Technical Note

A note on the occurrence of mycotoxins in cereals and animal feedstuffs in Kwazulu Natal, South Africa 1984–1993

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During the period 1984–1993 just over 1600 samples of agricultural commodities, comprising maize, compound animal feeds, oil seeds, soyabean, fishmeal and forage were examined for fungi and over 20 mycotoxins using a multi-screen augmented with individual assay. Aflatoxin had the highest incidence in over 14% of all samples examined followed by trichothecenes at 10% and then zearalenone at 4%. Since 1989 selected maize samples with high levels of *Fusarium* spp. have been examined for fumonisin B_1 and of these (n = 20) in 1993, 90% were positive. Because of this result and high incidence of *Fusarium* spp. (32%) in maize and maize containing feeds, which was higher than either *Aspergillus* spp. (27%) or *Penicillium* spp. (12%), concern is expressed with regard to the potential presence of fumonisin in the food chain.

Gedurende die tydperk 1984–1993 is net meer as 1600 monsters van verskillende landbouprodukte, wat, onder andere, mielies, gemengde veevoedsel, oliesade, sojabone, vismeel en kuilvoer insluit, ondersoek vir die voorkoms van swamme en net meer as 20 mikotoksiene. Die mikotoksiene is opgespoor deur gebruik te maak van algemene opsporingstoetse wat daarna deur spesifieke individuele toetse uitgebrei is. Die aflatoksiene het die meeste voorgekom en is in 14% van al die monsters wat getoets is, gevind, gevolg deur die trichothecene (10%), en zearalenone (4%). Sedert 1989 is mieliemonsters met hoë vlakke *Fusarium* spp. getoets vir die voorkoms van fomonisien B_1 en in 1993 is 90% (n = 20) positief getoets. As gevolg van die uitslag, asook die hoë voorkoms van *Fusarium* spp. (32%) in mielies en mielie-bevattende voere, wat hoër as beide *Aspergillus* spp. (27%) of *Penicillin* spp. (12%) was, word kommer uitgespreek ten opsigte van die potensiële voorkoms van fumonisien in die voedselketting.

Keywords: mycotoxins, fungi, food

In 1985 results were published of a multi-mycotoxin screening used to analyse samples of agricultural commodities sent in by farmers and commercial companies in Natal from 1981 to 1983 (Dutton & Westlake, 1985). Since that time the analytical service has continued with improvements resulting from increasing experience and the application of additional methods.

The introduction of immunoassay (Coker, 1984) and refinement of chromatographic methods (Chu, 1984) for analysing mycotoxins tend to have made thin layer chromatography (tlc) obsolete. Multi-mycotoxin screens can also be criticised because they are not optimum for any particular toxin and at best represent a compromise between the range of toxins analysed in developing countries. However, a multi-screen using tlc as its first line of detection, can be put to good service, because it requires the minimum of equipment, can detect a range of toxins in a short period of time, and can be made semi-quantitative with the use of standards. Lack of detectability is not a problem with most mycotoxins, as levels responsible for disease in animals can be detected, which is what the practical farmer needs to know.

Ranged against this is the importance of having an experienced evaluator who can interpret results correctly and can read indicative signs such as disease symptom, fungi present and condition of the sample, with a degree of insight. The necessity of modern instrumentation for definitive quantitation and identification is also a limiting factor, particularly as developed countries have laws governing permitted mycotoxin levels in imported produce. In order to give an assessment of one multi-mycotoxin screen, the results from analysing agricultural commodities provided mainly by farmers, feed companies and other commercial concerns for the period 1984 to 1993 are reported here.

Collection of samples

Samples were submitted for analysis, mainly from farmers, feed companies and poultry companies, usually together with a completed questionnaire (previously distributed to regular clients) giving details surrounding the circumstances of the sample, e.g., method of sampling, associated disease symptom(s) and condition of sample. Where possible 1 kg samples were submitted but there was as little as 100 g in some cases. The samples were all individually milled and thoroughly mixed prior to analysis. The major materials analysed were maize and its milled products, general feed, poultry feed and their components.

