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# REPORT ON THE THIRD SADC MYCOTOXIN TRAINING WORKSHOP

# AND

# THE FIRST WORKSHOP ON GRAIN STORAGE

**GABORONE, BOTSWANA**
**DECEMBER 1993**

# THE REPORT OF THE PROCEEDINGS OF THE THIRD MYCOTOXIN WORKSHOP AND THE FIRST WORKSHOP ON GRAIN STORAGE PROBLEMS, ORGANISED FOR SADC COUNTRIES BY THE UNIVERSITY OF BOTSWANA AND THE UNIVERSITY OF ZIMBABWE AND HELD DECEMBER 13 TO 17 1993 IN THE DEPARTMENT OF BIOLOGICAL SCIENCES, UNIVERSITY OF BOTSWANA CAMPUS, GABORONE

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DECEMBER 1993

## TABLE OF CONTENTS


| | Page |
|---|---|
| **Introduction** | i |
| **Organising Committee** | iii |
| **The Donors** | iii |
| **List of Resource Persons** | iv |
| **List of Participants** | iv |
| **Lectures and Outlines of Lectures Delivered** | |
| (i) Lectures on Grain Storage Problems | 1 |
| (ii) Lectures on Detection and Surveillance of Mycotoxins in Feed and Foodstuffs | 21 |
| **Summary of the Joint Discussion on Mycotoxin Surveillance and Grain Storage Problems** | 87 |
| **Appendices** | |
| I Addresses of Resource Persons | 92 |
| II Financial Report | 94 |

# INTRODUCTION

## THE TRAINING WORKSHOP ON GRAIN STORAGE PROBLEMS

The purpose of this training workshop was to provide an opportunity for grain storage personnel in the SADC countries to come together to:

- exchange information on their work in general;
- learn about changes that occur in grain during storage and transportation;
- be made aware of the effects of fungi (moulds) and insects on grain and seeds;
- learn the use of simple commercial kits (eg, Neogen AGRI-SCREEN and Idexx CITE PROBE) to detect and quantify aflatoxins in grain or flour; and
- evaluate methods for prevention and control of grain storage in the tropics.

## THE TRAINING WORKSHOP ON THE DETECTION AND SURVEILLANCE OF MYCOTOXINS IN FEED AND FOODSTUFFS

This workshop was for scientists working in the food industry and food laboratories in SADC countries, to enable them to come together and through teaching and discussion

- be made aware and appreciate the problem of feed and foodstuff contamination by fungi (moulds) and mycotoxins;
- learn the detection and quantification of mycotoxins in feed and foodstuffs by Thin Layer Chromatography (TLC) and the Enzyme Linked Immunosorbent Assay (ELISA) techniques;
- acquire information on mycotoxin surveillance and control methods and programmes;
- assess the state of mycotoxin surveillance in the southern African region; and
- acquire information they should use in dealing with food imports and exports.

## ATTENDANCE

Twenty participants attended the workshop on Grain Storage Problems, two of them were observers and eighteen bonafide students. Nine of the eighteen had never attended a workshop like this, but eight had attended from one to several such workshops and of these were the participants who were senior in their organisations. Four of the eighteen were female and the rest males.

Twenty-three participants attended the workshop on Detection and Surveillance of Mycotoxins; three were observers and twenty bonafide students. Of the twenty, only two had attended a workshop like this before, for the eighteen, this was the first one. The gender ratio was 1:1 (10 female and 10 male).

The two workshops ran concurrently, but in the last two days of the workshops, the participants in the Grain Storage Problem expressed strong desire to learn more about mycotoxins. The timetables had to be adjusted to enable them to attend lectures together with the mycotoxin class. This is a clear indication of the success of the workshops.

The participants were sensitised and they responded by wanting to know more. The distribution of the participants, according to country of origin was:

| | |
|---|---|
| Angola | 0 |
| Botswana | 28 |
| Lesotho | 1 |
| Malawi | 1 |
| Mozambique | 0 |
| Namibia | 2 |
| Swaziland | 2 |
| Tanzania | 1 |
| Zambia | 1 |
| Zimbabwe | 2 |
| Subtotal | 38 |
| Observers (all from Botswana) | 5 |
| | 43 participants in all |

## ORGANISING COMMITTEE

Dr F S Mpuchane
Dr L Marovatsanga (University of Zimbabwe)
Dr I M Zinzombe
Dr H K Taligoola (Co-ordinator)

Co-ordinator of the Workshop's address:

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## DONORS

United States Agency for International Development (USAID)
University of Botswana
Ministry of Agriculture - Botswana
United Nations Children's Fund (UNICEF)
The Third World Academy of Sciences
The British Council

*The organising committee is very grateful for your assistance and we believe your contributions will continue to serve mankind for many years to come*

## RESOURCE PERSONNEL (in alphabetical order)

[Full addresses are in Appendix I]

| | | |
|---|---|---|
| Dr Laurence Castle | - | Ministry of Agriculture, UK |
| Dr J M Chitsika | - | Chibuku Breweries, Zimbabwe |
| Dr L Marovatsanga | - | University of Zimbabwe |
| Dr F S Mpuchane | - | University of Botswana |
| Mr H Njapau | - | Research Council, Zambia |
| Prof D L Park | - | University of Arizona, USA |
| Mr E W Sydenham | - | Medical Research Council, RSA |
| Dr H K Taligoola | - | University of Botswana |
| Ms R Tanyongana | - | Grain Marketing Board, Zimbabwe |
| Dr J H Viljoen | - | Maize Board, Pretoria, RSA |
| Prof D M Wilson | - | University of Georgia, USA |
| Dr I M Zinzombe | - | University of Botswana |

## TECHNICAL AND SERVICE STAFF

Dr A Maekopo
Mr M Katomeni
Mr T Mahachi
Ms N Mokgethi
Ms Nteta (Secretary and Editor)

## PARTICIPANTS (those who were taught)

### (A) GRAIN STORAGE PROBLEMS WORKSHOP

Key: BAMB = Botswana Agricultural Marketing Board; MoH = Ministry of Health; MoLG = Ministry of Local Government

| | | |
|---|---|---|
| Mr T J Bandeke | - | MoH (Food and Nutrition Unit, Botswana |
| Mr I Chakona | - | Standards Association, Zimbabwe |
| Mr N Dube | - | BAMB, Botswana |
| Mrs N Dube | - | MoLG (Food Resources Dept), Botswana |
| Mr K S Gare | - | MoLG (Food Resources Dept), Botswana |
| Mr M D Jeremiah | - | BAMB, Botswana |
| Ms D Kgetlwe | - | MoLG (Food Resources Dept), Botswana |
| Mrs E Koosaletse | - | MoLG (Food Resources Dept), Botswana |
| Mr K Laletsang | - | MoAgric, Botswana |
| Mr C Moalosi | - | BAMB, Botswana |
| Mr O Motswasele | - | College of Agriculture, Botswana |
| Mr M Raboloko | - | BAMB, Botswana |
| Mr M E Semathane | - | BAMB, Botswana |
| Mr B K Thamae | - | BAMB, Botswana |
| Mr E Tshamekang | - | Thusano Lefatsheng, Botswana |
| Ms T S Walebowa | - | MoLG (Food Resources Dept), Botswana |

| | | |
|---|---|---|
| Mr H H T Tarimo | - | MoH, Botswana |
| Mr J F Tehinse | - | MoH (Community Health), Botswana |
| Dr N G Musonda | - | OBSERVER, College of Agriculture, Botswana |
| Mr M T Balke | - | OBSERVER, World Food Programme, Botswana |

## (B) MYCOTOXIN SURVEILLANCE WORKSHOP

| | | |
|---|---|---|
| Mr M N P Bannyaditse | - | Ministry of Agric Vet Animal Health, Botswana |
| Mrs H I Böckmann | - | Ministry of Agric Vet Lab, Namibia |
| Mr A M Dlamini | - | Ministry of Agric, Research, Swaziland |
| Mr D L M Jumbe | - | Zomba Municipality, Malawi |
| Mrs C Khupe | - | Ministry of Agric, Botswana |
| Mr M R Kgologolo | - | Food Technology Centre, Botswana |
| Ms K Lekaukau | - | Ministry of Agric, Botswana |
| Mr T Matlhare | - | Thusano Lefatsheng, Botswana |
| Mrs D L Müseler | - | Ministry of Agric, Namibia |
| Mr D M Muzungarabwi | - | MoH, Government Analyst, Zimbabwe |
| Mrs E Muzungaile | - | Council for Scientific Research, Zambia |
| Mr R Nsibande | - | MoAgric, Research, Swaziland |
| Ms M Phegelo | - | MoH, Family Health, Botswana |
| Mr R T Rakereng | - | Food Technology Centre, Botswana |
| Ms A Segwage | - | College of Agriculture, Botswana |
| Ms B Sekwate | - | Food Technology Centre, Botswana |
| Ms W Tabona | - | MoH, Community Health, Botswana |
| Mr I B Tshekiso | - | MoH, Community Health, Botswana |
| Mrs D A Tsiame | - | MoAgric, Nutrition, Lesotho |
| Mr E M Urio | - | Food Nutrition Centre, Tanzania |
| Dr Omo | - | OBSERVER, Food Technology Centre, Botswana |
| Ms F Chand | - | Student, University of Botswana |
| Ms R Oppong | - | Student, University of Botswana |

# LECTURES OR SUMMARIES OF THE

# LECTURES DELIVERED IN THE TWO WORKSHOPS

# PHYSICAL AND BIOLOGICAL CHANGES DURING GRAIN STORAGE AND QUALITY CHANGES DURING IMPORT AND EXPORT

**by J H Viljoen**

## INTRODUCTION

Grain is produced annually, but it is consumed throughout the year. Therefore, it needs to be stored until required for consumption. The ideal during storage, handling and transport, is to maintain grain quality as close as possible to what it was when the grain was harvested. During storage, certain physical and biological changes take place in the grain, which can severely affect its quality, particularly if the store manager is unaware of these and does not manage them correctly. Moreover, from harvesting to milling, grain needs to be handled and transported many times, and it is subjected to many processes, such as cleaning, pest control and weighing, all of which can have an effect on grain quality and on the quality of the product manufactured from it.

A chain of different parties are involved in this process of moving the grain from farmer to final consumer. Each of these links in the chain of parties perceives grain quality from his own viewpoint, depending on how it affects his profits. All wish to gain the highest income, while incurring the least costs during the time the grain is in his care. The first link is the farmer who grows, harvest and dries the grain and delivers it to a grain merchant, or sometimes directly to a miller. In southern Africa drying mostly takes place naturally in the field before harvesting, but in Europe and north and south America, the grain is harvested whilst still at a high moisture content and then dried artificially by blowing heated air through it. Sometimes, or in some countries, the farmer himself stores the grain for a considerable period before selling it to the grain merchant or miller. The second link in the chain is the grain merchant who stores the grain until he can sell it to a miller, he cleans it and sometimes he also dries it on his own behalf or as a service to the farmer. The third link is the miller. Usually, millers store the grain only for a short time, before milling it. Mostly, millers also clean the grain before milling takes place. The final link is the consumer. Where grain is imported from one or more foreign countries, there are several additional links in the chain of parties, and more handling of the grain takes place. Thus, grain quality is more severely affected before it reaches the consumer.

Grain quality means different things to different people and therefore creates much controversy in commercial circles. Grading is one method of describing grain quality and plays an important role in determining the value of grain. However, grading can be based only on a limited number of visible properties, and it therefore does not represent all the quality properties that are important to one or more of the parties involved in the commercial process. In a broad sense, quality can be defined as any property of the grain that affects:

* the aesthetics, smell and taste of grain;
* the presence of substances or organisms harmful to the grain itself or to consumers of the grain;
* the costs of storage, handling or processing; and
* the nutritional value of grain as food, or feed.

A more detailed breakdown of grain quality properties is given in figure 1.

## PHYSICAL PROPERTIES OF GRAIN AND CHANGES DURING STORAGE AND HANDLING

### 1 Bulk Density

Grain is a mixture of seeds and the intergranular spaces between them. The size of the seeds in relation to their weight (kernel density), together with the air spaces between them determine bulk density. The size and shape of grains and the smoothness of their outer surfaces determine the ratio of grains to voids. A bulk of grain consisting of smooth seeds will have less voids than rough seeds. Bulk density is the ratio of mass to volume of grain, eg, maize often has a bulk density of 75kg/100 1 (100 1 = 1hl). The bulk density of grain can vary from one production area to the next, depending on the growing conditions. Bulk density is severely affected by factors that prevent the grain from reaching physiological maturity, like early frost, and by fungal infection, resulting in significant lass of dry matter. Generally, grain with a high bulk density is considered to be of better quality. In a store, the grains "pack" during storage, thus reducing the void space between them somewhat. The degree of packing varies between grain types. Bulk density is measured in unpacked grain, by weighing the amount of grain that can be contained in a special device with a known volume. Bulk density is affected by grain moisture content: as the moisture content increases, the bulk density decreases and vice versa. This is because the seeds swell if they contain more moisture, and shrink as they dry out, and the increase or decrease in volume is much greater than the increase or decrease in mass as a result of the increase or decrease in moisture content.

### 2 Kernel Density

Kernel density is the mass per volume ratio of the grain kernel itself, independent of the intergranular voids. It is most commonly expressed as the weight per 1,000 kernels. Kernel density is one of the factors that determine bulk density. Kernel density is influenced by grain moisture content as described under bulk density.

### 3 Angle of Repose

When grain is poured in a pile, or when it flows from a silo, the grain surface forms a characteristic slope with the horizontal. The angle of the slope is determined by factors governing the flow of properties of the grain, such as the size, shape, moisture content and texture of the seeds. It is known as the angle of repose, and within certain limits, it is specific for the type of grain. Of all the factors that affect the angle of repose, grain moisture content is the most variable, and in addition, high moisture content may result in fungal development and caking, which adversely affect the grain flow properties.

### 4 Grain Flow from a Silo Bin

When the grain outlet of a silo bin is opened, the column of grain directly above the outlet flows out first. At the upper grain surface, the column widens and grain in the centre flows faster, so a cone-shaped depression soon forms at the surface. Grain at the edges of this depression flows into the depression, thus continuously widening the depression until it is as wide as the bin. Grain from the surface of the depression continues to flow down the grain outlet, until the bin is finally empty. Therefore,

grain in a bin empties from top to bottom, which means that grain last added to a bin, will be first to be discharged and that added first will be discharged last. The inload/outload sequence is not affected by storage conditions, but grain flow rate can be severely affected by changes in moisture content and fungal development in all, or parts of, the grain bulk during storage.

## 5 Grain Hardness, Stress Cracks, Breakage Susceptibility and Grain Dust

Grain hardness refers to the ratio of vitreous to floury endosperm in grain kernels. Some varieties have more vitreous than floury endosperm and are known as hard varieties. This applies to all grain types, but is of particular importance in wheat and maize.

Stress cracks are cracks in the endosperm which result from uneven drying of the kernel. During drying, the kernel shrinks. If the grain kernel is dried very slowly, such as under natural drying conditions, the entire kernel will shrink uniformly, with no or very few stress cracks developing. However, when dried rapidly, such as under artificial drying conditions, the outer layers of the kernel dry out more rapidly than the inside, and the difference in shrinkage between the outer and inner layers then results in cracks developing in the kernel.

Breakage susceptibility refers to the tendency of kernels to break up during handling. The percentage of broken kernels is a grading factor in most countries' grading systems. During grain handling, kernels collide numerous times against one another, and against concrete, metal or other surfaces, subjecting them to breakage. Breakage susceptibility depends on kernel hardness, percentage stress cracked kernels and grain moisture content. Of these factors, the percentage stress cracked kernels is by far the most important, followed by kernel hardness and moisture content. A low moisture content holds so many other advantages that it is not a viable option to handle and store grain at an increased moisture content as a means to limit kernel breakage.

Grain dust is primarily composed of starch granules and it mainly originates from broken up kernels. Therefore, soft grains with many stress cracked kernels tend to produce much more grain dust than hard grains with few stress cracks. The more times grain is handled, the more grain dust will be generated. The dust particles fill in intergranular spaces and affect the grain flow. Grain dust suspended in air in a confined space, such as in a grain silo, can explode violently if a spark is set to it.

## 6 Particle Distribution of Grain in Bulk Handling

When grain is poured into, or drawn from a bin, or when it is transported in a truck or on a conveyor belt, it tends to separate in heavier and lighter components. When a stream of grain falls into a bin, the heavier grain particles fall straighter and faster, while lighter particles float outwards because of air resistance. Thus chaff and dust accumulate near the bin walls. When the stream of heavy particles reaches the apex of the pile, smaller particles, such as weed seeds, and broken kernels are trapped between the kernels and remain at the centre, whereas free-flowing whole kernels tend to slide down the slope, creating a core of fines, called a spoutline. In a storage bin, the inlet spout stays fixed, but when grain is shipped for export into the hold of a vessel, it is moved to different positions from time to time to evenly fill the hold. This creates large spots in the grain bulk consisting mainly of peripheral fines,

spoutline fines and sound grain. During discharge from the vessel, whole rail or road trucks may be loaded with one or the other of these fractions, if care is not taken to avoid this, resulting in some buyers ending up with inferior grain, while others receive the best material.

When grain is transported in a truck, or on a conveyor belt, small particles, such as broken kernels, tend to sieve through the grain and concentrate at the bottom of the bulk. Large light objects, such as cocklebur seed, chaff and pieces of plant material separate to the grain surface and often give an exaggerated negative impression of the general quality of the consignment.

## 7 Grain Temperature

Grain kernels and the intergranular spaces do not allow a quick exchange of heat, therefore grain temperature adjusts only slowly to changes in the outside temperature. The development of insects and microflora in grain is closely associated with the grain temperature and moisture content. Grain harvested and brought into storage in the summer, or after having been artificially dried, will only slowly adapt to falling temperatures during the following winter. Insect infestations and fungal growth in such grain can therefore proliferate well into winter. Conversely, grain harvested in the winter will retain a low temperature well into summer and will allow only limited insect development. However, insects and fungi themselves generate heat, which may raise grain temperatures and enhance insect and fungal development. Grain managers should therefore always be on the lookout for signs of raised grain temperatures.

## 8 Grain Moisture Content

Grain always contains a certain amount of moisture. Insects and microflora are dependent on this moisture for their water requirements. Therefore, if the moisture content is lower than about 8 percent, insects rarely infest the grain. If the moisture content is below about 13,5 percent, fungi and other microflora cannot develop in the grain.

Moisture is easily added to grain, without leaving obvious signs that this has been done. It adds weight to grain and there is a definite temptation for farmers to deliver grain with as high a moisture content as possible. Artificial drying is costly, so, if farmers who have to rely on artificial drying succeed in selling off grain with a high moisture content, they also save on drying costs. There is also a temptation to unscrupulous parties in the grain trade to add a small amount of water - perhaps only 0.5 percent - to grain in their care in order to increase the weight for a consignment. In addition to the buyer having to pay the price of grain for water, such practices often result in fungal deterioration of the grain as the added water initially sits on the surfaces of grains, where it triggers germination of fungal spores.

On the other hand, where grain is stored in a relatively dry climate, the grain will dry out further during storage, even in silo bins. In South Africa, for instance, maize is received for storage at a moisture content of 14 percent, but the average moisture content at despatch is only 12.5 percent. The weights of all consignments are adjusted to 12.5 percent moisture content, therefore clients do not pay for excess moisture and the temptation to add moisture for a gain in weight is thus removed.

## BIOLOGICAL PROPERTIES OF GRAIN AND CHANGES DURING STORAGE

### 1 Infestation by Storage Insects

Through the ages, grain insects have become fully adapted to live in stored grain and are now an integral part of grain storage. For centuries storage insects have been distributed worldwide and are now universal. Grain insects depend on the grain they infest to provide them with food, water, shelter and a suitable temperature where they can live. At the same time, the insects affect the grain and cause changes to occur in the quality of the grain, its moisture content and temperature. When heavily infested, grain insects cause the grain to heat to a temperature of about 42°C. This happens in grain of which the moisture content is too low for fungi to start growing, therefore it is known as "dry grain heating". The hop spot can give rise to moisture migration, resulting in an area in the grain bulk with high moisture content, where fungi will start to develop.

At any one time, numerous species can be present in a bin of grain, but usually one or two occur in much larger numbers than the rest. Which species will dominate at any time, is very dependent on the grain temperature and as the temperature changes, the dominant species change concomitantly, until it becomes too hot in that area for any species to live there.

The infestation of grain, and the distribution of insects through the grain are determined by a number of factors. First, the size of the insect and how robustly it is built determine that the relatively large, frail moths infest grain merely near the surface of the bulk, while beetles, which are much smaller and more robust, can easily penetrate deep into the grain bulk. Secondly, the life expectancy in the adult stage allow moths only about a week to 10 days to penetrate a grain bulk, while most beetles live several months and have more time to move deep into a bulk of grain. Insects that spend their larval stage inside grains can easily infest grain whilst still in the field and are not so exposed to injury during harvesting and grain handling. These species can therefore be brought into the store with the grain and continue their development anywhere in the bulk. They can also infest the grain from outside, after intake. Insects that spend their larval stage free between the grains, are more limited and mostly infest the grain from outside, after harvest intake.

### 2 Infection by Microflora

Storage fungi mainly consist of a number of species of *Aspergillus* and *Penicillium* spp. The *Aspergillus* spp can grow in the absence of water, while the *Penicillium* spp mainly grow in grain with a high moisture content. Other fungi that will grow in grain at a high moisture content are *Mucor* and *Rhizopus* spp.

The different storage species of *Aspergillus* have different, and sharply delimited lower moisture content limits at which they can grow. At higher moisture contents each species is unable to compete with storage fungi to which that moisture content is more favourable. Therefore, by identifying the fungal species and the infection rate of storage fungi in grain stored for some time, it is possible to accurately estimate the moisture content of the grain during the course of storage. For example, *A halophilicus* can only grow in wheat at a moisture content between 13.8 and 14.3 percent, therefore, if this species is recovered from a significant number of wheat

kernels in a sample, then the wheat must have been stored for a considerable period at that moisture content. Likewise, *A restrictus* can only grow in wheat at a moisture content between 14.0 and 14.5 percent, the *A glaucus* group can only grow in starchy cereals at a moisture content between 14.5 and 15.0 percent, and *A flavus* in starchy cereals at a moisture content between 17.0 and 18.0 percent.

The grain moisture content is the main factor that determines whether fungi will start to grow in stored grain or not. The grain temperature, however, also plays a role. At temperatures below 10°C, *Penicillium* spp will grow faster than the *Aspergillus* spp, even if the moisture content is somewhat restrictive of the growth of *Penicillium* spp. Fungal growth generates heat, known as wet grain heating, and as the grain temperature increases, fungi more restricted by low grain temperatures will begin to grow faster and will eventually overtake the fungi that first developed.

