive results were given by 15 samples with an average level of 0.045 mg kg⁻¹ total. In four of these samples, aflatoxins B₁ and B₂ only were detected while the others contained all four aflatoxins.

Incidence of contamination in maize varies greatly world-wide. Many reports from largely underdeveloped countries show incidences of 30 to 90% positive samples with levels averaging greater than 0.2 mg kg⁻¹ total aflatoxins (Alpert *et al.* 1971; Shank *et al.* 1972; Cambell and Stoloff 1974).

Surveys covering all growing regions in the U.S.A. carried out by the Agricultural Research Service of U.S.D.A. during the years 1964-1969 showed an overall incidence of 2.6% positive samples with average levels of 0.013 mg kg⁻¹ (Shotwell, Hesseltine, Burmeister, Kwolek, Shanmore and Hall 1969; Shotwell, Hesseltine, Goulden and Vandegraft 1970; Shotwell, Hesseltine, Vandegraft and Goulden 1971).

However, a subsequent survey concentrating on the relatively smaller growing areas of the southern States (Shotwell, Hesseltine and Goulden 1973) showed higher incidences with 35% of samples positive and containing average levels of 0.066 mg kg⁻¹. In 1973, 38% of maize samples from South Carolina were found to exceed 0.020 mg kg⁻¹ (Lillehoj, Kwolek, Shannon, Shotwell and Hesseltine 1975). In 1977, heavy incidences of contamination were seen throughout

the southern growing regions of the U.S.A. In Georgia, most of the crop was contaminated with 107 out of 351 samples exceeding 0.3 mg kg^{-1} , while in Florida 48% of 143 samples exceeded 0.5 mg kg^{-1} total aflatoxins (Brown 1977).

Seen in this perspective, the incidence found in the present survey must be considered low. In only three samples were the Queensland regulatory limits of 0.02 mg kg^{-1} aflatoxin B_1 for stock feeds exceeded. After the truckloads were mixed in bulk storage, it is unlikely that overall levels would be detectable.

The American experience demonstrates how climatic and growing conditions can dramatically alter this picture. Further surveys should be extended to other regions of the State and the rapid screening test backed by chemical analyses would appear to be suitable for this.

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