Extraction, cleanup, thin layer chromatography and confirmation

The methods applied were basically the same as those used in the previous report (Dutton & Westlake, 1984). Briefly for the multi-screen, the sample was extracted with aqueous acetonitrile, defatted with iso-octane, treated with sodium bicarbonate which on extraction with chloroform gave respectively a neutral fraction and, after acidification with sulphuric acid, an acidic one. The neutral fraction was dialysed against aqueous acetone, re-extracted into chloroform and examined by tlc. The acid fraction was directly examined by tlc.

Several mycotoxins not amenable to the multi-screen analysis were independently assayed and these included: aflatoxin in groundnut (Stoloff & Scott, 1984) (other mycotoxins were not investigated in this commodity), ergot alkaloids (McLaughlin *et al.*, 1964) {extracted with ethyl acetate/ammonium hydroxide mixtures (Ware *et al.*, 1986)}; moniliformin (Scott & Lawrence, 1987); fumonisin B_1 (Sydenham & Thiel, 1992); gliotoxin (Richard *et al.*, 1989); and cyclopiazonic acid (Lansden, 1986).

Aflatoxins were detected with ultra violet light, and other mycotoxins with various spray reagents, as reported before (Dutton & Westlake, 1985) but zearalenone was located with diazotized dianisidine (in place of benzidine) (Malaiyandi *et al.*, 1983). Confirmation and semi-quantifition was done with authentic standards obtained from Sigma Chemical Co, St Louis or Makor Chemicals, Jerusalem or Council for Scien-

tific and Industrial Research, Pretoria or Medical Research Council, Tygerberg, by spiking and visual comparison.

Further confirmation and accurate quantifition of aflatoxin (Beebe, 1978) moniliformin (Thiel *et al.*, 1982) fumonisin B_1 Thiel *et al.*, 1991) patulin (Moller & Josefsson, 1981) and zearalenone (Prelusky *et al.*, 1989) were done using high performance liquid chromatography (Waters 501 or Spectra Physics P2000 both fitted with UV absorbance detector and fluorimeter) and for trichothecenes (Gilbert *et al.*, 1985) by derivatization with trimethylsilylimidazole followed by gas chromatography and mass spectrometric detection, gc/ms (Hewlitt Packard 5890 series II fitted with an m/s 5971 detector).

Mycological examination

Fungal contamination and viable propagule count was done as previously reported (Dutton & Westlake, 1985) using Ohio Experimental Station Agar (Kaufman *et al.*, 1963). For identification purposes individual colonies were transferred to other suitable agar media and where necessary sent to either the National Fungal Collection, Pretoria or to the Medical Research Council, Tygerberg (Professor W.F.O. Marasas) for species identification.

Although the total number of samples examined, 1602, was greater than in the previous report (794), it should be noted that the time period was 10 years as compared with two in the former. The lower annual average number is explained by the fact that included in the first report was the total turnover from a particular feed manufacturer wishing to ascertain the quality of their materials. In the current study the number of samples dealt with per year was fairly constant at between 100 and 200 per year up to 1993 when the number increased (Figure 1). This trend has continued for 1994 and by June had already reached the 305 mark (not presented in this report). The reason for this up-swing is difficult to explain but the increase in samples from the commercial sector (Figure 2) may be related to the re-entry of South Africa into world markets.