## QUALITY CHANGES DURING IMPORT AND EXPORT

### 1 Moisture Content, Fungal Infection and Heating

In Africa and Australia, grain is mostly dried naturally in the field, and stored in a relatively dry climate. When delivered for storage, the grain is therefore usually sufficiently dry for safe storage and dries further during storage. In the Americas and Europe grain is harvested when it still has a high moisture content and then artificially dried. Drying costs are high and farmers try to sell off the grain at as high a moisture content as possible. To be able to do this, yet meet official requirements for moisture content of traded grain, they often dry at a very high rate, reducing the moisture content of the outer layer of the grain kernel, while the inside still contains much moisture. Farmers also blend dry grain of the previous crop with high moisture new crop grain and manage to pass a moisture test, while fungi start developing in some of the grains with a high moisture content within weeks. It is clear, therefore, that moisture tests under such conditions often do not give reliable information about the moisture content of the grain. To further aggravate the situation, some exporters sprayed water on the grain during handling at terminal elevators for dust control. This practice has recently been banned in the USA, and currently legislation is being set up to also ban it in any stage between harvest and milling. During the sea voyage, moisture transfers occur in the grain, and the grain heats and cakes as a result of fungal development. To counter this problem may require to stipulate the moisture test method and the apparatus that should be used in import contracts in the future.

### 2 Mycotoxins

The conditions under which grain is grown in Europe and the Americas differ significantly from those in Africa, and different cultivars are planted. As a result, the field fungi infecting grain in the Americas differ from those in grain grown in Africa. The mycotoxins predominating in American maize, for instance, are fumonisins and aflatoxins and in Argentine maize, aflatoxins predominate. These mycotoxins are also found in African maize, but generally the levels, and frequency of occurrence in African maize tend to be much lower. The kinds and levels of mycotoxins vary from year to year, particularly in foreign grain. In some years fumonisins are found at very high levels in some parts of the USA, in other years aflatoxins are particularly troublesome. Recently, more rapid tests for various

mycotoxins become available, it may be possible to stipulate in the import contract the kinds and levels of mycotoxins acceptable, and the test methods to be applied.

## 3 Weed Seeds

Under certain growing conditions, the effectiveness of herbicides is diminished and more weed seeds may occur in grain. Some weed seeds are hazardous to the health of consumers. Import contracts normally make provision for the numbers and kinds of weed seeds acceptable, but sometimes, it is not possible to obtain grain that will meet the tolerances. Many weed seeds can be screened out of the grain, but some cannot. During grain handling, some weed seeds segregate from the grain and "float" on the grain surface. During grain sampling cognisance should be taken of these phenomena and care should be taken not to obtain an exaggerated impression of the amount of weed seeds in the grain.

## 4 Broken Grains and Foreign Material

During export/import, the grain is handled many more times than locally produced grain in the course of trade. Each time a kernel collides against another, or against metal and concrete surfaces, there is potential for it to break. Grain dried artificially develop stress cracks, thereby significantly increasing their susceptibility to breakage. Thus exporting/importing always results in a significant increase in the amount of broken kernels, and the more stress cracks there are, the greater the amount of broken kernels arriving at the final destination. Broken grains affect the grade and are lost during milling. This loss is seldom discounted against the cost of importing grain from overseas.

# INVASION/INFESTATION OF STORED GRAIN AND SEED BY INSECTS-PART 1

**by R Tanyongana**

## INTRODUCTION

The insects that commonly infest stored foodstuffs are extremely destructive in tropical climates. There are about 100 species responsible for damage to stored foods and about 20 of these are major pests with cosmopolitan distribution.

For effective pest control programmes, it is necessary to distinguish the major pests from those that are less important. It is also crucial to know a good deal about their biological habits which determine their importance as pests and also affect the measure employed for their control. Insect feeding and multiplying on stored foodstuffs causes weight loss and quality loss.

There are two orders in the class Insecta that are important pests. These are the Coleoptera (beetles) and Lepidoptera (moths and butterflies). The Coleoptera are undoubtedly the most common storage insect pests of the two orders. However, we still find important pests too in Lepidoptera.

It is convenient to describe insects that attack stored foodstuffs as either primary or secondary pests. Primary pests are those species which are capable of invading an undamaged commodity and establish an infestation. Secondary pests are restricted to commodities which have already been damaged. The damage may have been caused by man in the process of milling or grinding the product or by the activity of a primary pest.

## CEREALS' PRIMARY INSECTS

### Coleoptera

The important families in this order on stored product pests are: Curculionidae, Bostrichidae, Bruchidae, Dermestidae, and Tenebrionidae.

### Curculionidae (Weevils)

| | | |
|---|---|---|
| *Sitophilus zeamais* | - | maize weevil |
| *Sitophilus oryzae* | - | rice weevil |
| *Sitophilus granarius* | - | granary weevil |

Recognition

The adults are 2.5 - 4.5mm long. Distinguished from all other common storage beetles by having a well defined snout (rostrum) carrying the mouthparts. The antennae are elbowed and clubbed. The body colour can range from a tan brown to dark brown. *S zeamais* and *S oryzae* often have four large reddish-orange spots on the elytra.

Distribution

cosmopolitan

Hosts

Cereal grain (maize, wheat, sorghum, rice, etc)

Type of Damage and Importance

- On rice damage to kernels may cause up to 75 percent or more loss of their weights
- On maize losses of about 10 percent of more
- Damage to other grains is intermediate, more or less according to grain size
- Raise food moisture content by an increase of 5 - 6 percent during infestations

Habits and Life History

Some species like *S zeamais* are strong flyers and often infest commodities in the field before harvest. The female lays 100 - 150 eggs over a period of many weeks but most eggs are laid in the first weeks after emergence of the pupa. The eggs are laid singly in tiny holes, which the female gnaws/chews in the grain, and then sealed in the hole by a waxy secretion. Upon hatching the legless larva begins to feed in the endosperm producing a cavity in the grain as it increases in size. The larva remains inside the grain where it pupates and the adult which emerges bits its way out of the grain leaving a clean-cut emergence hole. Both adults and larvae feed and the adults are very long lived (several months - 1 year). Optimum conditions for the tropical species are close to 28°C (in the range 17° - 34°C) and 70 percent RH in the range 45 - 100 percent. The actual length of life cycle ranges between 31 - 37 days under optimum conditions depending on variety.

**Bostrichidae (Borers)**

| | | |
|---|---|---|
| *Rhizopertha dominica* | - | lesser grain borer |
| *Prostephanus truncatus* | - | larger grain borer |

Recognition

The adults are small cylindrical brown beetles 3-4mm in length. The head is flexed and more or less concealed from above by the prothorax which is roughened by ridges and tubercles. The elytra has well defined rows of punctures, apically the elytra are generally somewhat flattened and slope ventrally more or less steeply, ie, declivity.

Distribution

*Rhizopertha dominica* is cosmopolitan
*Prostephanus truncatus* - Americas and parts of Africa

Hosts

Cereal grains and cassava; development is possible in cereal flours.

Types of Damage and Importance

- major pests of cereals
- losses as high as 34 percent have been recorded but average loss of 10 percent in weight on farm storage is common

Habits and Life History

Both adults and larvae are voracious feeders

*R dominica*. The species is especially important as a pest of whole cereals even of rough rice which is resistant to the attack of most storage pests. The eggs are laid on the surface of, or among, cereal grains. Up to 500 eggs are laid per female over a period of three to six weeks. The larva emerges and eats its way into a grain where it feeds. The larvae have legs and may migrate from one grain to another but development usually takes place within a single grain. The species is adapted to rather higher temperatures and lower moisture content than *Sitophilus spp* and is therefore the dominant pest in areas which are hot, dry or both.

*P truncatus*. Adult females lay eggs in chambers bored at right angles to the main tunnels. Egg-laying on stabilised grain like that on the maize cob is more successful than on shelled grain. Larvae hatch from eggs after about three days at 27°C and seem to thrive on the dust produced by boring adults. The larvae pupates in dust. Development of larva through to adult at the optimum 32°C and 80 percent RH took only 27 days on a maize grain diet.

Types of Damage and Importance

Both *R dominica* and *P truncatus* create a lot of frass and dust as they feed because of the boring habits. With *P truncatus* losses of up to 40 percent have been recorded from maize cobs stored on the farm for 6 months in Nicaragua, and in Tanzania losses as high as 34 percent have been observed after 3 - 6 months from storage. Losses in dried cassava roots can be very high, these can be readily reduced to dust by adults boring and a loss of 70 percent has been recorded after only four months farm storage.

**Dermestidae**

*Trogoderma granarium* - khapra beetle

Recognition

Small oval beetle 1.5 to 3.0mm in length; densely covered with hairs. The elytra lightly clothed with fine hairs are mid-brown in colour or irregularly mottled. The antennae have distinct clubs. The larvae are straw-coloured with numerous tufts of hair and very long setae. Their cast hairy skins may cover the surface of infested grain.

Hosts

Groundnuts, cereals, pulses, and spices

Distribution

This species is favoured by hot dry climates around the world. It is widespread in the Indian sub-continent and adjacent areas. It is virtually cosmopolitan but absent from African countries including Kenya and Southern Africa. It is a prohibited pest in most African countries.

Types of Damage and Importance

Considered to be the most serious pest of grain stored in hot, dry conditions, oil seed, cereals and, to a lesser extent, pulses. Phytosanitary legislation has been introduced in several parts of the world to restrict its movements.

**Gelechiidae**

***Sitotroga ecrealella* (Angoumois grain moth)**

Recognition

Small pale yellowish brown moths. Forewing with over one or two small black dots. The hind wings carry a fringe of very long hairs and the apex is sharply pointed. The labial palps are long, narrow and pointed and curve upwards in front of the head.

Hosts

Rice, sorghum, maize, barley, and wheat

Distribution

Cosmopolitan

Type of Damage and Importance

Only the larvae feed, adults are short-lived. A serious pest of cereal grains, causing internal grain damage similar to that due to weevils but the emergence of individual grains of maize attacked by the species is just over 10 percent. Percentage loss from smaller grain (wheat and rice) is proportionately greater.

Habit and Life History

Infestation begins in the field before harvest. The eggs are laid in masses on the commodity. Upon hatching, the larvae bore into the grain and remain there feeding until fully grown. At this stage, it tunnels to the surface, leaving a thin layer of the seed coat intact. The pupa then forms and when the adult emerges it pushes open that thin area of seed coat prepared by the larva and leaves the characteristic "trap door" on the grain. Development from egg to adult takes about five weeks at 30°C. Minimum conditions of development are 16°C and 25 percent RH.

**PRIMARY PESTS OF LEGUME SEEDS**

**Bruchidae**

Have adapted to some of the chemicals contained in legumes which make them resistant to insect attack.

| | | |
|---|---|---|
| *Callosobruchus* spp | - | bean weevil |
| *Acanthoscelides* spp | - | dried bean weevil |
| *Caryedon* spp | - | groundnut beetle |

| | |
|---|---|
| | *C chinensis* |
| *Callosobruchus* spp | *C maculatus* |
| | *C rhodesiamus* |

Recognition

The adult is a robust beetle with body colours of greys and browns. The elytra cover all but the last abdominal tergum called the pygidium, which in some species are distinctly patterned, especially in females. Each hind femur bears two longitudinal ridges each of which bears a terminal spine.

Hosts

Cowpeas, pigeon peas, chickpeas, adzuki beans, peas, grains, and occasionally soya beans. They do not usually attack beans or butter beans.

Distribution

*C chinensis* originated in Asia but is now found throughout the tropics and sub-tropic.

*C maculatus* originated in Africa but is also now distributed throughout the tropics and sub-tropics.

*C rhodesianius* is generally restricted to Souther Africa, but occurs sporadically in West Africa and Kenya

Types of Damage and Importance

The adult beetles do not feed on stored produce and are very short lived (up to 12 days). Larvae develop within the beans causing serious damage and characteristic holes in the beans. Infestation begins in the field where eggs are laid on maturing pods.

Habit and Life History

The female lays eggs (up to 100) firmly glued to the surface of the host seed. Smooth-seeded varieties are more suitable for oviposition than rough seeded varieties. The eggs are domed structures with oval flat bases. Upon hatching the larva bites through the base of the egg, through the testa into the cotyledons. Detritus produced during this period is packed into the egg case as the insect hatches turning the egg while making it visible to the naked eye. The developing larva feed entirely within a single seed, excavating a chamber within the cotyledons as it grows. The optimum conditions for development are around 32°C and 90 percent RH 21 - 23 days.

**Acanthoscelides Obtectus**

Recognition

Adults 3.0 to 4.5mm long

Each hind femur of adult beetles has on the ventral side near the apex, a large tooth followed by two or three smaller sharp teeth. The main colours of the body are greys, browns, and

reddish-brown without any distinctive patterns. The antennae are dark grey except for segments 1 - 5 and two which are reddish.

Hosts

Beans (common beans, butter beans, or lima beans)

Distribution

Cosmopolitan

Type of Damage and Importance

Major pest; larvae develop within beans causing serious damage and characteristic holes in the beans. Infestation begins in the field.

Habit and Life History

Eggs are laid loosely but are often lodged under cracks in the bean testa. The newly hatched larvae immediately enter the seed and development to the adult stage proceeds within the seed. The pupation chamber is prepared in an area adjacent to the testa and at this "window stage" infestation can be detected by the appearance of a circular and somewhat translucent area of testa. Optimum conditions 30°C, 70 percent RH, can breed slowly at 18°C.

- highland areas of tropics as well as hotter regions
- some temperate

Minimum life cycle: $22^1/_2$ days.

**Caryedon Serratus**

Recognition

Large robust insect, reddish-brown in colour with irregular markings on the elytra. Each hind femur bears a conspicuous comb of the spines made up of one large spine and 8 - 12 smaller ones.

Hosts

Groundnuts and tamarind, wild tree legumes

Distribution

*C serratus* is of Asian origin but has become distributed to many tropical and sub-tropical regions of the world. It is prevalent in warm parts of Asia, North Eastern and West Africa, West Indies, parts of South and Central America, Mexico, and Hawaii. It is, however, a serious pest of stored groundnuts only in West Africa.

Habit and Life History

Prefers groundnuts stored in shells. Eggs are glued onto pods or kernels and infestation may take place as soon as the groundnuts are harvested and left to dry. The larvae bore into groundnut shells and feed on the seed. They usually leave the pod to pupate. The larva spins a paper-like pupal cocoon which it usually attaches to the outside of a pod. Optimum conditions 30 - 33°C, 70 - 90 percent RH, 40 - 41 days T° range 23° - 35°C.

**Zabrotes Subfasciatus**

Recognition

Oval-shaped beetles with two movable spurs attached too the apex of the tibia of each hind leg. These "calcaria" are reddish in colour and unequal in length. There are no teeth of the hind femur. The elytra of the female are strongly marked with white patterning on a dark background.

Hosts

Beans, butter or lima beans, cowpeas, bambara nuts

Distribution

Endemic to Central and South America. It is also found in many other tropical and sub-tropical regions especially Central and East Africa, Madagascar, the Mediterranean, and India.

Habit and Life History

Similar to that of *Callosobruchus* spp, the eggs which are hemispherical being stuck to the testa of beans or to pods. Optimum conditions 32°C and 70 percent RH, 24 - 25 days. To range 20°C - 38°C.

# INVASION/INFESTATION OF STORED GRAIN AND SEED BY INSECTS-PART II

**by R Tanyongana**

A large number of unrelated pests can be conveniently classified as secondary pests. They are predominantly associated with commodities which have suffered previous physical damage caused by a primary infestation or a milling process. Many are pests of cereal products, but others are associated with oil seed, spices, etc.

## A THE BEETLES

### Tenebrionidae

*Tribolium confusum* - confused flour beetle
*Tribolium castaneum* - red flour beetle

Recognition

Medium sized beetles of 3-4mm in length. They are dorso-ventrally flattened parallel-sided and brown in colour. The antennae have a distinct three-segmented clubs. The larvae are free living, cream or brown and are very active.

Distribution

Cosmopolitan - originated in India

Hosts

Cereals and milled cereals and products, oilseed cake, calee, groundnuts, spices, coffee, cocoa, dried fruit, etc.

Type of Damage and Importance

A major pest of damaged cereal grains and their milled products. Infestation can produce disagreeable odours and flavours in commodities due to the production of benzoquinenes from defense glands of the adults. Both adults and larvae feed.

Habits and Life Cycle

Up to 450 eggs are laid by the female over a period of many months. The eggs are laid loosely in the food stuff and hatch into cylindrical larvae. Pupation takes place in the food without formation of a cocoon. The emerging adults may live for as long as 18 months. Optimum conditions 35°C and 70 percent RH (20-30 days). Range 20-40°C and 10-90 percent RH.

*T confusum*. Biologically quite distinct. Optimum dev T%2-3°C lower and shows a greater preference for food with a high oil content.

**Cucujidae**

*Cryptolestes* spp - Flat grain beetles

Recognition

Small beetles 1.5mm-4mm, elongate, very flat and light brown in colour. The proterax has lateral ridges. The antennae are filiform and usually more than half the length of the body. The head and prothorax are large and account for half of the length of the body.

Distribution

Cosmopolitan

Hosts

Cereals, oilseeds, milled cereal products, cocoa, cowpeas, etc.

Type of Damage and Importance

Able to damage whole cereal grains to a limited extent, but more important as pests of damaged grain and milled products. Small larvae may enter cereal grains at points of minor damage especially in wheat where the embryo is often exposed. The embryo of cereals is often attacked preferentially, thus adversely affecting germination. Both adult and larvae feed.

Habit and Life History

Eggs are laid in cracks or splits in the grains and hatch into slender, straw-coloured larvae with a darker twin-pointed appendage at posterior end. When fully grown, the larvae construct cocoons to which particles of food adhere. Adults are long lived 6-9 months. Optimum conditions 30°C, 70 percent RH, 23 days.

Prefer high moisture content food. The presence of large numbers may indicate moisture problems.

**Silvanidae**

*Oryzaephilus surinamensis*
*Oryzaephilus mercator* - saw-toothed grain beetles

Recognition

Moderately small 2-4mm narrow flat parallel-sided beetles. Prothorax has six large teeth on each side and three ridges on the dorsal surface. Antennae have a compact club.

Distribution

Both species are cosmopolitan

Hosts

| | | |
|---|---|---|
| *O surinamensis* | - | cereals and milled cereals |
| *O mercator* | - | mainly oil seeds and oilcake but also rice, rice bran, spices and dried fruit |

Habit and Life History

About 300 eggs are laid loosely in the foodstuff over 10 weeks. These hatch into slender pale cream larvae with two slightly darker patches on each segment. The larvae are active and move about freely until fully grown. They then construct a silken cocoon in which to pupate.

Optimum conditions 30-35°C and 70-90 percent RH with *O surinamensis* preferring the hotter and more humid conditions. Tolerance range 18-38°C and 10-90 percent RH.

Types of Damage and Importance

Both adults and larvae feed. They can infest whole grains but are more important as pests of the milled cereals, copra, and oilseed cake.

## B THE MOTHS

### Pyralidae

*Ephestia cautella* - Tropical warehouse moth

Recognition

Medium sized moth with a forewing length of 6-13mm. In newly emerged adults the forewings are greyish-brown in colour, with an indistinct pattern. Older specimens which have lost most of their scales are dull grey in colour.

Distribution

Cosmopolitan

Types of Damage and Importance

A major secondary pest, causing very evident spoilage. Prefers the embryo of cereal grains and is therefore very destructive in seed grain.

Habit and Life History

The adult moth avoids strong light and rests in dark places during daytime. It has a flight rhythm giving periods of active flight around sundown and at dawn, when daily fluctuations in temperature and humidity occur. The eggs are laid loosely on the surface of the commodity up to 300 eggs. The larvae moves freely through the grain and contaminates it with webbing and frass. It then enters a wandering phase trying to locate a pupation site; spins a silken cocoon and pupates usually in cracks, curves

and frequently the gaps between bags.

| | | | |
|---|---|---|---|
| Optimum condition | - | 28°C | 70 percent RH (25 days) |
| Ranges | - | 15-30°C | 45-100 percent RH |

Newly emerged adults can mate within a few hours of emergence and eggs are laid soon after. Adult moths do not feed for less than 14 days.

Other species

| | | | |
|---|---|---|---|
| *E kuehniella* | 22% temperate | *E calidella* | } act as primary but do not get |
| *E elutella* | temperate | *E figulilella* | } established in store |

*Plodia interpunetella* - Indian meal moth

Recognition

The forewing of the adult is cream coloured in the basal third white, the rest of the wing is copper coloured with some dark grey markings. Wingspan 15-20mm and the labial palps point directly forward.

Distribution

Cosmopolitan

Host

Cereals and their products, groundnuts, dried fruit

Type of Damage and Importance

A serious pest in some areas. Webbing and frass produced in the infested material cause very evident spoilage.

Habits and Life History

Similar to *E cautella* - 500 eggs. The larvae feed first on the embryo of the grain and white eating. Spin a silken tread on which accumulates the larval droppings and particles of the produce. The larva can have a prolonged development by a pre-pupal resting stage of diapause and this complicates the life-cycle.

Optimum conditions 29-30°C and 70-75 percent RH (26 days)

*Corcyra cephalonica* - Rice moth

Recognition

In the adult moth the hind wings are mid-brown and the forewings mid-brown with lines of a darker shade along wing veins. Labial palps point straight forward.

Distribution

Cosmopolitan

Hosts

Rice, sorghum, millet, sesame, groundnuts, cocoa, copra

Type of Damage and Importance

A major pest especially in hot climates. The webbing together of food particles cause evident spoilage. In cases of heavy infestation the food material becomes very tightly matted together with webbing, cocoons, frass, and excreta. The contamination of food in this way may be of greater economic importance than larval feeding. Conway (unpublished) had found, in one instance, that the weight loss of some infested rice was 7 percent whilst the cost of cleaning the grain to make it saleable amounted to 9.4 percent of the value of the original stock.

Habit and Life History

Sexual activity may begin shortly after adult emergence. The sticky eggs - 150± - are laid on the food. There are 7-8 instars and the larvae are free living creating webbing and frass as they feed. Pupation takes place on either store structures, bag or actually in the food. The adult moth is necturial being most active at nightfall. They are shortlived and do not feed. Developmental period 4-5 weeks.

## C PESTS ASSOCIATED WITH DAMP AND MOULDY CONDITIONS

A number of pests are particularly associated with commodities that have a high moisture content or that have become mouldy. They are found on freshly harvested commodities, commodities which have been stored at an excessively high moisture content or commodities that have become damp and mouldy as a result of infestation by other species of insects.