During the period covered by this study, we have recorded all our results in data base using commercially available software together with the occurrence of the mycotoxins, their

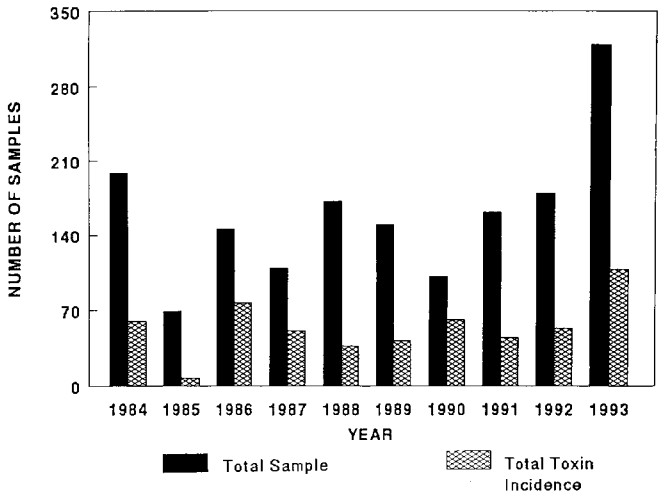

Figure 1 Number of samples per year and total mycotoxin incidence.

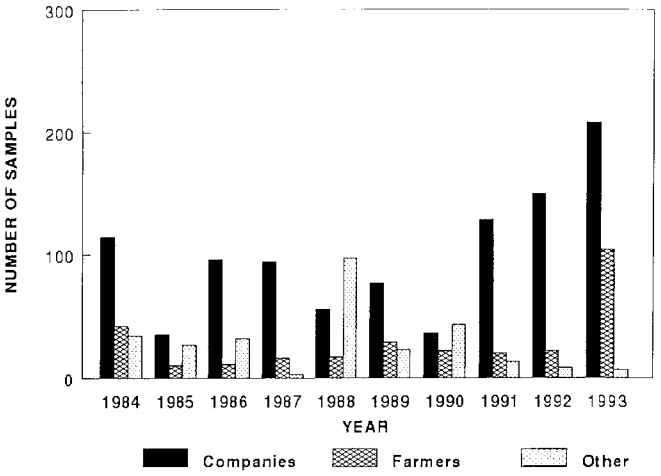

Figure 2 Sources of samples.

symptoms, and the fungi produced, which we obtained from the literature. This has proved very useful for generating statistics and directing which mycotoxins to look for based on information given by the client. The major commodities received were maize (408), general feed and components (346), and poultry feed (233). The latter figure was given separately to indicate the importance of mycotoxin analysis to the local poultry industry in Natal, where high production efficiency can be affected by the presence of sub-lethal levels of toxin in the feed. Minor but routine materials were groundnut (42), sunflower seed (22), cottonseed (38), soyabean (61); other cereals, mainly wheat (27); forage, mainly lucerne and hay (28); silage (8), poultry litter (37), fish meal (49), and various animal tissues and fluids (84). The latter were of both animal e.g., chicken liver and human, e.g., blood and urine origin. The balance was made up of food commodities (53) and miscellaneous items. Included in food commodities were 23 samples of apple juice which were examined specifically for patulin.

The source of the samples was of some interest. The main suppliers were poultry companies (346 samples), feed companies (299 samples), farmers (293 samples), and the University of Natal (121). The remainder in order of sample numbers came from food companies, co-operatives, insurance companies, millers, hospitals, rural homes, institutes, private individuals and supermarkets. The exact order, however, varied from year to year depending upon particular problems encountered.

Trends in mycotoxin incidence are similar to those observed in the previous report. Overall percentage of mycotoxin contamination was 35%. Allowing for double contaminations the figure is reduced to 30%, a similar figure (27%) to that found previously (Dutton & Westlake, 1985) (Table 1). Care should be exercised in interpreting these figures as they were biased for several reasons. Firstly there would have been a tendency towards finding mycotoxin contamination owing to the fact that the samples were drawn from suspect materials. Secondly where specific analyses had to be done, e.g., in the case of fumonisin, these were not carried out if the client did not request them (extra costs involved) or if there were no indicators such as symptoms or fungi to suggest such an analysis. This lead to an underestimation of contamination.