*Carpophilus* spp (coteoptera - Nitidulidae)

A common pest of mature maize before harvest and may persist in store if maize is stored on the cob and where moisture content remains high. It can also infest other cereals at harvest, cocoa and dried fruit.

*Cathartus quadricollis* (coleoptera - silvanidae)

Common on newly harvested cereals, cocoa and other crops and may persist for sometime in store. Feed on damp produce and may supplement their diet with moulds, and some feed exclusively on mould.

Other fungus feeders

*Ahasverus advena*
*Alphitobius diaperinus*
*Alphotobius laevigatus*

## PEST INFESTATION LEVELS

### INFESTATION %

| | Sorghum | Maize | Millet | Beans |
|---|---|---|---|---|
| Callosobruchis | 8 | 8 | 2 | 82 |
| Tribolium | 68 | 69 | 10 | 16 |
| Sitohilus | 70 | 73 | 34 | 13 |
| Rhizopertha | 29 | 19 | 16 | 3 |
| Oryzeaphilus | 22 | 31 | 10 | - |
| Sitotroga | 20 | 19 | 6 | - |

## SAMPLING AND INSPECTION

INSPECTION - The detailed visual examination of all or parts of a consignment. It may or may not include the taking and examination of samples

EQUIPMENT USED - Notebook, torch, knife and sampling probe, sample bags, labels and sieves, hand lenses are also useful

RECORDING INSECT INFESTATION - short notations are useful

| | | |
|---|---|---|
| Clear or none (C) | - | No insects found in the course of prolonged search |
| Few of light (F) or (L) | - | Small numbers of insects occurring irregularly |
| Medium (M) | - | Insects obvious, occurring regularly (sometimes in small aggregations) |
| Heavy (H) | - | Insects immediately obvious, ie, large numbers crawling actively over the whole of the outside of the stack |
| Very heavy (VH) | - | Insects so numerous and active |

If it is intermediate degree, M-H moderate to heavy, two methods:

General inspection - on bag stacks (GC or GH)
Sampling inspection - on soil bins (SC or SH)

SAMPLING INSPECTION - Normally four samples are collected one at the top of silo and three from the three outlet valves at the bottom. The sample should be approximately 1kg. It is sieved and the number of insects recorded

GENERAL INSPECTION ON BAG STACKS - This is mainly visual inspection with no sampling. But if sampling is required then this is done on all the four faces of the stack

## XEROPHILIC AND TOXIGENIC FUNGI
## (A laboratory display of these fungi growing naturally on various food commodities)

**by Dr H K Taligoola**

This was an extensive display of xerophilic and toxigenic fungi growing on twenty-five different samples of food crops; altogether there were 62 fungal specimens displayed.

The display was meant for (and was viewed for three days by) participants attending both workshops: the workshop on Detection and Surveillance of Mycotoxins and the workshop on Grain Storage Problems.

Xerophilic fungi were defined as fungi which can grow on dry organic matter such as dry cereal grains, flour, seeds or on substances (foodstuffs) with high sugar or salt concentration such as jam, jelly, syrups, salted meats, and insects like the *Phane* worms (processed caterpillars of the Emperor moth - *Gonimbrasia belina*). An example of this group of fungi is the *Aspergillus glaucus* group. Xerophilic fungi cause spoilage of grain and seeds during storage as pioneer colonisers of grain on which other types of fungi cannot grow. As the xerophilic fungi respire, they create pockets in grain bulk or sacks where both grain moisture and temperature start increasing. Their activities create conditions suitable for the less xerophilic fungi to invade and grow on grain and seeds.

Toxigenic fungi are those which, during metabolism, produce substances (metabolites) which are harmful to man and to livestock. These metabolites are generally called mycotoxins (fungus toxins). Examples of toxigenic fungi are: *Aspergillus flavus, A ochraceous, Penicillium viridicatum, Fusarium moniliforme* and *F graminearum*. It was emphasised to the participants that in considering food quality, all fungi (whether toxigenic or not) growing on feed or foodstuff alters the integrity of the foodstuff in many ways and production of mycotoxins is just one of those changes which include loss of dry matter, increase in fatty acids, changing the colour of the grain or foodstuff, flavour is also changed, seeds lose viability and disintegrate.

The participants were required to carry out three exercises on the fungal specimens displayed: (1) to examine the displays and be convinced that fungal contamination of feed and foodstuffs is indeed a problem; (2) to familiarise themselves with the gross appearance of various xerophilic and toxigenic fungi on grain and seeds. The colour of the fungus colony is very useful for practical purposes though not always 100 percent reliable.

Examples given included *Aspergillus flavus* whose colour is yellow-green, *Aspergillus glaucus* spp - usually blue, the sexual stage of *Aspergillus glaucus* spp usually yellow or reddish (Details of *Eurotium* spp were not mentioned). *Penicillium* spp usually blue, *Fusarium moniliforme*, whitish or pink and powdery. (3) to try and relate degree of fungal contamination of the commodity to the condition of the grain or seed. Whether there is insect damage, the presence of foreign or extraneous materials like other parts of the plant which are not eaten, and dust.

# FUNGAL INFECTION AND MYCOTOXIN CONTAMINATION OF COMMERCIAL GRAIN

**by J H Viljoen**

## INTRODUCTION

Fungi occurring in grain can broadly be separated into two groups: **field fungi** that mainly grow on grain plants whilst still in the field, and **storage fungi** that mostly develop only after the grain has been taken into storage. Stored grain almost always carry inactive spores of field fungi whilst in storage and some field fungi may continue to grow after the grain has been harvested and taken into storage, if favourable conditions for their development prevail during storage. Conversely, grain, whilst still in the field, is usually invaded to some small extent by storage fungi and these are the first to start growing if storage conditions are favourable for their growth. All storage fungi can seriously damage the quality of stored grain, and in addition they can, under certain conditions, produce toxic chemicals known as mycotoxins. With some important exceptions, field fungi often have comparatively little visible effect on infected grain, but similar to storage fungi, they also produce several mycotoxins which remain in the grain after harvest and which can be harmful to consumers of the grain. In this paper, the most important fungi and their mycotoxins occurring in grain will be discussed.

## FIELD FUNGI

### 1 Maize

The most common field fungi that invade maize are *Fusarium moniliforme, F subglutinans*, *F graminearum*, Diplodia maydis and *Aspergillus* flavus. *A flavus* is mainly a storage fungus, but in hot, dry weather it can invade maize in the field and becomes a field fungus. In South Africa, *F moniliforme* predominates in the western Transvaal and the north-western Orange Free State, and *F subglutinans* in the eastern Transvaal, Natal and eastern Orange Free State. *F graminearum* is of some significance only in maize from Natal. The predominance of *D maydis* varies from year to year between various production areas, but it is found frequently. *A flavus* is rarely found in South African maize, and only when the maize has been stressed by drought in mid summer.

In imported American maize the predominant field fungi are *F moniliforme* and *A flavus*. *F subglutinans* is also found, but slightly less often than in South African maize. In Argentine maize, *A flavus* predominates. *F moniliforme* is frequently found in dent Argentine maize from the southern production areas, but it is rare in flint types from northern production areas.

The mycotoxins produced by *F moniliforme* are the fumonisins, of which there are six related types. Of these, three are important to the health of consumers and are regularly found in maize. The highest level found in South African maize of apparently good quality in the last two years was about 4 parts per million. In imported American maize 10 parts per million, and in imported Argentine maize 6 parts per million were the highest levels found. *F subglutinans* produces moniliformin, and *F graminearum* produces three important mycotoxins, namely

deoxynivalenol, nivalenol and zearalenone. *A flavus* produces the aflatoxins, of which four types occur in grain. *D maydis* also produces mycotoxins, one or more of which the chemical structure has not yet been discovered. These mycotoxins cause a disease known as diplodiosis in sheep and cattle. *D maydis* causes ear rot in maize and can have a devastating effect on the crop. It occurs at very low levels in American and Argentine maize.

Horses and donkeys are particularly sensitive to the fumonisins, and a level of about 10 parts per million in their feed causes necrosis of their brains. In pigs fumonisins cause lung oedema at fairly high levels in the feed. In humans, fumonisins are suspected to cause oesophageal cancer. Deoxynivalenol causes vomiting and is also known as vomitoxin. Nivalenol is fairly toxic to animals, causing death in mice at a dosage rate of about 5mg/kg body weight. Both nivalenol and deoxynivalenol cause feed refusal, skin necrosis, and intestinal disorders. Zearalenone interferes with the sexual hormones, acting as an estrogen, particularly in pigs. Aflatoxins are the most potent carcinogens known and are a major cause of liver cancer in humans. It is acutely toxic to poultry at about 150 parts per billion. Cattle are less sensitive to aflatoxins than most other animals, but cows excrete aflatoxins in their milk, which can then poison humans. The highest level recently found in a single sample of South African maize was 20 ppb, in American maize it was over 100 ppb, and in Argentine maize it was 160 ppb. Average levels were well within the set tolerances. Most countries maintain legislation setting tolerance levels for aflatoxins, and strict control of aflatoxin levels in imported maize and oilseeds is of the utmost importance. Tolerance levels in South Africa are 10 ppb for humans, and between 20 and 50 ppb for various animals. American tolerance levels are considerably higher.

### 2 Wheat

The most common field fungus invading wheat, is *Alternaria* spp. Other field fungi regularly found on wheat are *Cladosporium* and *Drechslera* spp. *Alternaria* is unable to compete with storage fungi, therefore high infection levels of *Alternaria* in stored wheat sometimes is evidence of good storage. *Alternaria* does, however, produce a few mycotoxins, such as alternariol, the effects of which on consumers have not yet been fully investigated.

## STORAGE FUNGI

### 1 Conditions for Growth

With the exception of *A flavus* in maize during very hot and dry weather, storage fungi do not infect cereal grains to any significant extent in the field. The storage fungi mainly consist of a number of species of *Aspergillus* and *Penicillium* spp. The *Aspergillus* spp can grow in the absence of free water, while the *Penicillium* spp mainly grow in grain with a high moisture content. Other fungi that will grow in grain at a high moisture content are *Mucor* and *Rhizopus* spp.

The different storage species of *Aspergillus* have different, and sharply delimited lower moisture content limits at which they can grow. At higher moisture contents each species is unable to compete with storage fungi to which that moisture content is more favourable. Therefore, by identifying the fungal species and the infection rate of storage fungi in grain stored for some time, it is possible to accurately

estimate the moisture content of the grain during the course of storage. For example, *A halophilicus* can only grow in wheat at a moisture content between 13.8 and 14.3 percent, therefore, if this species is recovered from a significant number of wheat kernels in a sample, then the wheat must have been stored for a considerable period at that moisture content. Likewise, *A restrictus* can only grow in wheat at a moisture content between 14.0 and 14.5 percent, the *A glaucus* group can only grow in starchy cereals at a moisture content between 14.5 and 15.0 percent, and *A flavus* in starchy cereals at a moisture content between 17.0 and 18.0 percent.

The grain moisture content is the main factor that determines whether fungi will start to grow in stored grain or not. The grain temperature, however, also plays a role. At temperatures below 10°C, *Penicillium* spp will grow faster than the *Aspergillus* spp, even if the moisture content is somewhat restrictive of the growth of *Penicillium* spp. Fungal growth generates heat and as the grain temperature increases, fungi more restricted by low grain temperatures will begin to grow faster and will eventually overtake the fungi that first developed.

## 2 The Effects of Storage Fungi on Grain Seeds

***Reduced germinability***. Storage fungi first invade the germs of seeds and weaken and later kill the germ. This often happens with very little or no damage to the rest of the seed and very little discolouration of the seed. Germinability is of great importance in seed for planting and grain used for malting, such as sorghum and barley.

***Discolouration***. Both field and storage fungi can cause discolouration of whole seeds of parts of seeds, particularly the germ. According to most countries' grading systems for grain, discoloured seeds affect the grade. Discolouration caused by fungi is generally known as "heat damage", because of the dark colour the seeds attain, suggesting burning. However, although grain temperatures may rise to more than 60°C as a result of fungal growth, it is highly unlikely that the dark discolouration is actually caused by heat. Because the grade can be affected, invasion by fungi can have a direct effect on the commercial value of grain.

***Heating and moisture migration***. If grain in some parts of a grain bulk for some reason have a moisture content favourable for fungal growth, fungi will start growing there. Fungal growth increases both the grain moisture content and the temperature and usually there is a succession of fungi as the grain moisture content and temperature increase - first *A glaucus* which can raise the grain temperature to about 40°C, followed by *A candidus* and *A flavus* which can raise the temperature up to 55°C. The moisture produced by fungal growth is taken up in the intergranular air in the "hot spot" and because hot air rises, the moisture is carried upwards with the rising air through the grain. When this humid air reaches cooler parts of the grain bulk, especially the outer surface, the air cools down and its moisture holding capacity decreases. Grain in that area absorb some of this moisture and fungal growth may be started off there. At the surface of the grain, actual moisture condensation may take place if outside temperatures are significantly lower than that of the rising air. As a result, grain on the surface may sprout and fungal growth may result in a solid crust.

***Mustiness and caking***. Fungal growth in grain produces webbing in the grain that results in clumping and caking of the kernels. This can range from slight adhesion of kernels to solid clumps that do not break apart when handled. Caking therefore adversely affects the grain flow properties and handling characteristics of stored grain, which can considerably raise the costs of moving the grain. The fungal growth is accompanied by a musty smell, even where only little fungal growth is apparent, which makes the grain unacceptable for human consumption. Seriously caked grain is unacceptable for almost any use.

***Production of mycotoxins***. Aflatoxins are produced by storage fungi of the *A flavus* group, which includes *A flavus*, A parasiticus and *A nomius*. Cyclopiazonic acid is produced by *P cyclopium, P camembertii, P patulum*, and several other species of *Penicillium*, as well as by several species of *Aspergillus*, including *A flavus*, *A versicolor* and *A oryzae*. Cyclopiazonic acid is acutely toxic to chickens at a dosage level of about 19mg/kg body weight. Sterigmatocystin is produced by a large number of *Aspergillus* species, including *A flavus* and A versicolor, and also by some species of *Penicillium* and several other less common storage fungi. The toxic action of sterigmatocystin is similar to that of aflatoxins, but it is less toxic.

Ochratoxins, and penicillic acid are produced by several species of *Aspergillus* and *Penicillium*. Ochratoxins mainly affect the kidneys. Penicillic acid is carcinogenic in rats and mice and it kills poultry at a dosage rate of about 90mg/kg body weight.

Citrinin is produced by numerous *Penicillium* species, like ochratoxins it also affects the kidneys.

## 3 Prevention of Damage to Grain by Fungi

The combination of grain moisture content, temperature and storage time determines the amount of damage that can be caused to the grain by storage fungi. At a moisture content of 13.5 percent or less in starchy grains, the grain can be stored almost indefinitely, without any damage caused by storage fungi. The most important measure against fungal damage, therefore, is to make sure that grain moisture content is as close as possible to this level before it is stored. However, if it is not possible to dry grain to this level, then the grain can be stored for a limited period as long as the grain temperature is low - the lower the temperature, the longer the storage time possible without damage to the grain. If, however, it is also not possible to store moist grain at a low temperature, the grain should be consumed promptly, before fungal growth can damage the grain.

Often, the moisture content of grain appears to be within acceptable limits to allow storage, yet fungal damage soon occurs. There are two main reasons for this. The first is that moisture tests with electronic moisture meters under certain conditions give misleading results and the second is that electronic moisture meters are sometimes not sufficiently sensitive to determine the small differences in moisture content that will allow fungal growth, or safe storage. The conditions under which electronic moisture meters give misleading results about the moisture content of grain is where grain with a high moisture content has been blended with dry grain, and where grain has been artificially dried at a high drying rate. Where wet and dry grain have been blended, the moisture test often gives a result indicating that the grain can safely be stored. However, moisture exchange between kernels with a high, and

those with a low, moisture content only takes place slowly and fungi have ample time to start growing in the kernels containing high moisture, spoiling the entire bulk. Where grain had recently been artificially dried at a high drying rate, the outside kernels are much drier than the inside and in such cases some electronic moisture meters also seem to underestimate the grain moisture content. Care should therefore be taken that the moisture tests with electronic moisture testers are done under conditions that will give reliable results. If not, safety margins should be built into the moisture levels that are considered acceptable. As far as the sensitivity of electronic moisture meters is concerned, it is of value to thoroughly test a wide variety of models, and only accept as an official instrument one or more that give reliable results. Furthermore, the accepted moisture content of a grain consignment should never be based on the results of just one or two tests, but rather on multiple tests, and a calculation of the average.

# FUNGAL AND MYCOTOXIN CONTAMINATION OF FOOD AND FEED IN ZAMBIA

**by Henry Njapau, Elizabeth M Muzungaile, and Cheryl E A Lovelace**

## 1 FUNGAL CONTAMINATION OF AGRICULTURAL CROPS IN ZAMBIA

### 1.1 Occurrence

In Zambia, the commodity of concern has been maize, the major staple. Like elsewhere, the incidence of fungal infection in the field and storage has varied from time to time. The scourge was recognised as a potential problem in 1969 and 1974 when rains extended well into the harvesting season and more than 7 percent of the country's marketed crop was declared unfit for human consumption (MMISC, 1980). This situation prompted a more detailed examination of pre- and post-harvest fungal contamination by several investigators. Their findings have shown that the most important fungi infecting Zambian maize in the field are *Fusarium moniliforme* (Sheldon), *Diplodiamacrospora* (Earle), *Fusarium graminearum* (Schwabe) and *Diplodia maydis* (Schwabe) (FSCU, 1975/76). The prevalence of these two genera alternate depending on the climate with late rains favouring the predominance of *Fusarium* (FCSU, 1974). Among the *Fusarium* species, *F moniliforme* is the most prevalent pathogen of maize (Marasas *et al*, 1978 and 1979 and NCSR, 1989 (Table 1).

Soil, oil vegetation and infected stover are considered the main reservoirs of infection. A second source of inoculum has been identified as maize seed. In a study designed to investigate the transmission of infection, both dressed and undressed seed were found to be infected with *Acremonium, F moniliforme, F oxysporum, F graminearum* and *Diplodia* sp (MMISC, 1976). This study also showed that standard seed dressing (captan, benomyl and malathion) was not effective against some of the internal pathogens.

During storage, fungal build up will not present a serious problem when minimal moisture is maintained. In a 12 month study of traditional and improved storage structures where grain moisture did not appreciably increase, percent mould infection remained constant despite an increase in percent insect damage (0-10%) (Sakufiwa, personal communication). A state of "dormancy" of viable spores and mycelia is maintained until the environment triggers biological activity. Viable spores and/or mycelia of *Penicillium* sp (86%), *Rhizopus* (83%), *Aspergillus* sp 63%), *Aspergillus flavus* (57%) and *Fusarium* sp (14%) have been isolated from compounded poultry feeds (NCSR, 1984). Similarly, Marasas *et al* (1970) reported an increase in percent kernel infection by *F moniliforme* and *F graminearum* when moisture increased during storage.

A comprehensive handbook of fungi occurring on Zambian agricultural commodities has been published by the Plant Protection Research Programme of the Ministry of Agriculture (Raemakers *et al*, 1991). Potential mycotoxin producing species of *Fusarium, Aspergillus* and *Penicillium* are listed as

common pathogens of maize, groundnuts, wheat, sorghum, millet and cassava (Table 2). The handbook makes no mention of the genus *Diplodia*.

**TABLE 1: INCIDENCE OF *FUSARIUM* SPECIES IN MAIZE SAMPLES FROM THE MAZABUKA DISTRICT OF ZAMBIA (1986, 1987)[a]**

| FUNGAL SPECIES | NUMBER EXAMINED | NUMBER INFECTED |
|---|---|---|
| F moniliforme | 162 | 127 |
| F graminearum | 162 | 7 |
| F moniliforme and F graminearum | 162 | 13 |
| F moniliforme and F moniliforme var anthohilum | 162 | 1 |
| F moniliforme, F stilbeides and F moniliforme var subglutinans | 162 | 1 |
| F heterosporium | 162 | 2 |
| F | 162 | 1 |
| F graminearum and F lateritium | 162 | 1 |
| F moniliforme and F lateritium | 162 | 4 |
| F moniliforme var subglutinans | 162 | 1 |
| F oxysporum | 162 | 2 |

[a] NCSR Annual Report, 1989

## 1.2 Toxigenicity

Presented with the large quantities of grain (51,000MT) that was held for disposal or possible inclusion into stockfeed, it became imperative that an assessment of toxicity (or lack of it) of the grain be conducted. The assessment was conducted in the form of feeding trials (ad-lib) with ducklings, rats, mice, chickens, pigs and cattle.

Observed toxic response varied between test animals, sexes of the test animals, type of fungi and dose administered. Day old chicks showed a marked decline in weight gains while breeding sows fed 55 percent mouldy grains experienced growth retardation and delayed puberty. On the other hand, layers (40% inclusion), castrated weaners (55% inclusion) and steers (55% inclusion) displayed normal growth and productivity (NLRAC, 1980) (Table 2).

*F moniliforme* infected maize was acutely toxic to ducklings and rats at a lower inclusion rate (16%) (Marasas *et al*, 1978). Undiluted (100% inclusion) *Fusarium* infected maize caused mortalities in 25 percent of day-old chicks, whereas a 50 percent inclusion of the same maize resulted in only lower feed consumption and reduced weight gains (NLRAC, 1980).

# 2 OCCURRENCE OF MYCOTOXINS IN ZAMBIAN FOOD AND FEEDSTUFFS

Considering their historical position, easiness of assaying and probably their demonstrated carcinogenicity in laboratory animals, aflatoxins were the first to be investigated. Other toxins that have been isolated from Zambian food and feedstuffs are Zearalenone (F-2), Deoxynivalenol (DON) and Diplosporin. Research on isolation of actual toxins started in 1973, with studies by the Ministry of Agriculture Research Branch and by the University of Zambia. Although running concurrently with mycological investigations, only in one instance was fungi and toxin analysis undertaken simultaneously and a correlation established. The rest of the studies were restricted to toxin occurrence.

## 2.1 Aflatoxins in Human Foodstuffs

The aflatoxin programmes were perceived with a view of establishing an epidemiological relationship between exposure and the incidence of diseases and other reproductive inadequacies associated with them.

Initial studies did not establish the presence of aflatoxin in diseased maize (FCSU, 1974) and that stored in traditional structures over a 12 month period (Sakufiwa, personal communication). The first published account of the presence of aflatoxin was recorded in a maize-malt sample during an investigation of commercial and home-brewed opaque beer (Lovelace and Nyati, 1977).

The spectrum of commodities was broadened in two later studies separately conducted during the hot/dry and hot/wet seasons. Both placed emphasis on ready-to-be consumed commodities (plate foods). The hot/dry season study involved 200 samples (169 of them plate foods) collected from five villages during 1975 and 1977. Two of these villages were reported as having had documented cases of hepatoma within the previous five years. Six samples comprising groundnuts (1), cooked beans (1) and cooked green vegetables (4) were contaminated with aflatoxin at a level of 1-44$\mu$g/kg. Mean aflatoxin contamination was calculated to be 10$\mu$g/kg. Results from a parallel study for Hepatitis B antigens have not been made available.

The hot/wet season study was conducted in 1982 and 1983 in the same area but included food samples from the public institutions (hospitals, schools) within the locality. Of the 428 samples collected, 288 were plate foods (161 village and 121 institutional). The remainder were raw foodstuffs (whole maize, debranned maize, germinated maize, beer, groundnuts and millet). In addition, 101 samples of industrial food commodities were collected.

The pattern of contamination was similar to the previous hot dry season study although the incidence of contamination was higher (increased from 3 to 7%). Aflatoxin was not detected in the village plate foods but occurred variably in the rest of the tested items (Table 4). Aflatoxin $B_1$ and $G_1$ occurred in equal ratios. The most serious contamination was in germinated maize (58% incidence and 55$\mu$g/kg mean contamination). In the industrial category no aflatoxins were isolated from cow milk, peanut butter and groundnuts, but maize based meals were significantly contaminated. Thirty-eight percent of

the aflatoxin positive human food samples were contaminated above 20$\mu$g/kg (USA-FDA action level).

The results from plate food raised more questions than the answers sought. The non-occurrence of aflatoxin in the village plate food samples could not be attributed to articulate selection of "clean" materials based on historical experience. If that were the case, the people would have long abandoned the use of germinated maize. A laboratory based study was therefore initiated to assess the degree of decontamination arising from the preparation of the plate foods.

Results obtained to date indicate that a substantial reduction in aflatoxin content is achieved through the removal of bran (grain husks). The actual preparation of Nsima (plate food) does not seem to significantly alter the toxin level (work in progress).

**TABLE 2: SUMMARISED TOXICITY TEST RESULTS**

(a) **WHOLE MOULDY MAIZE**

| Test Animal | Dose (inclusion) | Response (effect) |
|---|---|---|
| Duckling | 25% | wt loss, necrosis |
| Layers | 40% | none |
| Weaners (pigs) | 55% | none |
| Sows (pigs) | 55% | retarded |
| Steers (cattle) | 55% | none |

(b) **PURE CULTURES OF FUNGI**

| Fungi | Test Animal | Dose | Response (effect) |
|---|---|---|---|
| Fusarium sp | Day-old chick | 100% | 25% mortality |
| Diplodia sp | Day-old chick | 100% | 100% mortality |
| F moniliforme | Duckling | 50% | 100% mortality |
| D macrospora | Duckling | 16% | 100% mortality |

MMISC, 1980: Masaras et al, 1978 and 1979

## 2.2 Aflatoxin in Body Fluids

It has been argued (Tannebaum and Skipper, 1984) that exposure data estimated from ambient levels of chemicals in air, water or food are inherently less valuable than those obtained from analysis of body fluids and tissues, because the latter give a more accurate dosimetry, a better measure of effective dose and an understanding of individual risk. To demonstrate exposure using body fluids, 219 human urine samples were collected from newly admitted patients at the central hospital in Lusaka (Lovelace *et al*, 1983). The samples were collected from patients showing their liver pathology, ie, hepatoma, cirrhosis and hepatitis, and those with clinically

normal liver functions. The only fungal metabolite detected was aflatoxin M, in 6 urine samples from control patients, and all but one positive sample occurred during the rainy season.

### 2.3 Aflatoxin in Livestock Feeds

Information on aflatoxin contamination of livestock feeds has been derived from two separate activities: samples brought to the laboratory (in-coming samples) by poultry farmers and an incidental survey of feeds obtaining in poultry houses. The farmers' reasons for requesting an analysis varied from routine check, growth retardation and occasional mortalities. There was no mechanism to verify the alleged symptoms. Aflatoxin was present in 40 percent of such samples (2-258$\mu$g/kg).

The poultry houses survey was conducted in the hot/dry (August/October) season of 1984 in response to an unusual outcry about growth retardation. Samples were collected from 36 poultry houses around Lusaka and assayed for aflatoxin, diplosporin, crude protein, calcium, phosphorus and magnesium. The incidence of aflatoxin was high (83%) despite a low concentration (2-27$\mu$g/kg). Diplosporin was present in 64 percent of the samples (0.3-3.6$\mu$g/kg). In additions, fifty percent of the samples contained less than recommended levels (Zambia Bureau of Standards, 1986) of crude protein, calcium and phosphorus. Veterinary diagnosis associated the poor performance to mostly Vitamin E deficiency.

### 2.4 Other Mycotoxins

In addition to the aflatoxin and diplosporin, Zearalenone and Deoxynivalenol have been detected in Zambian maize and maize based animal feeds. Zearalenone alone (FCSU, 1974), and together with deoxynivalenol (Siame and Lovelace, 1989) have been isolated from mouldy maize and poultry feeds. The incidence varied from 15 to 30 percent for zearalenone and was 1.7 percent for deoxynivalenol.

Lovelace and Nyati (1977) also reported the occurrence of zearalenone in 75 percent of maize malt and traditional beer samples. The process of brewing beer did not seem to effect any reduction in the quantity of the toxin.

## 3 DISCUSSION

From mycological analysis Zambian maize is predominantly infected by *F moniliforme* and *D macrospora*. The degree of infestation varies and the two alternate in prevalence depending on climatic conditions in a particular year. Zambia is overall a dry country and the "storage fungi" *Penicillium* and *Aspergillus* may proliferate during the rainy season when storage is inadequate.

The influence of mycotoxins on human health in Zambia, however, remains to be determined. Incidences of acute aflatoxicosis such as those recorded in Kenya (Ngindu *et al*, 1982), India (Krishnamachari *et al*, 1975), Uganda (Alpert *et al*, 1971) and Thailand (Shank *et al*, 1972) have not been reported in Zambia, either because they have not occurred or been associated with other causative agents. Aflatoxin

levels in the foodstuffs associated with the incidences cited above were in some instances lower than those detected in germinated maize. In some parts of Zambia, germinated maize is used in the production of traditional beer and sweet beverages often consumed by infants, yet germinated maize contain high levels of aflatoxin. Recently, a team (Dendy and Trotter, 1988) of experts visiting the SADC region recommended the use of fermented and germinated maize as a weaning food. The absence of hydrocyanic acid was used as a measure of safety, consequently, this potentially hazardous material which could result in acute responses has been adopted in Zambia (National Food and Nutrition Commission, 1993).

The poultry industry is the second largest consumer of cereals in Zambia (CSO, Lusaka, 1992). Poultry feed contains 60-70 percent maize, a staple food, and as human population increases so will its food requirements. With grain production lagging behind, population growth (FAO, 1992), only grain declared unfit for humans and possibly containing mycotoxins will be diverted into stockfeeds. Growth retardation is the commonest manifestation of aflatoxins in poultry (Willie and Morehouse, 1978). The levels encountered in Zambian feeds are, however, much lower than those reported to elicit such symptoms in other regions of the world (Ostrowski-Messer, 1984). Nutrient imbalances in the feed may result in similar symptoms (Quaraishi, 1992). Excess or inadequate protein, calcium and magnesium have a depressing effect on body weight. Coccidiosis, Gumboro, NewCastle and Mycoplasmosis are the most prevalent poultry diseases in Zambia and trace amounts of aflatoxins have been associated with susceptibility to coccidiosis (Smith, 1984). The combination of one or two mycotoxins and inappropriate nutrient composition of the feed may be more hazardous than aflatoxin alone. Poultry mycotoxins in Zambia should, therefore, be viewed in the light of these facts, in that admissible levels of aflatoxin set elsewhere may not be applicable.

## 4 CONCLUSION

The mycotoxicology programme in Zambia was charged with examining the risk exposed by mould infection, and the prevention or control of mycotoxicoses in human and animals. The various studies that have been executed have identified the commodity, fungi and probable mycotoxins of concern. Maize, the staple for man, poultry and pigs is a target commodity and so are the aflatoxins and toxins elaborated by *F moniliforme* and *D macrospora*.

The feeding trials and pilot surveys for aflatoxin contamination have not yielded the exact correlations sought because of varied responses and the unavailability of data on diseases. It is therefore impossible to make a true appraisal of the problem. Nevertheless, they have provided an insight into the dimension and complexity of the situation. What is required now is an expansion of the exposure assessment base through the intensification of surveillance activities, and a definite identification of trends in associated disease incidences. An interesting addition could be the role of immunosuppressive mycotoxins as potentiators of epidemics and infectious diseases prevalent in Zambia. This epidemiological information, together with toxicological data, will assist the nation make scientifically plausible conclusions as regards the level of concern (risk) and priority of mycotoxins. Prevention and control strategies become an automatic second step.

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# ECONOMIC AND NUTRITIONAL CONSEQUENCES OF INSECT DAMAGE TO CEREALS AND SEEDS

**by R Tanyongana**

## WEIGHT LOSS

- Storage insects are generally small, most are about 2-3mm long. The damage done by a single insect in terms of actual food loss is therefore very little. In the grain weevil, for example, it amounts only to some 10-20mg per insect. Therefore, it is the capacity for extremely rapid population growth that makes insects major causes of food loss in storage.

- A population of 10 weevils in a 100kg bag of grain at about 27°C can increase in 2-3 months to about 100,000 weevils that will have consumed about 1kg of grain. The next generation of that population may consume more than 10kgs of the grain in 4-5 weeks. The contamination of commodities by residual waste matter and dead insects add to the spoilage and can lead to considerable further loss of food value and cash value.

- The small size of these insects also makes them relatively inconspicuous except when very numerous. A population of up to 10,000 weevils in a bag of grain would not be very evident to a casual observer and the probability that one or more insects might be seen in a small sample drawn from the bag is not high.

- In order to deal effectively with insect infestation and prevent the problem caused by it a good basic knowledge of insect behaviour and ability to recognise the various insects that occur in storage places are necessary. Such knowledge and ability will permit the discovery of potentially serious infestations before it reaches the level at which loss and spoilage are significant.

**STORAGE LOSS SMALL SCALE (9-10 MONTHS) in SORGHUM** (Scrimshaw, 1978)

| | | | |
|---|---|---|---|
| Zimbabwe | 5-10% | Swaziland | 3.66% |
| Zambia | 1.7-5.6% | Kenya | 4.54% |
| Malawi | 2-3% | Tanzania | 8.7% |
| India | 35% | Nigeria | 46% |

Work done in Kenya in 1978 estimated that the annual losses in subsistence maize due to insects was 4.54% and that due to rodents 1.45%. This loss of approximately 6.0% is equivalent to US$12 million (C P F De Lima, 1978).

## WEIGHT LOSS

Weight loss may be defined as the loss in weight of the commodity over the period under investigation. It is essential to distinguish between the two types of weight loss, apparent and real.

Apparent. The difference in weight of the commodity before and after the particular post harvest activity under study.

In commercial practice, a weight loss may to some extent be hidden by an increase in moisture content and by transformation of usable material into dust and frass which, since it remains within the bag, may still be a usable commodity in transactions.

Real. The apparent loss with correction applied for any change in moisture content, plus weight of dust, frass and insects.

In experimental work, the loss is often given in terms of dry weight to avoid the problem of change in moisture content but for commercial practice it is suggested that figures for weight loss be quoted for commodity at a particular moisture content eg, the loss in maize (at 10%mc) was estimated as 5%.

## NUTRITIONAL LOSS

Any loss in weight of the edible matter will inevitably lead to a nutritional loss. Therefore, weight losses can be used to estimate gross food losses. In the majority of situations this is the only method of estimating nutritional loss because the analysis of the various nutrients requires elaborate equipment and is expensive and time consuming. It is said that of what insects eat, 10 percent is converted to insect body and 90 percent to heat and water. Therefore, heavy infestations of insects cause rises in moisture contents and temperature and this causes further nutritional loss by allowing the growth of micro-organisms that cause food spoilage as follows:

Unacceptable food - altered in colour, texture or flavour

Unpalatability due to:

- Fermentation - acid and gas produced from carbohydrates
- Putridness - objectionable flavours and odours produced from protein breakdown
- Rancidity - fats hydrolysed and/or oxidised

Toxic Food

- Carcinogenic - due to cancer-producing metabolites formed in the food eg, mycotoxins

Chronic or acute toxicity due to toxic metabolites (Rajan et al, 1975-76)

Another indicator of nutritional loss is the protein efficiency ratio. PERs of uninfected maize and cowpeas were 1.49 and 2.22 compared with 1.16 and 1.47 for infested maize and cowpeas respectively, as a result of damage by *S oryzea* and *C chinensis*. Chick peas 2.21 to 1.83 attributed to an increase in kernel damage from 2 percent to 15 percent. Pigeon peas 2.04 to 1.66 due to infestation.

## QUALITY LOSS

The presence of damaged grains, dust and contaminants such as insects, rodent hairs and pesticidal residues within the commodity contributes to a loss of quality which in many cases will lead to a loss in monetary value of the commodity. Similarly, changes in the biochemical composition may rank as losses in quality eg, an increase in free fatty acids

content. The evaluation of such losses is simplified in cases where specific quality standards are prescribed for commodity.

## LOSSES IN VIABILITY

Loss in viability in seed crops is one of the easiest losses to estimate but may not be immediately apparent in situations where there is replacement seed grain available. Some insects have the preference for the embryo of grains thus adversely affecting germination.

It has been shown through research that *Trogoderma granarium* single larva destroyed the germ of 1.55 wheat seed and a larva of *Tribolium castraneum* destroyed the germ of 12.28 seeds.

## CONTROL STRATEGIES FOR INSECT PESTS OF STORAGE

The provision of pest control is an important integral component of the operation and management of grain handling and storage systems. There are two main groups of insecticides that are used in chemical control methods.

a) **Fumigants**

These are insecticides which act in the gaseous phase as respiratory poisons and which can, therefore, penetrate to all parts of infested produce rather than only those which are accessible. However, they provide no lasting protection to the produce so that rapid reinfestation can occur. For example, methyl bromide, aluminium phosphide.

b) **Contact insecticides**

These are solid or liquid formulations which exert their toxic effect only when insects come into direct contact with them. Examples are:

| | | |
|---|---|---|
| Fenitrothien | - | both ec and dust |
| Pirimiphos methyl | - | " " " " |
| Chlopyrifos methyl | - | " " " " |
| Methacrifos | - | " " " " |
| Tetrochlorvinphos | - | " " " " |
| Etrimfos | - | " " " " |
| Dichlorvos | - | " " " " |
| Deltamethrin | - | " " " " |
| Permethrin | - | " " " " |
| Bioallethrin | - | " " " " |

### Methods of Application

Fumigants: Create airtight environments with grain inside and apply the gas and maintain airtightness for a specified time to kill insects.

| | | |
|---|---|---|
| Contacts: | a) admixtures | - dusts and sprays |
| | b) sprays | - ec surface spraying |

# PEST CONTROL IN BAG-STORED GRAIN

**by J H Viljoen**

## INTRODUCTION

Stored grain invariably becomes insect infested during the course of storage. Some insects, particularly those that develop inside the seeds, can infest the grain whilst still ripening in the field, and be brought into the grain store with the grain. All insects may infest the grain whilst in storage, as most of them can fly. Stacks of bagged grain are particularly exposed to insect attack, because of the large surface area. On the other hand, stacked grain can be inspected easier for insect infestation than grain stored in bulk.

Insects develop faster the warmer it is, therefore the build-up of insect numbers is most rapid during summer. The highest insect numbers are reached about March/April, when the need for pest control is greatest. During winter, insect numbers are usually low, but good pest control then will help to contain rapid build-up of insects as soon as warmer weather sets in.

In this paper, inspection of bagged grain for insect infestation, fumigation and the spraying of bag stacks with pesticides, controlled atmosphere storage of bagged grain, and the use of natural enemies of storage pests for pest control in bag-stored grain will be discussed.

## INSPECTION OF BAG STACKS FOR INSECT INFESTATION

The bag probe used for taking grain samples from bags for grading is completely unreliable as a tool for determining insect infestation. Even at very severe infestation levels, very few insects are found in grain samples taken from bags using the probe. Therefore more laborious methods must be applied to inspect the bag stacks for the presence of storage insects.

The differences in size, form and habits of the various insect pests, and the different developmental stages in the life cycle of each species are important considerations when applying methods of examining stacks for the presence of insects. Examination of the sides of a stack and the surrounding area in a store, is useful to reveal the presence of adult moths and moth larvae in particular, and to a more limited extent also of flour beetles and rusty grain beetles. However, infestations of most of the beetles, and of weevils in particular, only become evident in this manner once the infestation has already grown heavy. This is because mostly adult insects are seen on the outside of stacks, while the larvae, pupae, and eggs inside the bags are rarely seen. In the case of weevils, the larvae, pupae and eggs are inside the grain kernels, where they cannot be seen at all, even if the grain is screened to separate insects and grain. Inspection of the outside of stacks is, nevertheless useful if concentrated on the sides of stacks closest to the east and west walls of the store, as these areas get morning and afternoon sun, and become the warmest. Also, the tops of stacks, closest to the roof will often reveal infestations first, particularly of moths. Generally, the bottom layers in a stack are more heavily infested by weevils. In outside stacks, on the other hand, the shaded parts should be examined more closely, as parts that get direct sun are too hot directly below the tarpaulins for insects to live there and the heat drives them deeper into the stack.

Infestation by beetles in general, and by grain weevils in particular, are best revealed by screening whole bags of grain selected from the stack. The main limitation is that only bags from the top layers can be selected if the stack must still remain intact, and weevils most often infest the bottom layers. When the stack is to be broken for dispatch, more bags can be screened as discharging progresses, to avoid complaints and claims for compensation from buyers.

The number of bags to be screened depends on the size of the stack. The absence of insects in one or more bags does not mean there are no insects in the stack, therefore, if circumstance indicate that a stack is likely to be infested, more bags must be screened. The larger the number of bags screened, revealing no insects, the smaller the likelihood of an infestation remaining undiscovered somewhere in the stack.

Insects breed very rapidly and surprisingly high numbers can be built up very quickly. Inspections should therefore be carried out on a regular, scheduled basis, eg, every week during summer, and once a month during winter. Records of the results should be kept. During inspections, sources of possible infestation should be identified, and cleaned up. These include all possible breeding and hiding places for insects inside and outside grain stores, such as spilt grain, sweepings, screenings, used and new empty bags and dunnage poles or pallets.

## FUMIGATING BAG STACKS

Poisonous gases are one of the very few means available to us to reach and kill insects inside a grain bulk. Only gases that can penetrate into the grain without harming the grain, leaving no poisonous residues, are easy to work with, and which will not harm the environment when released into the atmosphere after the fumigation, are suitable as grain fumigants and therefore very few fumigants are available today. The two gases currently widely in use are methyl bromide and phosphine gas. Methyl bromide has recently been discovered to deplete the ozone layer in the atmosphere, which protects us all against harmful ultraviolet radiation and therefore the use of methyl bromide will in future become more restricted. It is possible for insects to develop resistance against fumigants, and to prevent this, fumigations should always be carried out very thoroughly. The most important requirement is to maintain a very high standard of gas tightness in the stack being fumigated.

### 1 Fumigating with Methyl Bromide

#### 1.1 Fumigation equipment

*Gas tight fumigation sheets.* Canvas tarpaulins are unsuitable as fumigation sheets, because they are not gastight. PVC or polythene sheets, with a thickness not less than 0.09mm and not more than 0.3mm are suitable, but they puncture and tear easily and do not last very long. Woven nylon cloth, coated on one or both sides with PVC or neoprene are far superior, but expensive. Fumigation sheets should be ordered in sizes suited to the sizes of stacks to be fumigated. Stack sizes should therefore be standardised.

*Sand or "sand snakes".* This is used to weight down the fumigation sheets on the floor around the stack, and to provide a gastight seal there. Loose sand works best, but tubes of hessian with a diameter of about 150mm, loosely filled with sand ("sand snakes") are also used. Rocks or other small

objects are unsuitable because a continuous, wide seal is required.

***Pipe system.*** This is made up of black, thick-walled polythene piping (inner diameter 6.35mm), threaded T-junctions, and spray nozzles. Rubber pipes and fittings are unsuitable, because methyl bromide deteriorates rubber very quickly. The function of the pipe system is to release liquid methyl bromide in equal quantities simultaneously at many different points in the stack. The make-up of a pipe system is shown in figure 1.

***Gas cylinder couplings.*** This is a special device that screws onto the methyl bromide cylinder and to which the pipe system is connected. It consists of valves to control the flow of methyl bromide through the pipe system, a Schrader valve and a pressure gauge. In the past, two couplings were required, one to connect the gas cylinder to the pipe system and the other to connect the gas cylinder to a compressor to add pressure to the cylinder. Currently gas cylinders are pressurised in the gas plant after filling and the use of a compressor is no longer required.

***Halide detector lamp.*** This is used to test the air for the presence of methyl bromide as a means to detect leaks and to safeguard the fumigator and his workers against the gas. It consists of a kind of blow lamp that heats a copper element. Methyl bromide reacts with the heated copper and the reaction causes the flame to change colour from almost colourless to green and thence gradually to bright blue, depending on the gas concentration in the air.

***Gas masks.*** These are essential to protect anyone that may be exposed to the gas from poisoning. Wherever there is the slightest possibility of exposure to methyl bromide fumes, a full face mask, equipped with the appropriate filter canister, must be worn. Filter canisters can only absorb a limited amount of gas, therefore they are effective only at low concentrations, and for a short exposure time. This stresses the need to regularly replace filter canisters. Where protection against higher concentrations, or for longer periods is required, a self contained breathing apparatus, similar to those used by underwater divers or firemen, must be used.

***General equipment.*** This includes tools, ladders, a tape measure, ropes, warning notices and a scale.

## 1.2 Fumigation technique

***Determine the stack volume.*** In the case of flat-topped stacks, measure the length, width and height of the space that will be enclosed by the fumigation sheets when the stack is eventually sealed off, and multiply these three factors to obtain the volume. Thus:

$$\text{Volume} = \text{width} \times \text{length} \times \text{height}$$

The volume of ridged stacks, such as those in the open, is determined by measuring the width and length as above, but the height is measured to the shoulder and then vertically from the shoulder height to the top of the ridge (see figure 2). The volume is then calculated thus:

Volume = width x length x (shoulder height + $^1/_2$ shoulder to ridge height)

***Calculate the quantity of gas required.*** Methyl bromide is applied at 48g per cubic meter to bag stacks. Multiply the stack volume (calculated in cubic meters) with 48g to obtain the total quantity of methyl bromide that must be applied to the stack, eg, stack volume is 1250 cubic meters x 48 = 60,000g or 60kg methyl bromide will be required for the fumigation.