Table 1 Number of positive incidences of mycotoxins in the total number of samples analysed from 1984 to 1993

Mycotoxin	Incidence	Level PBB	Comment
Aflatoxin B_1/B_2	114	1–500	Both toxins
Aflatoxin (All)	115	1–500	All 4 toxins
Cyclopiazonic acid	1	ND	Insensitive
Fumonisin B_1[b]	43	TR–1000	By tlc
Gliotoxin[b,c]	1	ND	
Kojic acid	34	ND	
Oosporein[c,e]	4	ND	
Patulin	33	1–280	
Penicillic acid	6	ND	
Sterigmatocystin	4	ND	
Tenuazonic acid	2	ND	In litter
Trichothecenes			
Deoxynivalenol	22	ND	
Diacetoxyscirpenol	19	ND	
Nivalenol[c]	10	ND	
Trichothecene[c,d]	100	ND	Unknown
T-2 Toxin	3	ND	
Zearalenone	67	50–8000	
Total	594		

[a]Out of a total number of samples of 1602

[b] Analysis of these done separately and commenced in 1989

[c] Unconfirmed because of lack of standard

[d] Unknown metabolite which gives characteristic colour reactions of a trichothecene and is found in maize

[e] Oosporein associated with the presence of *Acremonium* spp. and appears as a yellow visible spot on tlc

[f] It has recently been determined that the multi-screen is insensitive to cyclopiazonic acid and a separate assay is now done when appropriate fungi are found

TR = trace < 200 ppb; ND = not quantitated, > 200 ppb

Another difficulty was that of sampling, as this was done by the person supplying the sample. Hence there was no guarantee that the results have any statistical validity, although efforts were made to ensure that representative random samples were submitted. In these cases a mixture of the samples was analysed. Lack of sensitivity would also overlook low level contamination by certain toxins, e.g. cyclopiazonic acid, the detection limits of the dialysis method for mycotoxins being given by Patterson (Patterson & Roberts, 1979). Finally it was not possible to screen for all known fungal toxic metabolites.

In order to address the latter problem we have now set up a cell toxicity test based on human cell lines and a tetrazolium salt assay coupled with microscopy (Robb *et al.*, 1990; Smith *et al.*, 1992). This has already proved its worth recently by showing cattle feed extracts to be highly cytotoxic in spite of the absence of detectable mycotoxins.

Aflatoxin B_1 was the most prevalent mycotoxin contaminating 14.3% of total samples, 7.2% of these containing all four aflatoxins, with trichothecenes next at 8.5% and then zearalenone (4.2%). A major contributor to the trichothecene figure is an unknown compound which gives positive response to the spray reagents used to detect these metabolites, i.e., 50% sulphuric acid in methanol, chromotropic acid reagent (Baxter *et al.*, 1983) and Kato's sulphuric acid in methanol chromotropic acid reagent (Takitani *et al.*, 1979). Initial gc/ms results support its identity as a trichothecene but no further identification was possible owing to a lack of a coincidental spectrum or standard. The incidence of the fumonisins should not be overlooked as these were not screened for until 1989. As these have to be analysed separately, not all samples are examined, as clients have the option to have this analysis at extra cost. Maize examined in another programme (Dutton *et al.*, 1993) was found to have a 50% incidence of fumonisin B_1 contamination and in this study it had an incidence of 90% (18/20) of samples examined in 1993. The result is biased, however, as the samples analysed were selected as being suspect. Considering the relative ease of detecting aflatoxin with respect to fumonisin it seems very likely that the latter is at least as ubiquitous in South African products as aflatoxin.

Other minor mycotoxin contaminants were patulin and kojic acid both at just over 2% of all commodities examined. The significance of these fungal metabolites is difficult to assess, as both have low toxicity, although patulin has been implicated as a carcinogen in the past and is now back in the news owing to its prevalence in apple concentrates. Minor incidences of other mycotoxins included cyclopiazonic acid, sterigmatocystin, gliotoxin, tenuazonic acid, and oosporein, although the latter two could not be confirmed owing to lack of standards. A detailed record of their incidence is included in Table 1.

By far the most predominant fungus found in the samples was *Fusarium* spp. (507 samples positive = 31.3%) which were mainly either *F. moniliforme* or to a lesser extent *F. subglutinans* as indicated where the fungus was identified down to species level. This reverses the order of prevalence between this genus and *Aspergillus* spp. as noted in the previous study (Dutton & Westlake, 1985), the latter incidence being 435 (26.9%) of which 284 belong to the *A. flavus* group. The third major genus was *Penicillium* spp. which had an incidence of 156 (9.6%). Other fungal genera included *Acremonium* (60; 3.7%), *Diplodia* (19; 1.2%), *Paecilomyces* (8; 0,5%), *Rhizopus* (24; 1.5%) and a group of unknown fungi with white non-sporing mycelium (68; 4.2%). There were 420 incidences where more than one fungal species occurred in a sample.

The high incidence of *Fusarium* spp. in samples indicated the common use of maize in South African feeds and other commodities, for it is likely that this is the source of *F. moniliforme* and *F. subglutinans* which are common contaminants of this cereal. This finding could have important repercussions as they are a source of fumonisin. The significance of this for human and animal health is not at the present clear but it seems likely that it may clarify previously unexplained animal disease conditions and production losses.

The distribution of mycotoxin incidences amongst commodities is given in Table 2. The results follow the pattern exhibited in the previous 1985 report (Dutton &

Table 2 Incidence of mycotoxins in South African agricultural commodities 1984–1993

Commodity	Mycotoxin (No. samples positive)[a]						
	Aflatoxin	Trichothecene	Kojic acid	Fumonisin B_1	Zearalenone	Patulin	Other[d]
Maize	35	56	17	10	21	3	6
Other cereal	7	4	0	0	2	1	0
Oil seed	37	2	0	0	3	1	0
Animal feed	57	69	9	14	31	2	7
Poultry feed	46	26	7	20	7	4	7
Fish meal	4	0	0	0	0	0	0
Animal tissue	0	0	0	0	0	0	
Forage[b]	6	1	0	0	0	0	2
Soyabean	9	0	0	0	0	1	0
Miscellaneous[c]	18	11	1	0	3	21	2
Total	229	169	34	44	67	33	20

[a] Total number of samples assayed = 1602. [b] Includes: hay, lucerne, and poultry litter. [c] Includes: bagasse, beer, brewer's grains, dry beans and food. [d] Includes: gliotoxin (1), cyclopiazonic acid (1), penicillic acid (5), oosporein (6), tenuazonic acid (2), sterigmatocystin (4).

Westlake, 1985).

After using the dialysis clean-up method for a multi-mycotoxin for over 13 years we have found that with modifications we can routinely assay for over 20 of the more commonly occurring mycotoxins. The method is, however, limited to the more non-polar toxins, and fumonisins and moniliformin, for example, have to be analysed using other clean-up methods.

For precise results the method is poor compared with specifically designed methods coupled to modern instrumental methods, as recoveries can be as low as 50% for certain toxins or even lower., e.g., cyclopiazonic acid. In experienced hands, however, and with a knowledge of the limitations of the methods, levels of toxins can be effectively gauged by visual comparison with known levels of standards run under similar conditions. Often this is all a local farmer or miller needs to know so that the correct remedial action can be taken. Where more precise figures are needed, samples can then be forwarded to centres which have more sophisticated methods at their disposal.

To screen for over 20 toxins, estimate the magnitude of contamination, and identify the major fungal contaminants costs R58 ($17) per sample for consumables using fairly basic laboratory equipment. We feel that this makes it highly suitable for use in Southern Africa where more fully equipped laboratories are not available for routine mycotoxin analysis.

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