***Determine the number and sizes of fumigation sheets required.*** All around the stack, at least one meter of sheeting must remain on the ground to be sealed off tightly with sand. Where two or more sheets are needed to cover a stack, the adjoining sheets must also overlap by at least one meter of each sheet. Thus 2m of the width, and 2m of the length of each sheet must be "set aside" beforehand to provide for seals.

The total width of sheeting that will be required to cover a stack is from the ground up to the full height of the stack, over the width of the stack, and down the full height again to the ground on the opposite side. Likewise, the total length of sheeting that will be required is from the ground up to the full height of the stack, over the length and down the full height of the stack to the ground on the other side again. Keeping in mind the 2m of the length and width of each sheet "lost" to provide for seals, the dimensions of the available sheets are compared with the length and width to be covered, and the best choice of number and size of sheets is made accordingly.

***Determine the number of "fumigation channels" to be constructed on the stack.*** Channels are constructed on top of the stack by rearranging some of the bags in the stack, to provide an open space where liquid methyl bromide from each jet in the pipe system can evaporate, without the liquid coming into contact with grain bags or fumigation sheeting. Channels also promote even distribution of the fumes through the stack. The proper arrangement and dimensions of channels are shown in figure 3.

***Lay down a pipe system and position the jets.*** Having completed the channels, a pipeline is laid down in each channel. The distal ends of each pipe is sealed tight. The pipeline is firmly anchored under the top layer of bags on one side of the channel, so that each jet is at least 30cm from any object. The arrangement and dimensions of pipe systems are illustrated in figures 4 and 5.

Having completed the pipe system, air is pumped through, and each jet is tested to see that it is completely unblocked. Joints and sealed pipe ends are at the same time inspected for undesired leaks.

***Prepare the floor around the stack for proper sealing of fumigation sheets.*** The surface onto which the fumigation sheet is to be weighted down, must be smooth and even to ensure a gastight seal. This can be achieved by sweeping up stones and spilled grain and spreading a 30cm band of fine sand on the floor around the stack.

***Place the fumigation sheets in position over the stack.*** Fumigation sheets are heavy and difficult to handle on top of the stack, therefore it must beforehand be folded in such a way that it can easily be spread open. This is done by repeatedly folding the fumigation sheet from each side to the middle, until it finally takes shape as a long tube. These tubes are hung over the stack in their approximate positions, so that, when they are pulled open, each will cover its designated portion of the stack. The sheets are then pulled open starting at one side of the stack, so that remaining tubes can be moved closer or further away as the need may be. After a sheet had been spread open, at least one meter of it on the side where it will be adjoined by the next sheet, is folded back over itself. The adjoining sheet is then spread open so that it overlaps the folded back portion of the first sheet. The two sheet edges are then rolled tightly together, until all the slack of the overlay has been taken up. This provides a totally gastight seal between adjoining sheets. The roll must be weighted down to ensure that it will stay intact during the fumigation. This process is repeated until the stack is fully enclosed in a gastight cover of fumigation sheeting.

On the floor around the stack, the sheeting is weighted down using sand or sand snakes, to form a continuous seal about 30cm wide. Rolls are weighted down with sand snakes or bags of grain. At the stack corners, the surplus sheeting is folded back tightly and smoothly against the stack and held in position by propping up wooden poles against it, or with ropes or rope nets. The purpose is to prevent the sheeting from being ripped open by wind gusts, allowing the gas to escape.

Fumigation sheeting should always be handled with great care, as it is easily punctured by stones and other hard objects, are difficult to repair and expensive to replace.

***Apply the gas.*** Place the warning notices in position around the stack, don a gas mask, and screw the cylinder coupling onto the gas cylinder, making sure that all connections are firm and gastight. Place the gas cylinder on the scale and screw the pipe system into the coupling. In figure 6, the cylinder with coupling is represented schematically. Weigh the gas cylinder and subtract from that the amount of gas to be applied to the stack. That gives the weight the cylinder should be after the full quantity of gas has been applied. Now light the gas detector lamp and make sure that valve C is closed and then open valve A slowly and immediately close again. With the halide detector lamp, test for leaks in the coupling between valves A and C. If there are no leaks, open valve C and test the junction of the pipe system with the coupling for leaks. Adjust valve A to maintain a pressure of 2 bar on the pressure gauge (B). As soon as the required amount of gas has been applied, close valve A. Allow the pressure to drop to 0, then close valve C. Unscrew the cap on the Schrader valve (E), and connect the compressor pipe to the valve. Open valve C slowly and leave it open 5 to 10 minutes to clear the pipe system of all liquid methyl bromide. Then unscrew the pipe system from the coupling and stopper it to prevent dirt entering it. Unscrew the coupling and replace the stoppers and protective cap on the cylinder. Test for gas leaks around the stack and seal where necessary. Leave the stack to stand for the required fumigation period - usually 24 hours with methyl bromide.

### 1.3 Aeration after fumigation

Workers wearing gas masks lift the sheets from under the sand seal and allow half an hour for the gas to dissipate, while working in another part of the store, or at another stack. Tests for gas should frequently be done to safeguard workers against exposure. Finally, the sheets are removed and folded as described before.

## 2 Fumigating with Phosphine

When fumigating with phosphine, sealing the stack is done exactly as for methyl bromide. In this case, however, gastightness is of much greater importance, because the gas exposure period is a minimum of five days, during which time the gas must be retained within the stack. On the other hand, application of the gas is much simpler and involves distributing the gas generating tablets or sachets evenly over the stack followed immediately by sealing off the stack with fumigation sheets. The tablets or sachets are applied at a rate which will give 1g of phosphine gas per cubic meter of stack volume. The chemicals contained in the tablets and sachets react with moisture in the air, forming the poisonous phosphine gas. This takes a few hours to complete, depending on air humidity and the temperature. Each tablet produces 1g and each sachet 11g of phosphine gas.

## 3 Controlled Atmosphere Storage of Bagged Grain

In this case, the stack is permanently stored in an atmosphere rich in carbon dioxide gas. Carbon dioxide is only mildly poisonous to insects, but it can be very effective for pest control if the grain is stored permanently in an atmosphere which contains more than 30 percent carbon dioxide.

Gastight sealing is even more important here than when fumigating with phosphine and special sealing techniques are therefore required. The first step is to sweep the floor very clean and then spread a canvas sheet on the floor where the stack will be constructed in the future. On top of this is spread a plastic, gastight sheet. The purpose of the canvas sheet is to protect the plastic sheet from punctures. The plastic sheet should cover an area about 1m larger on all four sides than the area that will be covered by the stack. The stack is then built on top of these sheets. After completion, the stack is covered with a plastic sheet, tailored to fit exactly over the stack. The overlaps are glued together to provide an absolute gastight seal. A gas inlet is cut into the top sheet, and an outlet at the opposite side. Carbon dioxide is introduced through the inlet and allowed to displace the atmosphere inside the stack through the outlet. As soon as the atmosphere under the sheeting contains 70 percent or more carbon dioxide, the in-and outlets are sealed and the stack is left like that. More carbon dioxide is introduced if test show that the carbon dioxide concentration has fallen to less than 30 percent.

## SPRAYING BAG STACKS WITH RESIDUAL INSECTICIDES

Fumigants can kill insects inside bag stacks, where they cannot be reached by other means, but once the gas has disappeared, the grain can quickly be reinfested. In an effort to prevent reinfestation, it has become customary to spray the outer stack surfaces with pesticides that leave a toxic residue. Pesticides that are in common use for this, are malathion, pirimiphos-

methyl, and chlorpyriphos methyl. These pesticides are relatively safe for humans at the levels of contamination of the grain that normally takes place. Care should be taken when spraying bag stacks that heavy deposits harmful to people who will eat the grain are not built up in the grain by repeated spraying.

In practice it has been found that spray treatments are not as effective as expected in preventing or delaying reinfestation. The problem is that the pesticide not only kills pest insects, but also many of the parasites and predators of pest insects. These natural enemies of pest insects help to keep the numbers of the pest insects down, and if they are killed, this benefit is lost. Moreover, the pest insects, within just a few years, build up a tolerance against the insecticide, and infest the grain in spite of the spray treatment. Moths, like the tropical warehouse moth and Indian meal moth in particular, have become especially troublesome, since the spraying of bag stacks has become common practice. In recent years, other methods of protecting the fumigated grain from reinfestation have therefore been investigated. These include the use of timed space fogging with dichlorvos, and the use of natural enemies of pest insects.

## INTEGRATING FUMIGATION WITH TIMED SPACE FOGGING TO CONTROL MOTHS IN BAG-STORED GRAIN

This control programme is primarily aimed at reducing the number of moths from laying their eggs in fumigated bag stacks soon after the fumigation sheet is removed. During fumigation, many moths escape fumigation by leaving the stacks when disturbed. Also, many migrating larvae pupate in the fabric of the store, or in cracks in dunnage poles, and the adults emerge from pupae between about 12h00 and 17h00. They mate the same day at about sunset, and start laying eggs that night, continuing every night for about 10 days. Each female lays about 400 eggs. This control programme endeavours to kill all adults after they emerged from the pupae, before they can mate and start laying eggs. Fogging is therefore carried out at sunset, and starts when the fumigation is in progress. It is continued every evening for as long as dead moths are found on the floor the morning following the fogging treatment - usually 3 to 4 weeks.

In Africa, there are four generations of moths per year. Therefore, moths may at any time fly into the store from other breeding places and reinfest the grain. To avoid this, moth traps are hung in the store to reveal the reappearance of moths. As soon as this is observed, a further series of daily fogging is started and continued for as long as dead moths are found on the floor the following morning.

This control programme has proved to be very effective, but it is expensive and demanding, as it involves fogging everyday, including weekends and holidays, and it has to be done after normal working hours.

## INTEGRATING FUMIGATION WITH BIOLOGICAL CONTROL TO CONTROL MOTHS IN BAG STORED GRAIN

This programme makes use of a small wasp that parasites the larvae of the tropical warehouse moth and Indian meal moth. The wasp is harmless to humans. The adult female wasp first stings and paralyses a number of almost fully grown moth larvae, feeds on some of them, then lays eggs on most of them to produce about two or three wasp progeny per moth larva. The female wasp will penetrate into the bags and parasitise larvae inside.

Paralysed larvae stop feeding and eventually die. The wasps are bred artificially and adult wasps are released in grain stores at an average of about 1 wasp per week per ton of grain in storage. Weekly releases should start in early spring and should be maintained continuously throughout summer and autumn. Moth populations should also be monitored on a weekly basis with the help of moth traps and the rate of wasp releases should be increased when a build-up of moth numbers is evident.

# PHYSICAL STRUCTURES FOR STORAGE OF GRAIN AND SEED AND THEIR MAINTENANCE

**by J H Viljoen**

## INTRODUCTION

Grain is produced seasonally, but it is consumed throughout the year. Therefore, grain stocks need to be stored from one crop to the next, and sometimes for longer periods. The main aim during storage is to maintain the quality of the grain as closely as possible to what it was when the grain was freshly harvested. To achieve this, the grain store must perform various functions: it serves as a container to hold the grain, and it must keep the grain cool, dry and out of reach of pests such as storage insects and rodents. Sometimes the grain store doubles as a fumigation chamber. Often, storage takes place in such a manner that it facilitates drying of the grain.

Through the ages, and in different parts of the world, various methods have been applied to store grain. In arid parts, and particularly in ancient times, grain was often stored underground in airtight pits, out of reach of insects and rodents. In humid areas, keeping the grain dry, or facilitating further drying during the initial stages of storage, is of overriding importance and pests must be dealt with in another way. It is clear, therefore, that the grain store has to fulfil various needs to satisfactorily maintain the quality of the grain during the course of storage. When planning a grain store, all these functions must be taken into consideration, else the quality of the grain will deteriorate. The same applies to grain handling techniques. Grain should not be handled in ways that will destroy the grain quality, as that defeats the objectives of storage. In this paper, current grain storage and handling facilities, their maintenance and their effects on grain quality will be outlined.

## GRAIN STORAGE METHODS

Grain can be stored threshed or unthreshed. Unthreshed grain is usually stored for only a relatively short time, to facilitate drying before the grain is threshed and then stored in another type of storage for the rest of the time it needs to be stored. Threshed grain can be stored either in bags, or in bulk. Threshed grain stored in bags, can be stored inside warehouses, or in the open under tarpaulins for protecting the grain against the elements. Threshed grain stored in bulk can be stored in upright or silo-type bins, in flat bins or in temporary bunkers. In each of these types of stores, specific means of handling the grain can be applied. We will discuss each of these storage facilities and their maintenance in more detail.

### 1 Storage of Unthreshed Grain

Where a combine harvesting machine for harvesting the grain is unavailable, grain is often stored unthreshed for a very short time in the field, sometimes under some type of temporary protection. Grain may also be stored unthreshed for a longer period, where there is a need to allow the grain to dry further after harvesting. IN some parts of the world, the grain is only threshed shortly before it is processed for consumption. Most often, however, the grain is threshed as soon as it is dry enough, and then stored in bags or in bulk until needed.

When unthreshed grain needs to be stored for a longer time to allow it to dry further during storage, it is removed from the field and stored in a structure specially built for storage. The most common case of storing unthreshed grain like this, is that of maize, where the ears are picked, de-husked, and then stored in specially built cribs. The cribs are round or rectangular structures raised from the ground on posts. Cribs are constructed of woven sticks, wire mesh, or any other suitable material that will allow free air passage through the grain. The roof consists of any suitable waterproof material, such as thatch or metal sheets with a large overhang to prevent the crib sides from getting wet during rain storms. The main purpose of crib storage is to allow the grain to dry. In a crib the grain is very vulnerable to insect and rodent attack, and precautions are necessary to limit damage. To prevent invasion of rodents, rodent guards are installed on the posts that raise the structure from the ground. Insect attack is limited by keeping storage time in the crib as short as possible, and limited to the coldest part of the year, when insect activity is low.

*Maintenance*

Maintenance of cribs involves clearing away grain residues of the previous season, keeping roofing material in a waterproof state, maintaining a proper overhang, mending holes in the wire mesh or woven material and keeping the rodent guards in effective working order.

## 2 Storage of Threshed Grain

### 2.1 Bag-storage

Bags of grain are neatly piled under suitable shelter to protect the grain against the weather. Bag-storage is very common throughout Africa and other places where only limited equipment for handling and transporting grain are available and manpower costs are low. In more developed regions, the costs of storing grain in bags are becoming prohibitive and the move is towards storing grain in bulk. To store grain successfully in bags, good planning is essential, and it should start long before the grain is actually received for storage. This includes estimating the amount of grain likely to be stored the coming season and keeping the new stacks as far as possible away from stacks of the previous season.

#### 2.1.1 Bag stacks in the open tarpaulins

Marshy terrain is unsuitable for grain storage and it should only be done on gravelly or sandy soils. The slope should be taken into account and stacks should be laid on lengthwise along the slope, so that they will not seriously impede the flow of rain water away from the storage area. The size of stacks should be standardised as far as possible in accordance with the sizes of the available tarpaulins for protecting them against the weather, and also in accordance with the available fumigation sheets. Stacks should not be too large and should therefore not contain more than 1,300t of grain. Stack width should not exceed 8m, the length 35m, and the height at the ridge, 8m.

Bags should be stacked on at least two, but preferably more than two layers of dunnage. The dunnage poles should not protrude from underneath the bags. It is best to spread tarpaulin over the dunnage poles, after these had been laid down in accordance with the planned size of the future stack. The tarpaulin protects the bottom layer of bags against dampness from the soil. A tarpaulin about one-and-a-half meters on each side larger than the area covered by the dunnage poles is used, so its sides can be turned upwards against the sides of the stack, and fastened over the top, once the stack is completed. This protects the lower sides of the stack very effectively from rainwater splashing up against the bags during rain storms.

After the stack has been completed, wide, shallow drainage canals are constructed around it, so that rainwater will not run underneath the stack. The area around the stacks need to be cleared of all vegetation, so as to limit any fire hazard.

*Maintenance*

Dunnage poles not in use, should be sprayed regularly with a registered insecticide to kill insects hiding in the cracks. Holes and tears in tarpaulins should be mended before the start of the new storage season, stacks should be regularly inspected for tears and holes, and for worn rope ties, and these should be mended or replaced. The drainage canals around stacks should be maintained in effective order, and the area around the stacks should be kept clear of vegetation.

### 2.1.2 Warehouses for Bag stacks

Most warehouses for bag storage are constructed of a steel or wooden frame, clad with corrugated iron sheets, but some have brick walls with an iron roof. The floors may be of concrete, tar or earth.

Bags are piled in neat stacks in a similar way to bag stacks in the open. However, space is more limited inside a warehouse and there are more physical obstructions that may inhibit fumigation and other pest control measures. Therefore, planning where stacks are to be placed, their size, and so on, is even more important than with outside storage of bags.

Bags should be stacked on two layers of dunnage poles, even on good concrete floors, and the floor must be swept clean before the dunnage is laid out. The stack should nowhere touch against another stack, or against upright or horizontal roof supports, walls, concrete footings or any other obstacle that may obstruct the proper sealing off of the stack with a fumigation sheet. the clear distance between stacks should be at least 1m to allow frequent inspection of the stacks, fumigation and spraying. The size of stacks should be in accordance with the sizes of the available fumigation sheets. Stacks should not be higher than 8m, longer than 35m, and should not contain more than 1,850t of grain.

*Maintenance*

Warehouses should be maintained in a waterproof state, by repairing all leaks due to damage or loose screws. Wire netting to keep birds and rodents out, should be kept in good repair. Doors should be kept rodent proof.

Dunnage poles should regularly be sprayed with registered insecticides to kill insects in cracks in the wood.

## 2.2 Bulk storage

### 2.2.1 Silos

Silos are designed and built in various configurations of size, shape and construction material. The common feature of all silos is that their height is always greater than their diameter or width. Silos are mostly circular, but some with hexagonal and rectangular shapes are also in use. They range in capacity from a few,to as much as 14,000 tons, with diameters from two or three, to 25 meters, and heights from two or three to over 50 meters. The most common size in Africa is a diameter of 15.2m, height of about 30m, and grain capacity of about 4,000t.

The most common construction material used, is concrete reinforced with steel, particularly for large structures. Bolted steel, and welded steel silos are also fairly common, particularly for smaller silos.

Basically, a silo complex consists of one or more rows of bins, all served by a common grain conveyor at the bottom, and another one at the top. Grain is elevated to the top by means of the bucket elevator. Usually there is also a shorter elevator to carry the grain to the hopper scale and the cleaning machinery. Grain delivered by road is dumped in receival hoppers, from where a short conveyor takes it to the bottom of the elevator. It is carried to the top and dumped onto the top conveyor, which carries it to the bin in which it will be stored. A tripper throws it off the conveyor into the bin. The tripper can be moved between bins, as needed.

When grain is discharged, the grain outlet at the bottom of the bin is opened, and the grain flows under gravitation onto the bottom conveyor. This carries the grain to the short elevator, which takes it to the cleaning sieves and the scales to be weighed. From there the grain flows into a chute that deposits it into the rail or road truck parked in the appropriate position.

Grain storage in silos has been automated to a considerable degree, and most of these operations can be put into action from a central control room.

*Maintenance*

Concrete is generally porous, therefore rain water may penetrate the walls in places where it is particularly porous. Where the water can penetrate to the inside of the bin, the leaks need to be repaired to prevent damage to the grain. Such leaks are evident where rotten grain cake against the wall. Also, where the water can penetrate to the steel reinforcement inside the wall, the leak needs to be repaired, as rusting of the steel wall will severely reduce the structural strength of the concrete and reduce the life expectancy of the silo. Such leaks are evident by the rusty discolouration of the concrete. Expansion joints in the floor, and at the floor/wall joint, as well as the roof/wall joint need from time to time to be cleaned out and filled with a pliable filling material to maintain the silo watertight and gastight for fumigation, and to limit the movement of storage insects from one bin to another.

The grain handling machinery, aspiration system and electrical system need to be maintained in perfect working order on an ongoing basis to prevent any possibility of fire or grain dust explosion. Steel chutes need to be replaced from time to time, where they are worn thin by abrasion.

Ongoing cleaning everyday is required to eliminate the possibility of grain dust explosions and to keep insect development to the minimum.

### 2.2.2 Flat bins

Flat bins provide large storage capacity at relatively low cost. The floor is of concrete and at ground level and is usually flat, but some flat bins have floors sloping to the centre to assist discharge. The walls are also of concrete and strong enough to contain the side pressures of the grain. The walls are usually less than 10 meters high. The roof is mostly of corrugated iron on a wide span iron frame and tends to follow the natural slope grain forms when heaped in a pile.

The grain handling equipment usually consist of an overhead belt conveyor for grain intake, and sometimes a conveyor is used for grain discharge as well. Most often, however, the floor is flat and power shovels or front end loaders are used to convey the grain towards a bucket elevator or an auger for loading the grain in road or rail transport.

*Maintenance*

Maintenance is similar to silos.

### 2.2.3 Temporary bunkers

Bunkers are low cost temporary structures used for long term storage of grain in the open. The only permanent feature of a bunker is the

specially prepared floor. The floor consists of a rectangular, raised area, slightly rounded and paved with tar or concrete. The floor looks very similar to a section of paved road surface, but is wider than most roads. The edges of the bunker are lined with collapsible A-frame sides clad with corrugated iron, which can easily be folded flat and stored in piles when not in use. After having been set up, the A-frame edges are lined on the inside with plastic sheeting extending about one-and-a-half meters over the floor. The grain is piled in a rectangular heap within the A-frame edges and when the bunker is full, the heap is covered with gasproof tarpaulins. The edges of the tarpaulins are glued and stitched to the edges of the plastic sheeting lining the A-frame edges, using a suitable adhesive. Thus the entire bulk of grain is enveloped in a weather and gastight cover. Short slits are cut in the tarpaulins for the introduction of fumigants, and sealed afterwards with adhesive tape.

Special machinery, such as grain lobbers or very tall augers are used with very wide bunkers to pile the grain high enough to cover the full width of the bunker. Discharge is done with power shovels or front-end loaders to work the grain towards an auger for loading road or rail trucks.

### *Maintenance*

Floors should be kept in good condition, and should be swept clean before grain is taken in to avoid stones and grit in the grain. Tarpaulins should be maintained in absolutely gastight condition and all holes mended.

## REVIEW OF MYCOTOXINS

**by David M Wilson**

Mycotoxins were introduced in this presentation. Mycotoxins were defined and their production in foods and feeds were discussed. The reasons that mycotoxins in foods and feeds are of importance in animal and human health were discussed in the introductory lecture. Mycotoxins are secondary metabolites of fungi that exhibit toxic responses in animals ingesting them as components of foods and feeds. These compounds can affect human and animal health because they can be acutely toxic, cause chronic disease or be carcinogenic. Therefore, mycotoxins are of great public health concern because of their importance in insuring a safe food supply.

The first mycotoxins known were the ergot alkeloids which cause ergotism. Other important mycotoxins include the aflatoxins (produced by fungi in the *Aspergillus flavus* group), the ochratoxins (produced by several *Aspergillus* and *Penicillium* species), zearalenone, the fumonisins and the trichothecenes (produced by *Fusarium* species.

The conditions leading to growth of toxigenic fungi in foods and production of mycotoxins were discussed. The chemistry of the mycotoxins were briefly described in order to expose the participants to the diversity of the chemistry of mycotoxin production, analysis and toxicology. The aflatoxins were discussed in detail, beginning with the human health risk because of their toxicity and cancer providing ability. The mechanisms allowing aflatoxin contamination in maize and groundnuts were discussed and methods for eventual control were considered. Strategies for minimising contamination were presented in relation to developing suitable control measures. The FAO text provided to the participants is an excellent resource and this was used as well as original literature in preparation for this important workshop. This will serve to reinforce the workshop participants knowledge and provide an effective avenue for communication.

## INCIDENCE OF MYCOTOXINS IN FOODS AND FEEDS

**by Douglas L Park**

Many foods and feeds can be contaminated with mycotoxins before harvest, during harvesting and in storage. Aflatoxins, potent hepatotoxins and carcinogens, are metabolites of *Aspergillus flavus* and *A parasiticus*. The principal commodities affected include corn, peanuts, treenuts, and cottonseed. Aflatoxin metabolites can occur in tissues of animals exposed to aflatoxins in feeds, ie, milk, eggs, etc.

Besides the aflatoxins, several other mycotoxins may contaminate foods and feeds. These include sterigmato-cystin, produced by *Aspergillus* and *Penicillium* spp, trichothecenes, produced by *Fusarium, Cephalosporium, Myrothecium, Stachybotrys* and *Trichoderma* spp, zearalenone, produced primarily by *F graminearum*, ochratoxins, produced primarily by *A ochraceus*, and many others. Fumonisins are newly described mycotoxins produced by *F moniliforme*. Primary commodities affected by mycotoxin contamination include cereal grains, animal feeds, oil seeds, fruit and dairy and egg products.

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## "OCCURRENCE, SIGNIFICANCE AND DETECTION OF FUMONISINS"

**by Eric W Sydenham**

Since their isolation in 1988, the fumonisin mycotoxins have generated an enormous amount of interest world-wide. The major reason for this interest stems from the fact that the fumonisins are produced by, amongst others, *F moniliforme*, a major fungal contaminant of maize which is itself a cereal crop consumed in large quantities by both animals and humans. Six fumonisin analogues have thus far been isolated and chemically characterised, but only three of these - *viz* fumonisins $B_1$ ($FB_2$), $B_2$ ($FB_2$ and $B_3$ ($FB_3$) appear to be produced at significant concentrations under natural conditions.

The major analogue ($FB_1$) acts both as a cancer-initiator and promoter, and it has been shown to be the causative agent in several animal and plant disease syndromes. Of particular concern is the fact that both $FB_2$ and $FB_3$ exhibit cancer-initiating potentials similar to those observed for $FB_1$, suggesting that total $FB_1$, $FB_2$, and $FB_3$ concentrations will have to be both determined and taken into consideration. To date, seven *Fusarium* related species have been shown to have fumonisin-production capability, but only two of these *viz F moniliforme* and *F proliferatum* should be of major concern due to their prevalence in maize. Exposure assessment studies have clearly indicated that the fumonisins are ubiquitous contaminants of maize and maize-based products world-wide. In home-grown maize produced in the Transkei, human populations are exposed to combined fumonisin levels that are considered to be potentially harmful to both horses and swine. HPLC methods for the determination of the fumonisins in maize have been developed and improved on over the past few years. One of these methods, which uses a combination of iron-exchange clean-up of samples, derivatisation and fluorescence detection has been subjected to an international collaborative study. The results have indicated that the method is highly reproducible, allowing for the determination of fumonisin levels as low as 50ppb, however, several factors such as pH, solvent strength, flow rates and derivatisation can affect the performance of the method. Several other analytical methods have been developed for the determination of the fumonisins. These have included TLC, GC, ELISA, and MS. It should be remembered however, that when correctly performed, TLC can be used both quantitatively and effectively for the analytical determination of $FB_1$, $FB_2$, AND $FB_3$ (using anion-exchange chromatography for sample preparation) at concentrations at the 1ppm level.

# MYCOTOXIN SCENARIO IN SOUTH AFRICA

**by Eric W Sydenham**

This lecture covered several mycotoxin topics of recent interest to PROMEC scientists. Visual assessment of recently imported maize was characterised by a wide distribution in the size of the participate matter. In a study concerning a series of selected samples, removal of "fine" particulate matter (those fractions having a diameter of $\leq$ 3mm) resulted in significantly decreases in combined fumonisin levels of between 27 and 70 percent. Alkali-treatment of fumonisin-contaminated maize (at pH13) results in the hydrolysis of the fumonisin molecule to yield the aminopolyol (AP) and tricarballylic acid (TCA) moieties. Similar treatment of ground maize with a slurry solution of OIM calcium hydrocide ($Ca[OH]_2$), also results in the removal of the fumonisin toxins from the solid maize fraction to the aqueous phase, and their subsequent hydrolysis to their corresponding AP and TCA moieties. $Ca(OH)_2$ treatment of maize kernels has been shown to reduce fumonisin levels by about 95 percent. Recent metabolism studies on fumonisin $B_1$ ($FB_1$) have been carried out using both rats and velvet monkeys $FB_1$. In the rat model, $FB_1$ is both rapidly absorbed (½ life in blood = 18 minutes) and similarly excreted, predominantly in the faeces. Studies using $^{14}C$-labelled $FB_1$ have shown that relatively low concentrations are retained in the liver, kidneys and red blood cells. Slight differences were observed in the velvet monkey model, however, partial hydrolysis of $FB_1$ occurs in this model, but the resultant moieties are found in the faeces. The final topic of interest is that concerning patulin, a potential carcinogen produced by *Penicillium expansum* that is a major contaminant of apples, and their products. One major source of patulin in apples stems from the sporadic contamination of "good" or "healthy" apples with "rotten" or "damaged" apples. This sporadic contamination highlights the problems associated with mycotoxin levels in various commodities, and illustrates the importance of sampling. Preliminary studies have also shown that internal infection of apples can lead to patulin levels of up to 10ppm in single apples. Removal of damaged fruit, prior to further processing, can reduce patulin levels. Further studies will be carried out in South Africa during 1994, aimed at continued reduction of patulin levels in apple juice products.

# STATUS OF MYCOTOXIN SURVEILLANCE IN SADC COUNTRIES

**by Henry Njapau and Elizabeth M Muzungaile**

## ABSTRACT

The climatic conditions of the SADC region are conducive to fungal growth and mycotoxin production. Surveys have shown that aflatoxin contamination is widespread in all agricultural commodities. Of the 10 member countries, only Malawi and Zimbabwe have legislation limiting acceptable aflatoxin levels in foodstuffs. For the other countries, monitoring is restricted to export commodities. The lack of aflatoxin monitoring activities in the region is largely a result of insufficient exposure data. Risk to human health resulting from aflatoxin consumption is therefore accorded a low priority by policymakers. The situation is unlikely to change unless the high risk to children is highlighted and widely publicised.

## INTRODUCTION

Human food and animal feed become contaminated by mycotoxins either by direct fungal (mould) growth on the food or feed, or as a result of use of mould-contaminated ingredients in their production. Once moulds have invaded food crops they may produce mycotoxins given the right conditions, with temperature and substrate-moisture playing a major role. The potential problem of mycotoxins was recognised soon after Britain encountered the Turkey X disease whose origin was associated with Brazilian peanut meal (Allcroft and Carnaghan, 1963). Subsequent investigators established the worldwide occurrence of mycotoxins in food crops, and a host of diseases and other physiological malfunctions were associated with the consumption of such crops (Wyllie and Morehouse, 1976).

Short exposure to large doses produces acute toxicity which may be lethal. The frequently fatal Alimentary Toxic Aluekia (ATA), Reyes syndrome and acute fatal hepatic necrosis are but a few examples of acute toxicity known to be a result of consumption of mycotoxin contaminated grains. Exposure to small doses over a protracted period of time may be carcinogenic (van Rensburg, 1985) and may reduce resistance to infection by bacteria, fungi, viruses (Hendrickse, 1984). It is now generally acknowledged that mycotoxins have an impact in areas as varied as agriculture, public health, and commerce.

With the exception of Namibia, 70 to 80 percent of the SADC regions' over 90 million inhabitants are involved in agriculture (FAO, 1992). Maize, cassava and sorghum comprise the major components of the human diet. Other crops are wheat, rice, millet, groundnuts, cotton and sunflower. These crops or their by-products are also extensively used in the production of feeds for poultry, pigs and, to a lesser extent, supplements for dairy cattle.

Consumption of mycotoxin contaminated feed by the various categories of livestock may impair their productivity as well as result in the transmission of metabolites, particularly those of the aflatoxins, into their tissues (Hsieh and Wong, 1982).

The climatic conditions are suitable for fungal growth and the crops are acknowledged substrates of mycotoxin producing fungi (Norred, 1986). The human population in the region is, therefore, likely to encounter mycotoxins from multiple sources - food crops and animal products. Many countries in the world have taken up the challenge by conducting surveillances for mycotoxins in the feed supply channels, whose underlying purpose has been consumer protection from both acute and sub-acute hazards.

## SURVEILLANCE

Surveillance can be classified into two phases, the first being primary surveillance as it encompasses those activities conducted in pursuit of knowledge of the existence and distribution of an identified potential hazard over food commodities (assuming a laboratory hazard evaluation has been made). It also involves an estimation of the extent of the risk by determining whether and to what extent the potential hazard is realised in the real world, through indicators such as extent of exposure, number, and probably nature of persons exposed (infants, adults, etc). The ultimate goal is the determination of a level of concern for the hazard. Many SADC countries are probably in the initial stages of primary surveillance.

The second phase (secondary surveillance) aims at limiting exposure and is ordinarily based on a quantifiable amount of the toxic agent related to some level of risk as a function of exposure (level of concern). It is often governed by legislation covering identified commodities and the particular toxin(s). This form of surveillance is commonly conducted in the developed countries (Japan, USA, and Western Europe).

Mycotoxin surveillance in the SADC region has largely been at the primary level. Several surveys of differing magnitude and scope seem to have been conducted to determine the incidence and degree of mycotoxin contamination in raw (field and storage) and dietary (plate foods) food items (Appendix 1). As happened elsewhere in the mid-sixties and early seventies, an additional stimulus for conducting surveys in countries like Mozambique and Swaziland was the high incidence of primary liver cancer in the areas, and the incrimination of aflatoxin as a causative agent. No efforts were made to establish any causal relationships with other diseases, nutritional or otherwise. The surveys covered maize, sorghum, groundnuts and beans as raw and cooked dietary items (Tables 2 and 3) on the realisation that as means of assessing exposure, raw commodities are conceptually inferior to dietary items. Other frequently consumed items like beer and milk were also included.

Results from the surveys indicate widespread aflatoxin contamination in all but milk samples. The incidence of contamination reveals a variation between studies (6.8 and 4.7% in Swaziland, and 2.9 and 1.9% in Zambia - Table 3). The incidence in a single survey in Mozambique was estimated at 8.2%. Mean aflatoxin contamination was moderate except for groundnuts and beer. Traditional village beer is often made from germinated maize, an item which was found to have high levels of aflatoxin in a Zambian survey.

Consumption of aflatoxin contaminated material was further demonstrated in studies that focused on human body fluids and tissues. Such studies examined human urine (Zambia - Lovelace *et al*, 1982, and Zimbabwe - Nyati *et al*, 1987), breast milk (Zimbabwe - Wild *et al*, 1987) and liver autopsy samples of normal, Marasmic and Kwashiokor children in Zimbabwe (Hendrickse, 1984). Aflatoxins were isolated from urine, breast milk and the liver autopsy samples of all classes of children. The ratio of liver samples from which aflatoxins were detected was higher in Marasmic Kwashiokor (6/8) and Kwashiorkor (10/21), than normally nourished children (11/35).

Regarding other mycotoxins, only Zearalenone (Zambia and Lesotho) and Deoxynivalenol (Zambia) have been reported to occur in raw and processed cereal-based commodities.

As concerns regular monitoring to limit exposure (secondary surveillance), emphasis is placed on export commodities like groundnuts which are tested to satisfy requirements of

importing countries. Regarding monitoring foodstuffs for local consumption, only Malawi and Zimbabwe have legislated maximum limits for aflatoxin. In Zimbabwe, 20$\mu$g/kg total aflatoxin is the maximum tolerated level in groundnuts, maize, sorghum, peanut butter, other cereals, flour and bread, whereas Malawi's law stipulates that all foods should not contain more than 35$\mu$g/kg (van Egmond, 1989). The rationale for setting these limits and their enforcement mechanism could not be obtained. In the rest of the SADC member states, mycotoxin contaminated foodstuffs could probably be placed under the American (US) classification - GRAS - generally recognised as safe, because no legislation appears to be in place.

## CONCLUDING CONSIDERATIONS

Surveillance in terms of limiting exposure is a regulatory response made to an assessed risk. The degree of effort expended ought to relate in some concrete way to the likelihood that the substance poses a potential health risk to the public. The assessment of the risk is therefore paramount and, if done in a sound and logical manner, justifies the need for surveillance as a means of control.

Discussing standards and guidelines for toxicity testing, Harris and Morgenroth (1981) make reference to a quality indice they termed "core quality standard", which they define as the minimum data required for the acceptance of a study. Using such a measure they argue, precludes use of data from studies which fail to meet the required standard. Although such measures of quality may not exist for studies of a survey nature, it is only logical that the adequacy or inadequacy of available data be examined in a similar way to explain, at least in part, the non-existence of mycotoxin surveillance activities in the region.

The studies conducted at the primary level, in the three countries, were few, isolated and not systematic. They were not coherently focused on a general understanding of the occurrence and distribution of the aflatoxins. There was a general lack of continuity making the accumulated data of less value in terms estimating average daily intakes of aflatoxin. The narrow scope (numbers and time frame) of the Zambian studies, for instance, fails to provide sufficient exposure data on a national basis. Consequently, the data generated is insufficient as a measure of exposure. It can only be used as a basis for expansion of activities related to exposure evaluation.

The other aspect in the assessment of risk is the demonstration of the hazardous nature of the toxic agent. In this respect, the "religious-like fervour" about cancer has overshadowed other vital areas of aflatoxin toxicity. It should be realised that cancer is a "dread disease", in communities where it is well publicised and quantified, and the population appreciates or understands the statistics. It does not make a similar dramatic impact on the other less enlightened societies - societies with a skeptical perception of the disease itself.

Acute toxicity (though occasional) and the immune suppression phenomena are probably more important in the latter societies particularly where it concerns children, than malignant neoplasms. Infections (bacterial, viral) and nutritional (marasmus, kwashiorkor) diseases are endemic in developing countries, accounting for over 60 percent of the deaths of children under the age of five years (UNICEF, 1993). Furthermore, it is estimated that 30 percent of the children in the SADC region are malnourished (Dendy and Trotter, 1988), and it has been demonstrated that such children have an impaired capability to quickly metabolise aflatoxin (Hendrickse, 1984), increasing exposure duration at the target organ. Children consume more food per kilogram body weight and are therefore likely to be more at risk

from aflatoxin contamination than adults. A consideration of the role of mycotoxins in child development is likely to have a bigger impact in developing countries, hence generate sufficient concern by policy and financial regulators to institute surveillance activities.

Finally, the assertive reason that resource-poor developing nations are not regulating mycotoxin contamination because of hunger and the lack of policy and necessary institutions, should be gauged in the light of the fact that these countries seldom know the degree and extent of contamination of their foodstuffs and hence, the risk. In reality, they lack a basis for instituting such activities.

Therefore, until a sound rationale for legislating mycotoxin regulatory measures is established, Hendrickse's (1984) statement that, animals in developed countries are currently better protected from mycotoxin contaminated food than people in tropical developing countries will remain valid. This is a painful fact for a region that has served as one of the world's "field laboratories" for research on mycotoxins.

## APPENDIX 1: MYCOTOXIN RELATED LITERATURE FROM SADC COUNTRIES

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Nil

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C LESOTHO

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Nil

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## TOXICOLOGICAL EFFECTS OF MYCOTOXINS

**by Douglas L Park**

The presence of mycotoxins in animal foods and human foods pose a risk to animal and human health. Pathological effects include hepatotoxicity (aflatoxins, sterigmatocystin), nephrotoxicity (citrinin, ochratoxin A, patulin, penicillic acid), hemorrage (aflatoxins, cyclopiazonic acid, patulin, trichothecenes), carcinogenesis (aflatoxins, ochratoxin A, patulin, penicillic acid, sterigmatocystin), teratogenesis (ochratoxin A), estrogenic effects (zearalenone), digestive disorders (trichothecenes), tremors (penitrem), and many other disorders. Aflatoxin B, binds to DNA disrupting genetic coding thus promoting tumorgenicity. Sub-molecular aflatoxin metabolites can either be activation (toxicity, carcinogenicity) or detoxification products. Some postulated human mycotoxicoses include aflatoxins, AIDS, alimentary toxic aleukia, nephropathy, kwashiorkor, reye's syndrome, pellagra, ergotism, encephalopathy, and hepatic, cervical, renal and oesophageal carcinogenesis.

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# SAMPLING METHODS

**by Prof D L Park**

## INTRODUCTION

The testing or estimation of mycotoxins in bulk food and feeds is often difficult because of large variability associated with testing procedure which generally consists of the following steps:

i) Taking sample from the lot (bulk)
ii) Grinding sample and taking sub-sample
iii) Extraction and quantifying of mycotoxins in the sub-sample

At each stage, random variation occurs and hence it is difficult to determine mycotoxin concentration with 100 percent certainty.

## VARIATION AMONG TEST RESULTS

A study by Velasis et al (1975) involved taking 20 replicated samples from each of 10 contaminated cotton seed lots. Each test result was obtained by

i) Taking 2.27kg sample of dehulled kernels
ii) Grinding the sample and taking a 100g sub-sample
iii) Quantifying the aflatoxin by densitometry

The following observations were made:

i) A wide range among replicated test results from same lot was reflected by large variances and coefficient of variation (CV)
ii) As the lot concentration increased the variance among the test results increased but the relative variance (CV) decreased
iii) The distributions of the test results were negatively skewed at low concentrations and became more symmetrical as the lot concentration increased. The skewness was also found to be greater for small sample sizes than large ones.

The total variance (Vt) described here, is the sum of sampling variance (Vs), sub-sampling variance (Vss) and analytical variance (Va).

$$Vt = Vs + Vss + Va$$

## SAMPLING VARIABILITY

The largest source of variation in the testing procedure is the sampling step. The variation is more pronounced for small sample sizes. Sampling variance is large because

a) aflatoxin is found only in small percentage of the particles in the lot/bulk, and
b) the concentration of aflatoxin in a single particle may be extremely high thus giving high testing values if the particle is sampled.

An example of studies in peanuts showed that for a contaminated lot at 20ppb, the number of contaminated kernels will be equivalent to less than one kernel per thousand kernels. Or a 5kg sample of peanut kernels with a single kernel containing 106ng aflatoxin will have a sample concentration of 200ppb.

In the same study, it was observed that the percentage of contaminated particles in a lot are a function of the aflatoxin concentration, increasing with increase in lot concentration.

Since lot/bulk contamination depends on (i) percentage of contaminated particles and (ii) range in aflatoxin concentration among individual particles, therefore variation among replicated samples is large. Several studies have also indicated that sampling variance is a function not only of lot aflatoxin concentration but also of type of commodity.

## SUB-SAMPLING VARIABILITY

For granular products/commodities, the entire sample must be comminuted in a suitable mill before a sub-sample is removed. The distribution of contaminated particles in a comminuted sample is assumed to be similar to the distribution of contaminated particles in the lot.

In general, the sub-sampling variation is smaller than the sampling variation, however, the sub-sampling variability increases with aflatoxin concentration in the sample. For a given commodity, the sub-sampling variance is reduced by reduction in particle size or by increasing the number of particles per unit mass.

## ANALYTICAL VARIABILITY

Testing procedure of aflatoxins usually involves several steps such as (i) solvent extraction, (ii) centrifugation, (iii) drying, (iv) dilutions, and (v) quantification. Considerable variation may arise among replicated analyses on the same sub-sample. Studies have shown that analytical variance is a function of aflatoxin concentration of the sub-sample and analytical method employed. Of the three commonly used analytical methods, TLC has shown greatest variability whilst HPLC has shown least variability. Variability associated with immunoassay has been found to be less than TLC but slightly more than HPLC.

## REDUCING VARIABILITY OF TEST RESULTS

Total variance can be reduced by reducing individual variance components associated with each step of the test procedure as follows:

(i) Sampling variance can be reduced by increasing sample size
(ii) The sub-sampling variance can be reduced by increasing size of sub-sample or by increasing the degree of comminution
(iii) Analytical variance can be reduced by increasing number of aliquots quantified or by using more precise quantification methods such as HPLC.

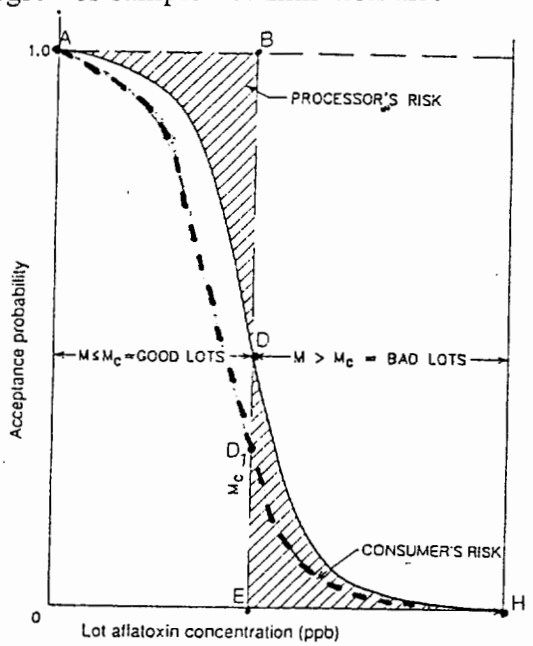
## DESIGNING AFLATOXIN TESTING PROGRAMMES

Bulk food and feed is often tested for aflatoxins. If the estimation of aflatoxins exceeds a defined limit (Mc) then the consignment is rejected, however, because of large variability on test results, good lots may test as bad (processor's risk) or bad lots may test as good (consumers' risk).

A lot is defined as "bad" when sample aflatoxin result X is above some predefined critical level Xc, and is defined as "good" when X is less than Sc. For a given testing design Xc could be less equal or greater that Mc. Lots with aflatoxin concentration M will be accepted with certain probability P(M). A plot of P(M) versus M is called an operating characteristic (OC) curve. The figure below shows a typical OC curve with shaded consumers' and processors' risks. The shape of OC curve is defined by

(i) sample size
(ii) sub-sample size
(iii) number of analyses
(iv) critical level Xc

Increasing all these parameters reduces both consumers' and processors' risks. Increasing degree of sample comminution also decreases both consumers' and processors' risks.




When Xc is reduced (dotted line), the consumer's risk is reduced (Area DEH→D,EH) and processor's risk is increased, (Area ABD→ABD,)

Figure 1

When Xc is reduced (dotted line), the consumer's risk is reduced (▒ area) and processor's risk is increased. When Xc is increased, the consumer's risk is increased and processor's risk reduced.

## SELECTING SAMPLES

(i) Sample selection should be unbiased
(ii) Since contaminated particles may not be distributed uniformly throughout the lot, the sample should be an accumulation of small portions taken from many different locations
(iii) In general, 0.5kg sample should be drawn from 1,000kg feed
(iv) Sampling should be from ¼ of the total number of sacks or bins for small samples OR square root of the number of sacks for large lots
(v) If access is limited, then sampling should be drawn when the commodity is being removed
(vi) Automatic cross-cut samplers could be used for sampling a moving stream of product

## CONCLUSION

Sampling is usually the largest source of variability for small sample sizes.

## CONSIDERATIONS FOR QUALITY ASSURANCE

**by Lawrence Castle**

The presentation dealt with certain aspects of laboratory finality assurance under the headings:

- making sure you get the correct result
- convincing others that your result in correct

Areas covered included:

- proper identification of the putative mycotoxin(s)
- correct quantitation
- laboratory accreditation
- validated methods
- standard reference materials
- performance assessment schemes

The basic steps of a typical analytical procedure for mycotoxins were described from receipt of the sample in the laboratory and ending with the final determination, by TLC or ELISA, for example, and confirmation of presumptive positives. For each step, the pitfalls that could compromise accuracy and precision were highlighted. The application of validated methods and standard reference materials to ensure correct results for unknown samples, was discussed.

Finally, validated methods and standard reference materials along with laboratory accreditation and especially performance assessment schemes[1], were recognised as valuable tools whereby the reliability of a laboratories results could be both ensured and demonstrated to others.

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[1] For example, the check sample exercise organised for mycotoxin analysis by IARC (Int Agency for Cancer Research). Also, the Food Analysis Performance Assessment Scheme (FAPAS(, MAFF Food Science Lab, Norwich, UK

# RATIONALES FOR REGULATORY PROGRAMMES FOR MYCOTOXINS
## (Perspectives on Aflatoxin Control for Human Food and Animal Feed)

**by Douglas L Park and Bailin Liang**

Aflatoxins are potent carcinogenic, mutagenic and teratogenic metabolites produced by molds that grow on food and feed. Their toxicity has caused severe health and economic problems worldwide. Human exposure to aflatoxins can arise from the direct consumption of contaminated commodities such as corn (maize), peanuts or figs, or from the consumption of foods from animals previously exposed to aflatoxin in feeds (milk and egg products). Food safety monitoring programmes for aflatoxins have been established for raw and finished products; these include establishment of regulatory limits or guidelines, monitoring programmes for aflatoxin levels in susceptible products, and decontamination procedures and/or the diversion of contaminated products to lower-risk uses.

Aflatoxins, potent carcinogenic, mutagenic and teratogenic metabolites produced by the fungal species *Aspergillus flavus* and *Aspergillus parasiticus*, can contaminate human foods and animal feeds. Such contamination is the result of (currently unavoidable) invasion by molds before and during harvest, or because of improper storage of agricultural commodities. The major agricultural commodities affected with aflatoxins are corn (maize), peanuts, figs, tree nuts, rice, dried fruit, pumpkin seeds and cottonseeds. Aflatoxin residues can also occur in the milk of lactating dairy animals following the ingestion of aflatoxin-contaminated feed. Human exposure to aflatoxins can result from the direct consumption of contaminated commodities, or from the consumption of foods from animals previously exposed to aflatoxin in feeds (milk and egg products). The significance of the risk due to aflatoxin contamination is dependent on the toxicological properties of the particular compound (acute, subacute, reproductive or long-term toxicity; mutagenicity; teratogenicity) as well as on the extent of the exposure (occurrence, incidence, level of contamination).

Unquestionably, prevention is the best method for controlling mycotoxin contamination. However, pre-harvest invasion of the mold and its subsequent production of aflatoxin are currently unavoidable.

Hazards associated with the toxins must be removed if the products are to be used for food or feed purposes. Mycotoxin control programmes include the establishment of regulatory limits or guidelines, monitoring programmes for mycotoxin levels in susceptible products, and decontamination procedures and/or strategies for the diversion of contaminated products to lower-risk uses. Research into the development of decontamination procedures has been underway for over 20 years. The decontamination procedures currently used are based on physical or chemical inactivation. The ammoniation of corn, peanuts, cottonseed and meals to alter the toxic and carcinogenic effects of contaminating aflatoxins appears to have practical applications and has been the subject of intense research efforts by scientists in various government agencies and universities worldwide.

This review describes the acute and chronic toxicity/carcinogenic potential of aflatoxins, the rationale for the control of human exposure, and monitoring and decontamination programmes that have been put into use.

## Health Hazards to Humans from Aflatoxins

Acute toxicity potential of aflatoxins in humans

There is ample epidemiological evidence that humans are not immune to acute aflatoxicosis, and the US Food and Drug Administration (FDA) has established "action levels" for aflatoxins in various foods and feeds (Table 1). The first report of acute intoxication with aflatoxin came from Taiwan in 1967.

A few years later, in Uganda, a 15-year old boy died two days after hospitalisation. *Post mortem* examination found organ and cellular damage that was similar to that described for aflatoxicosis in the African monkey; the examination of foodstuffs in the child's home revealed moldy cassava containing 1700$\mu$g/kg aflatoxin.

In 1974, in India, there was an outbreak of hepatitis affecting 397 patients in 200 villages and resulting in 106 deaths. The outbreak lasted for two months and was confined to tribal population groups whose staple food was corn. Adverse rains had drenched the standing crop and the outbreak began a few weeks after harvesting. Aflatoxin $B_1$ was detected in seven serum samples. Daily aflatoxin $B_1$ intakes from the corn were estimated to have been at least 55$\mu$g per kilogram of body weight for an undetermined number of days.

An additional outbreak of aflatoxicosis occurred in 1982 in the Machakos area of Kenya, where 20 patients developed hepatitis; 12 died. Aflatoxin $B_1$ was detected in the livers of two patients at levels of 39$\mu$g/kg and 89$\mu$g/kg. Levels of aflatoxins $B_1$ found in corn samples from affected households were high (3200-12,000$\mu$g/kg); unaffected homes had levels not exceeding 500$\mu$g/kg.

**Table 1. Current aflatoxin action levels established by the US Food and Drug Administration**

| Food/Feed | Action Level ($\mu$g/kg) |
|---|---|
| Human Foods (except milk) | 20 |
| Milk | 0.5 |
| Animal Feeds (except as listed below) | 20 |
| Cottonseed meal (for mature beef, swine & poultry) | 300 |
| Corn (for breeding beef cattle, swine or mature poultry) | 100 |
| Corn (for finishing swine) | 200 |
| Corn (for feedlot beef cattle) | 300 |

Chronic toxic/carcinogenic potential

Aflatoxins, although highly carcinogenic in all experimental animal species studied, and tumorigenic in many organs, act predominantly in the liver and biliary tract, after oral, subcutaneous or intraperitoneal administration.

Although a direct cause-effect relationship has not been demonstrated, there is a general correlation between the incidence of liver cancer in humans in specific areas of Africa and Asia and dietary exposure to aflatoxins.

Several epidemiological studies have shown that there is a positive correlation between aflatoxin levels in the diet and the incidence of primary hepatocellular carcinoma (PHC) in specific regions of the People's Republic of China, Kenya, Mozambique, The Philippines, Swaziland, Thailand and Transkei in South Africa. However, the prevalence of hepatitis B virus (HBV) infection was also found to be correlated to liver cancer incidence in these regions. In contrast, low PHC incidence was observed in certain regions such as the south-eastern states of the USA, where the estimated dietary exposure to aflatoxin was high. Although it is difficult to differentiate the roles of aflatoxins and HBV on the pathogenesis of PHC in humans, recent studies have indicated that aflatoxin exposure is more important than the prevalence of HBV. In Swaziland, where there is little geographic difference in HBV exposure throughout the area, PHC incidence was strongly associated with the level of aflatoxin exposure in the subregions. Studies in Fusui of the People's Republic of China, where the incidence of PHC is among the highest in the world (120 cases/10), also revealed the existence of a sub-population in which there was a positive and almost perfectly linear relation between dietary aflatoxin $B_1$ level and PHC death rate.

Several epidemiological studies have been published on the correspondence between aflatoxin exposure and liver cancer in Africa. In one of these reports, Bulatao-Jayme *et al* compared the mean dietary intake of 90 age-sex-matched controls. By using dietary recall, the frequency and amounts of food items consumed were calculated into units of aflatoxin load per day using a standardised Philippine table of the aflatoxin levels in these items. Of the subjects' total aflatoxin load, 51.2 percent came from cassava, 20.3 percent from corn, 6.8 percent from peanuts and 5.8 percent from sweet potato. The mean aflatoxin load per day for the liver cancer patients was found to be 440 percent that of the controls. Furthermore, dietary aflatoxin loads and alcohol intakes were subjectively allocated into "heavy" and "light" exposure groups; comparison of the numbers of patients versus controls in each group generated a measure of the relative risks of developing primary liver cancer from the ingestion of contaminated foods. The following foods were found to be statistically significant in the following order of rank: cassava > peanuts > sweet potato > corn > alcohol. Boiled rice, which had negligible aflatoxin content, gave no difference in risk.

Indirect human exposure to aflatoxins

Aflatoxins can also cause significant health problems in animals. The premise "until demonstrated otherwise, any animal carcinogen should be considered a human carcinogen, and that, as an unavoidable carcinogenic contaminant, aflatoxin should be controlled at the lowest practical level" has been applied to the regulation of aflatoxin in animal feeds and requires that levels in feeds result in toxicologically insignificant levels of aflatoxin and/or its metabolites in edible tissues and dairy products. Extensive studies on feed-to-tissue transfer rates have been conducted. Knowledge of these ratios played a major role in the FDA's decision to increase the allowable levels of aflatoxins in cottonseed meal. The highest feed-to-tissue transfer rates (ie, the ratio of the level of aflatoxin $B_1$ in the feed to the level of aflatoxin $B_1$ and its metabolites in the edible tissue) are 75:1, 800:1 and 2200:1 for milk (dairy cows), liver (swine) and eggs (poultry), respectively (Table 2). Aflatoxin $M_1$ is the primary metabolite found in milk; aflatoxicol has also been identified in milk. Aflatoxicol has been shown to be almost as carcinogenic as aflatoxin $B_1$ in the rat and in rainbow trout; however, the feed-to-tissue transfer rate is high (195,000:1). Thus, aflatoxin metabolites in

milk, particularly aflatoxin $M_1$, are of potential concern for human health. Because of the presence of aflatoxins and metabolites in foods and feeds and strong evidence of their association with human aflatoxicosis and carcinogenesis, aflatoxins are still a serious threat to human health even after almost 30 years of research.

**Table 2. Relationship between aflatoxin levels in feed and aflatoxin residue levels in edible tissues**

| Animal | Tissue | Aflatoxin | Feed: tissue ratio |
|---|---|---|---|
| Beef cattle | Liver | $B_1$ | 14,000 |
| Dairy cattle | Milk | $M_1$ | 75 |
| | | Aflatoxicol | 195,000 |
| Swine | Liver | $B_1$ | 800 |
| Layers | Egg | $B_1$ | 2,200 |
| Broilers | Liver | $B_1$ | 1,200 |

Occupational exposure to aflatoxins

The possible role of aflatoxins as an occupational risk factor has been considered in only a few studies. In a registry-based analysis of occupational risks for primary liver cancer in Sweden, significantly increased risk was observed in both male and female workers in grain mills; this finding was associated with potential exposure to aflatoxins. In a follow-up study of 71 Dutch oil-press workers exposed to aflatoxins, mortality from all cancers and from respiratory cancer was higher than expected. Although no cases of primary liver cancer were recorded, two of the 30 deaths were due to an unspecified liver disease. In addition, case reports have been published that provide circumstantial evidence for an association between human cancer and the inhalation of aflatoxin-contaminated dust.

A study of cancer risk among male employees at 241 livestock feed-processing facilities in Denmark was conducted utilising the data linkage system established at the Danish Cancer Registry for the detailed investigation of occupational cancer, providing employment histories back until 1964. Crops imported for feed production were often contaminated. Although aflatoxin concentrations were highly variable, the estimated average concentration of $\sim 140\mu g/kg$ aflatoxin $B_1$ in prepared mixed cattle feed yielded a daily intake for workers via the respiratory route of $\sim 170ng$. The calculated risk was established on the basis of the number of cancer cases among male workers whose employment with one of the companies was the single job that they had held for the longest time since 1964. A two- to three-fold increase in the risk for liver cancer and for cancers of the biliary tract was observed. Exposure to aflatoxins in the imported crops was judged to be the most probable explanation for these findings, although the influence of lifestyle factors (eg, alcohol consumption) on the results cannot be fully disregarded. Increased risks for salivary gland tumors and multiple myeloma were also detected.

## Rationale of Aflatoxin Control

Mode of contamination

Aflatoxins occur worldwide. *Aspergillus* species are capable of growing on a variety of substrates and under a variety of environmental conditions. Therefore, most foods and feeds

are susceptible to invasion by aflatoxigenic *Aspergillus* species at some stage during production, preharvesting, processing, transportation and storage. The presence of aflatoxigenic molds on a substance does not automatically result in the presence of aflatoxin. Conversely, the absence of aflatoxigenic molds does not guarantee the absence of aflatoxins, since the toxins may have diffused into the substrate. Aflatoxin formation in agricultural commodities is likely to occur in warm and moist weather conditions, and in faulty or inadequate storage facilities. The most important factors influencing growth of, and aflatoxin production by, *A flavus* and *A parasiticus* are the relative humidity surrounding the substrate, which in most cases is 88-95 percent, and a storage temperature of 25-30°C.

The life cycle of aflatoxigenic fungus consists of entry into the plant, seed infection, and finally aflatoxin formation in the seed (the inoculum may be from primary or secondary sources). The potential sources of primary inoculum in the form of spores of *A flavus* in the spring appear to be propagules in the soil, mycelium that overwinters in plant debris and litter on the soil, insects, or sclerotia of *A flavus* in the soil. The production of conidia on floral parts and leaves may be an important source of secondary inoculum, while insects as vectors can cause infestation of the plant.

Meteorological factors, potential insect vectors and the plant interact to allow for the formation of aflatoxins prior to harvest. Attempts to develop aflatoxin-resistant hybrid corn crops have been disappointing due to the difficulty of selecting for drought-stress resistance applicable to various different geographical locations. Biological control agents provide a promising approach to preventing the preharvest formation of aflatoxin. Certain bacteria, and a highly competitive, non-aflatoxin-producing strain of *A parasiticus* have been shown to replace or inhibit wild toxic strains in peanut test plots. Integrated pest management programmes, which through the use of pesticides and other agronomic procedures effectively control insect populations, have also been effective in reducing preharvest formation of aflatoxin. None of these programmes, however, completely eliminate the possibility of aflatoxin formation in selected field crops.

Risk assessment

Unquestionably, prevention is the best method for controlling mycotoxin contamination. Should contamination occur, however, the hazard associated with the toxin must be removed if the product is to be used for food or feed purposes. As described by Park and Stoloff, the ideal process is usually divided into four components: hazard evaluation, exposure determination, risk determination and management of the risk.

A hazard evaluation for a toxin provides a description of the expected toxic effect and of the dose-response relationship. Ideally, the test animal used should be the target animal of interest. The most important "target animals" from a public health agency's viewpoint are humans, but domestic animal welfare also comes within the regulatory jurisdiction of public health agencies. Since experimentation with humans is not a viable option, and unless the target animal is some species of livestock, laboratory animals must be used. It must be determined, usually be comparative toxicology, whether the test animal is an appropriate surrogate for the target animal, and a scaling factor must be established for translation of the dose-response data from test species to target species. When the toxic agent is a recently recognised component of the environment, the laboratory data must be examined in relation to epidemiological evidence of past exposure to the agent.

After the development of suitable analytical and sampling procedures, a determination must be made of the geographic and temporal distribution of the toxic agent in each major commodity subject to contamination. Combined with commodity consumption data, this information can lead to an estimate of exposure to the toxic agent by various human and animal populations. Method development and the surveys by which such data are accumulated are usually more costly in both time and resources than is generally acknowledged.

With dose-response, scaling and exposure data in hand, an estimate of risk to the exposed population can be made. For the newly recognised natural toxicant, regardless of the theoretical basis for calculation, the risk based on laboratory data must be compared with the observed risk from epidemiological data. When the two approaches produce divergent results, comparative toxicology observations may shed light on the reasons for the difference and lead to a regional conclusion.

Risk management decisions are usually made with an inadequate knowledge of all the foregoing factors. Decisions can be forced by societal or political pressures, economic necessity, or legal mandates, and can be molded by the same factors. The management plan can take the form of maximum tolerated levels that are legally enforced to limit human exposure to these toxins (Table 1), educational programmes leading to voluntary compliance by the industry, and research programmes leading to a better understanding of how and where the toxicant occurs, with prevention of the occurrence or removal of the toxicant as the ultimate goals. Risk analysis and management should be an evolving process as more and better knowledge is acquired.

Monitoring programmes

In addition too the establishment of regulatory limits, monitoring programmes to identify high-risk products and decontamination procedures to reduce the risks associated with aflatoxin contamination are integral parts of an aflatoxin control programme. For example, the Arizona Department of Health has had an extensive monitoring programme for aflatoxin in milk for almost two decades (Collier, R, Arizona Dairy Commissioner, pers. commun). However, during the year 1978, Arizona dumped almost 910,000lb (409,500kg) of milk with contamination levels as high as 10$\mu$g/kg aflatoxin $M_1$. As a result of this contamination event, the State instituted a comprehensive programme to monitor aflatoxin levels in whole cottonseed and cottonseed products, the primary source of aflatoxin contamination within the State. All cottonseed produced in the state is tested for aflatoxin content. The maximum size of each lot tested is 100t and the testing is conducted in state-certified laboratories. The end use of the product is dictated by the aflatoxin levels found. Cottonseed lots testing over 20$\mu$g/kg aflatoxin are usually treated with ammonia to reduce aflatoxin levels. Cottonseed products containing less than 20$\mu$g/kg aflatoxin, either with or without ammonia decontamination treatment, can be used for dairy rations.

Since the passage of statutes allowing the use of ammonia to reduce aflatoxin levels in the State of Arizona, the destruction of milk due to violative levels of aflatoxin $M_1$ ($>0.5\mu$g/kg) was ordered only once; it was later determined that the event had resulted from a feeding error, in which a pile of cottonseed that had been designated for ammonia treatment was fed before it could be treated. A summary of the results of testing for aflatoxin residues in Arizona milk show that from 1979 to 1982 and in 1989, no samples contained aflatoxin $M_1$ at a level above 0.5$\mu$g/kg; however, detectable levels of aflatoxin $M_1$ were found in 11-28 percent of the samples. The use of ammonia to treat cottonseed feed for lactating cows has

been a significant benefit, keeping Arizona's milk supply free from aflatoxin contamination. The key aspect of the aflatoxin decontamination programme is that the product is tested for aflatoxin levels both before and after the ammonia treatment, as appropriate.

Many organisations worldwide continually monitor aflatoxin-susceptible commodities for the incidence and levels of aflatoxin contamination. Many different commodities are susceptible to aflatoxin contamination, and include corn, corn-based products, peanuts, and peanut products which are important.

Significant aflatoxin contamination levels in corn and corn-based commodities have been reported in Latin America and the Caribbean. Mexico, Guatemala and Costa Rica have the three highest frequencies (87.9%, 54.0% and 50.0%, respectively); the range of aflatoxin levels was 0-1650$\mu$g/kg, with most of the samples containing $>20\mu$g/kg aflatoxin. Other reports from Canada, France, Senegal, Japan, the UK and Italy have shown that Senegal, Italy and France are the three countries with the highest frequencies (60%, 45% and 23% respectively). High incidence levels are evident in tropical regions worldwide. Relatively lower levels of aflatoxin contamination occur in temperate regions.

The results of a FAO/WHO/UNEP (Food and Agriculture Organisation/World Health Organisation/United Nations Environmental Programme) monitoring programme for the years 1976-1983 indicated that Kenya had the highest contamination level; 90 percent of the samples contained 30-1920$\mu$g/kg aflatoxin, compared to $<4\%$ in Canada. Again higher levels of aflatoxin were evident in tropical regions.

US FDA compliance data for milled corn products (meal, grits, hominy and hominy grits) obtained from retail outlets show that aflatoxin contamination levels in these products in the Southeastern states is significantly higher than in other states. From 1980 to 1983, 72%, 21%, 8% and 41% of the samples from the Southeast contained aflatoxins, whereas for other states no more than 19 percent of the total samples were contaminated. The average contamination levels in the Southeast were 27, 21, 34 and 16$\mu$g/kg, respectively for these years, much higher than that in other states ($<15\mu$g/kg). In 1983, 45 percent and 43 percent of samples of shelled corn from the Southeast and South contained aflatoxins. FDA compliance data for 1986 for shelled corn, milled corn products and manufactured corn-based products revealed that 32%, 28% and 47% of the samples, respectively, contained aflatoxins. Again this indicates that the Southern and Southeastern regions of the USA have the highest contamination levels.

Although aflatoxin contamination can occur at significant levels in the raw product, processing procedures such as screening and milling can reduce these levels. This is evident from the results of USDA aflatoxin tests of processed corn products for 1989-1991, which revealed negative screening results ($<20\mu$g/kg aflatoxins) for 14,013 analyses. However, it is not clear whether the levels are reduced to a similar extent by processing in lesser-developed countries.

Significant aflatoxin contamination levels in raw peanuts are evident in Latin America, Caribbean regions and West Africa. Canada, France and Japan, which import peanuts, show comparatively low levels of aflatoxins in finished products. Hydar *et al* reported aflatoxin $B_1$ and aflatoxin $B_2$ levels of $<3\mu$g/kg for raw and roasted peanut products from Syria. The results of 188 analyses for aflatoxin in edible peanuts imported into the UK during 1982-1984 show that 74 percent had aflatoxin $B_1$ levels of $<5\mu$g/kg (Ref 35). The incidence of measurable aflatoxins in consumer peanut products in the USA for the time periods 1980-

1984 and 1986 varied from year to year, but the overall incidence of products containing violative levels (total aflatoxins $>20\mu g/kg$) as low.

Other commodities, including figs, almonds, pecans, walnuts, raisins and spices are considered to have a lower risk of aflatoxin contamination. Soybeans, beans, cassava, grain sorghum, millet, wheat, oats, barley and rice are moderately susceptible to aflatoxin contamination in the field. However, it should be reiterated that resistance to aflatoxin contamination in the field does not guarantee that the commodities remain free of aflatoxin contamination during storage. Thus, the analysis of stored susceptible commodities should also be included in monitoring programmes.

# DECONTAMINATION METHODS FOR MYCOTOXINS
## (Approaches to Reduction of Aflatoxins and Feeds)

**by Timothy D Phillips, Beverly A Clement, and Douglas L Park**

Mycotoxins are fungal poisons that occur as frequent contaminants of grains and result in substantial losses to agriculture (Stoloff, 1976; Council for Agricultural Science and Technology [CAST], 1989). The most thoroughly studied and best understood of the mycotoxins are the aflatoxins. *Aspergillus flavus* and *Aspergillus parasiticus* (the fungi primarily responsible for the production of aflatoxins) are widespread and especially present a problem in corn and peanuts during periods of prolonged drought. Fungal growth on host plants in the field is favoured by warm ambient temperatures and plant stress; fungal growth on grain in storage is favoured by warm temperatures and high humidity (Diener *et al*, 1979). Four afltoxins are elaborated by these fungi as common "invisible poisons" in foods and feeds. Of the aflatoxins, aflatoxin $B_1$ has invoked the most concern since it is a potent animal carcinogen (Carnaghan, 1965; Dickens and Jones, 1965; Lewis *et al*, 1967; Wogan and Newberne, 1967; Lancaster, 1968; Wieder *et al*, 1968; Berry, 1988).

Every year a significant percentage of the world's grain and oilseed crops is contaminated with hazardous mycotoxins such as the aflatoxins. Unfortunately, discontinuing the feeding of aflatoxin-contaminated grain is not always practical, especially when alternate feedstuffs are not readily available or affordable. Thus, these poisons frequently are detected in animal feeds and result in substantial economic losses to the poultry and livestock industries (Nichols, 1987; Shane, 1991).

Also, the prevalence of aflatoxins in a variety of foods destined for human consumption is a major concern. For example, dairy animals can secrete a carcinogenic metabolite of aflatoxin $B_1$ (afltoxin $M_1$) in their milk after ingestion of aflatoxin-contaminated feed. Aflatoxins are heat stable and survive a variety of processing procedures. These compounds have been found as natural contaminants (sometimes at very high concentrations) in food crops and foods such as peanut butter and other peanut products, breakfast cereals, corn and cornmeal, dairy products, and other processed foods (Diener, 1981; Jelinek, 1987, CAST, 1989, Wood, 1989). More importantly, aflatoxin $B_1$ has been implicated as a factor in human liver cancer and classified as a probable human carcinogen (International Agency for Research on Cancer [IARC], 1987). In light of these important findings, clearly viable strategies to reduce the levels of aflatoxins in foods and feeds are critically needed. The reader is referred to several excellent reviews on the subject for details (Goldblatt and Dollear, 1977, 1979; Diener *et al*, 1979; Marth and Doyle, 1979; Anderson, 1983; Palmgren and Hayes, 1987; Park *et al*, 1988; CAST, 1989; Park and Rua, 1990; Cleveland and Bhatnagar, 1991; Darrah and Barry, 1991; Hagler, 1991; Phillips *et al*, 1991). Categories of aflatoxin reduction strategies include: (1) food and feed processing, (2) biocontrol and microbial inactivation, (3) structural degradation after chemical treatment, (4) dietary modification of toxicity, and (5) reductions in bioavailable aflatoxin by selective chemisorption.

## AFLATOXIN REDUCTION STRATEGIES

### Food and Feed Processing

Foods processed from peanuts, corn, and milk have received the most attention concerning the stability of aflatoxins; although corn and peanuts are probably the commodities of greatest worldwide concern since they are staple foods for many countries and usually are grown in climates that are favourable to fungal growth and aflatoxin production (CAST, 1989).

#### Thermal Inactivation

Aflatoxins are resistant to thermal inactivation and are not destroyed completely by boiling water, autoclaving, or a variety of food and feed processing procedures (Christensen *et al*, 1977). Aflatoxins may be destroyed partially by conventional processing procedures such as oil- and dry-roasting of peanuts to be used as salted nuts, in confections, or in peanut butter. Lee and co-workers (1969, reported a 45-83% reduction in aflatoxin that was dependent on roasting conditions and initial aflatoxin concentrations in raw peanuts. Comparable results were obtained in an investigation by Waltking (1971). In other studies, roasting conditions resulted in a significant decrease in the aflatoxin content of nuts, oilseed meals (Escher *et al*, 1973; Marth and Doyle, 1979), and corn (Conway *et al*, 1978). The degradation of aflatoxins was a direct function of temperature, heating interval, and moisture content (Mann *et al*, 1967).

Only partial destruction of aflatoxins in contaminated wheat occurs during the various stages of bread making (Jemmali and Lafont, 1972). Baking temperatures do not significantly alter the level of aflatoxin $M_1$ in freeze-dried milk (resulting from pasteurisation, sterilisation, preparation of evaporated milk, roller drying, and spray drying) was reported by Purchase *et al* (1972). Detoxification was confirmed by the duckling bioassay. Also, aflatoxins were not detected in cottage cheese (prepared from the contaminated milk), but were present in the whey. In other studies, aflatoxin $M_1$ was apparently stable in raw milk and was resistant to pasteurisation and processing (Stoloff *et al*, 1975; Stoloff, 1980).

Evidence suggests that aflatoxin may be "masked" chemically in alkaline-processed corn (ie, tortillas and corn chips) and, thus, may escape analytical detection. More importantly, reformation of parent aflatoxin may occur under the acidic conditions that are found in the stomach (Martinez, 1979; Price and Jorgensen, 1985; Bailey *et al*, 1990, 1991).

Specific criteria have been established for evaluating the acceptance of a given aflatoxin reduction or decontamination procedure. The process must: (1) inactivate, destroy, or remove the toxin; (2) not produce or leave toxic residues in food; (3) retain nutritive value and feed acceptability of the product; and, if possible, (5) destroy fungal spores (Park *et al*, 1988). Although some destruction aflatoxins in feed-grade meals has been achieved by conventional processing procedures, heat and moisture alone do not provide a very effective method of detoxification.

#### Irradiation

Studies have demonstrated that exposure of contaminated peanut oil to short-wave and long-wave UV light causes a marked reduction in the concentration of aflatoxins (Shantha and Sreenivasa, 1977). A 14-hr exposure to sunlight destroyed approximately 77-90 percent of aflatoxin $B_1$ that was added to groundnut flakes, whereas only 50 percent of the toxin was

destroyed in naturally contaminated product (Shantha *et al*, 1986). Gamma irradiation did not result in degradation of aflatoxin in contaminated peanut meal, and UV light produced no observable change in flourescence or toxicity of the treated sample (Feuell, 1966). Exposure of contaminated milk to UV light for 20 minutes at 25°C decreased the aflatoxin $M_1$ content by 89.1 percent in the presence of 0.05 percent peroxide, compared with 60.7 percent in peroxide-free milk (Yousef and Marth, 1986). Aflatoxins produce singlet oxygen after exposureto UV light and singlet oxygen further activates these chemicals to mutagens.

Solvent Extraction and Mechanical Separation

Several suitable solvent systems are capable of extracting aflatoxins from different commodities with minimal effects on protein content or nutritional quality (Rayner *et al*, 1977; Goldblatt and Dollear, 1979). These systems include 95 percent ethanol, 90 percent acetone, 80 percent isopropanol, hexane-ethanol, hexane-methanol, hexane-acetone-water, and hexane-ethanol-water. However, current extraction technology for the detoxification of aflatoxin-contaminated oilseed meals appears to be impractical and cost prohibitive (Shantha, 1987). Most of the aflatoxin associated with contaminated corn or peanuts can be found in a relatively small number oof kernels or seeds, providing an excellent opportunity to reduce the level of aflatoxin contamination by a varietyof separation approaches. For example, peanuts and Brazil nuts are sorted (electronically and manually) to reduce the levels of aflatoxins successfully, although complete removal of all contaminated particles is not achieved by these methods (Dickens and Whitaker, 1975; Natarajan *et al*, 1975).

Conventional methods used to clean corn (eg, dry cleaning, wet cleaning, density separation, and preferential fragmentation) are somewhat effective in reducing the aflatoxin content (Brekke *et al*, 1975a). Milling of corn resulted in better results. The distribution of aflatoxins was apparently low in grits and high in the germ, hull, or degermer fines of dry milled corn (Brekke *et al*, 1975b). Aflatoxin occurred mainly in the steepwater and fiber of wet milled corn, with smaller amounts present in the gluten and germ (Yahl *et al*, 1971).

Adsorption from Solution

Adsorbent materials including activated carbon (Decker, 1980) and clay and zeolitic minerals (Masimanco *et al*, 1973) have been shown to bind and remove aflatoxins from aqueous solutions such as water, Sorensen buffer, Czapek's medium, Pilsner beer, sorghum beer, whole milk, and skimmed milk. The adsorption of aflatoxins by clay (which is incorporated to remove pigments from crude oils) is a major factor resulting in the significant reduction of aflatoxins in refined peanut and corn oils. Reports indicate that a phyllosilicate clay (HSCAS or NovaSilTM) effectively removes aflatoxins from contaminated peanut oil and prevents its mutagenicity and toxicity *in vitro* (Machen *et al*, 1988, 1991). Ellis and co-workers (1990, 1991) reported that HSCAS, at levels as low as 0.5 percent (w/w), resulted in significant removal of aflatoxin $M_1$ from naturally contamination skim milk, with negligible change in nutritional quality (ie, total milk solids, lactose, and protein) as determined by proximate analysis.

**Biocontrol and Microbial Inactivation**

Established techniques for the management of aflatoxin contamination during crop production have not adequately insured aflatoxin-free commodities prior to harvest (Cole *et al*, 1989; Cotty, 1989). A new preharvest strategy for the prevention of aflatoxin contamination of peanuts and cottonseed is the use of nontoxigenic strains of *A parasiticus* and *A flavus* fungi

to compete with (and exclude) toxin-producing strains. Initial studies have shown that bioprevention can be used to reduce preharvest aflatoxin contamination in peanuts and cottonseed significantly. Additional studies are justified to address human health and environmental impact concerns of this technology (Cole and Cotty, 1990).

A promising new approach to the prevention (or elimation) of preharvest aflatoxin contamination is the molecular regulation of aflatoxin biosynthesis by toxigenic *Aspergillus* fungi. Enzymes that govern multiple steps in the biosynthetic pathway of aflatoxin have been purified (Bhatnagar *et al*, 1989; Bhatnagar and Cleveland, 1990), providing investigators with the key to characterising aflatoxin pathway genesand their regulation at the molecular level (Cleveland and Bhatnagar, 1991). A complete understanding of the molecular regulation of aflatoxin biosynthesis may lead to enhanced food safety through selection of crops that minimise expression of aflatoxin pathway genes (Cleveland *et al*, 1990; Cleveland and Bhatnagar, 1991).

Microorganisms (including yeasts, molds, and bacteria) have been screened foor their ability to modify or inactivate aflatoxins. *Flavobacterium aurantiacum* (NRRL B-184) was shown to remove aflatoxin from a liquid medium significantly without the production of toxic by-products (Ciegler *et al*, 1966). The same investigators also found that certain acid-producing molds could catalyse the hydration of aflatoxin $B_1$ to $B_{2a}$ (a less toxic product). The applications of microbial detoxification of aflatoxins have been reviewed (Ciegler, 1978; Marth and Doyle, 1979). Hao and co-workers (1987) reported that *F aurantiacum* removed aflatoxin $B_1$ from peanut milk. This bacterium grew in both defatted and partially defatted peanut milk and was not inhibited by the presence of aflatoxin.

Fermentation of contaminated grains has been shown to degrade aflatoxins (Dam *et al*, 1977), but ensiling aflatoxin-contaminated high-moisture corn was apparently not as effective. This difference may be attributed to the production of insufficient acid to catalyse the transformation of aflatoxin $B_1$ to aflatoxin $B_2a$ by this method (Lindenfelser and Ciegler, 1970).

**Structural Degradation Following Chemical Treatment**

A diverse group of chemicals has been tested for the ability to degrade and inactivate aflatoxins. These chemicals include numerous acids, bases, aldehydes, bisulfite, oxidising agents, and various gases (Feuell, 1966; Trager and Stoloff, 1967; Mann *et al*, 1970; Goldblatt and Doellar, 1977, 1979; Anderson, 1983; Park *et al*, 1988; CAST, 1989; Park and Lee, 1990; Hagler, 1991; Norred, 1990; Park and Rua, 1990; Samarageewa *et al*, 1991). A number of these chemicals can react to destroy (or degrade) aflatoxins effectively but most are impractical or potentially unsafe because of the formation of toxic residues or the perturbation of nutrient content, flavour, odour, colour, texture, or functional properties of the product. Two chemical approaches to the detoxification of aflatoxins that have received considerable attention are ammoniation and reaction with sodium bisulfite.

Ammoniation

Treatment of grain with ammonia appears to be a viable approach to the detoxification of aflatoxins. Ammoniation (under appropriate conditions) results in a significant reduction in the level of aflatoxins in contaminated peanut and cottonseed meals (Dollear *et al*, 1968; Masri *et al*, 1969; Gardner *et al*, 1971; Park *et al*, 1984) and corn (Brekke *et al*, 1977, 1979). The ammoniation process, using ammonium hydroxide or gaseous ammonia, has

been shown to reduce aflatoxin in corn, peanut-meal cakes, and whole cottonseed and cottonseed products by more than 99 percent. If the reaction is allowed to proceed sufficienty, the process is irreversible. Primarily, two procedures are used: a high pressure andd temperature process (HP/HT) at feed mills or an atmospheric pressure and ambient temperature procedure (AP/AT) on the farm.

The State of Arizona (USA) permits the use of ammonia to reduce aflatoxin levels in whole cottonseed and cottonseed meal and is studying the feasibility of using the process for aflatoxin-contaminated corn. Two processes have been approved: (1) HP/HT for whole cottonseed and cottonseed meal and (2) AP/AT for whole cottonseed. The HP/HT process involves the treatment of the contaminated product with anhydrous ammonia and water in a contained vessel. The amount of ammonia (0.5-2%), moisture (12-16%), pressure (35-50 psi), time (20-60 min), and temperature (80-120°C) varies with respect to the initial levels of aflatoxin in the product. The AP/AT process also uses a 13 percent ammonia solution, which is sprayed on the seed as it is packed into a plastic silage-type bag (approximately 10 feet diametre by 100 feet long). After this procedure, the bag is sealed and held at ambient temperature (25-45°C) for 14-42 days. The holding time will vary according too the ambient temperature, that is, a lower ambient temperature will require a longer holding time. The amount of ammonia (1-5%), moisture (12-16%), and time (14-42 days) will vary according to the initial levels of aflatoxin present. With this process, the bag is probed and tested periodically until test results show that aflatoxin levels are equal to or below 20 ppb.

The use of aqueous or gaseous ammonia, as well as other chemical treatments for the detoxinfication of aflatoxins, has been reviewed thoroughly (Goldblatt and Dollear, 1979; Anderson, 1983; Palmgren and Hayes, 1987; Park *et al*, 1988; Park and Lee, 1990). Because of the severity of aflatoxin contamination in selected agricultural commodities from various locales, specific decontamination procedures have been approved and put into use. In the United States, Arizona, Texas, and California permit the ammoniation of cottonseed products and Texas, North Carolina, Georgia, and Alabama have approved ammoniation for corn. Peanut meal is a frequent component of animal feeds in Europe and elsewhere. Consequently, ammoniation is used routinely in France, South Africa, Senegal, and Brazil and soon will be utilised in the Sudan and India to lower aflatoxin contamination levels. Several member countries of the European Economic Community import ammonia-treated peanut meal on a regular basis (Park and Lee, 1990).

Several studies indicate that peroxide and heat enhance the destruction of aflatoxin $B_1$ by sodium bisulfite added to dried figs (Altug, *et al*, 1990). Clearly more research is warranted in this area to determine the potential of bisulfites to reduce or inactivate aflatoxins in food and feed (Hagler, 1991).

**Reduction in Bioavailable Aflatoxin by Selective Chemisorption**

A phyllosilicate clay (HSCAS or NovaSilTM) currently available as an anticaking agent for animal feeds has been reported too (1) tightly bind aflatoxins in aqueous suspensions; (2) markedly diminish aflatoxin uptake by the blood and distribution to target organs; (3) prevent aflatoxins in farm animals, including chickens, turkey poults, goats, pigs, and mink; and (4) decrease the level of aflatoxin $M_1$ residues in milk from lactating dairy cattle (Davidson *et al*, 1987; Kubena *et al*, 1987, 1988, 1989, 1990a,b,c,d, 1991a,b; Phillips *et al*, 1987, 1988a,b, 1989, 1990a,b, 1991, 1992; Harvey *et al*, 1988a,b, 1990, 1991a,b,c; Colvin *et al*, 1989; Beaver *et al*, 1990; Chung *et al*, 1990; Bonna *et al*, 1991). In other studies, Ellis *et al* (1990, 1991) reported that HSCAS effectively removed aflatoxin $M_1$ from contaminated

skim milk.

Several test compounds with one or more of the functional groups in common with aflatoxin were reacted with HSCAS *in vitro* in an attempt to elucidate the specificity and mechanism of tight binding (or chemisorption). Test compounds that were homologous to $AFB_1$ and $AFG_1$ (ie, $AFB_2$, $AFG_2$, $AFB_{2a}$, $AFG_{2a}$, $AFQ_1$, $AFP_1$, $AFM_1$, $AFM_2$, and $AFM_{2a}$) were bound tightly to HSCAS, whereas those that contained a dihydrofurofuran fused to an aromatic ring did not show any significant interaction and binding. Coumarin, 4-methyl umbelliferone, esculetin, xanthotoxin, aflatoxicol, and tetrahydrodeoxyaflatoxin $B_1$ were bound initially but were desorbed significantly from the HSCAS pellet. These findings suggest that the ß-dicarbonyl system contained in the aflatoxin B and G series is essential for chemisorption by HSCAS.

## CONCLUSIONS

Although good crop management and surveillance programmes are essential for the control of aflatoxin contamination, they do not resolve the mycotoxin dilemma. In other words, aflatoxin levels can be lessened in food and feed by these strategies, but not eliminated completely. Since aflatoxins are produced from ubiquitous fungi as unavoidable adulterants of food and feed and are very potent carcinogens, they are regulated explicitly in the United States. Therefore, viable strategies for the effective remediation of aflatoxins are critically needed.

Several potentially feasible strategies for the reduction or inactivation of aflatoxins have been reported in the scientific literature. Some of these methods are clearly more effective and practical than others, but most result in a degree of reduction in levels of parent aflatoxins or in a modification of the toxicity associated with these poisons. These approaches include (1) food and feed processing (eg, thermal inactivation, irradiation, solvent extraction and mechanical separation, density segregation, and adsorption from solution), (2) biocontrol and mocrobial inactivation, (3) structurall degradation following chemical treatment (eg, ammoniation, bisulfite, catalytic adsorbents), (4) dietary modification of toxicity, and (5) reduction in bioavailable aflatoxin by selective chemisorption.

The method that has received the most attention is the treatment of aflatoxin-contaminated feeds (primarily cottonseed, corn, and peanut products) with ammonia (ie, ammoniation). The ammoniation process has been used successfully for many years in the United States, France, and Africa but has not yet been sanctioned by the US Food and Drug Administration. However, the use of ammonia to cottonseed for dairy cows has been a significant factor in keeping Arizona's milk supply free from major aflatoxin contamination. Current research supports the use of ammoniation in an effort to reduce markedly the risk posed by aflatoxin contamination of grains and oilseeds.

Presently, no "silver bullet" exists that can be used to eliminate the aflatoxin problem. Newer strategies for the prevention of aflatoxins in grains and the modification of toxicity (and carcinogenicity) in animals show considerable promise and innovation, but more research is warranted to establish their individual efficacy and safety. Perhaps in the future, a combination of various reduction strategies will lead to the elimination of aflatoxins in foods and feeds or to the prevention of aflatoxicoses.

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## INTRODUCTION TO TLC METHODS

**by Lawrence Castle**

This lecture followed closely the introduction to thin layer chromatography (TLC) techniques given in the course manual[2]. Discussion focussed on the general principles of chromatography and drew specific examples from, mainly, aflatoxin analysis.

The physio-chemical basis of chromatography separation mechanisms was discussed with emphasis on absorption chromatography. Use of silica stationary phases as thin layers on glass, aluminium or plastic plates is of greatest utility for aflatoxin analysis and one- and two-dimensional techniques were reviewed. Precautions needed to obtain reliable results were outlined. Attention was drawn to the rather special facility in TLC analysis to perform reactions with the components on the plate. Thus, in its simplest form, spray reagents or chemical vapours can be used to facilitate visualisation or other detection means for components. Reaction followed by TLC analysis for specific and characteristic products was another facility. Here, reaction *in Situ*(?) on the plate can either be performed on the mixture before solvent development or can be performed between the first and second development in a two-dimensional analysis. Finally, the ten basic steps in chromatography were presented to summarise:

1. Define problem
2. Select absorbant
3. Select format
4. Select developing solvent(s)
5. Apply sample(s) and standard(s)
6. Develop
7. Visualise
8. Detect
9. Estimate (calculate)
10. Confirm

[2] Food and Nutrition Paper 14. Manuals of Food Quality Control 10. Training in Mycotoxin Analysis. FAO (1990) pp 83-88

## ELISA AND OTHER MYCOTOXIN DETERMINATION METHODS

**by David M Wilson**

Detection methods for mycotoxins include chemical methods such as minicolumns, TLC, HPLC and GC as well as the immunochemical methods utilizing antibodies prepared to recognise individual mycotoxins. This portion of the training workshop emphasised the immunochemical methods. ELISA (Enzyme Linked Immunosorbent Assay) methods were described as well as immunochemical affinity columns. The mycotoxins have to be chemically bound to protein in order to produce the antibodies. The antibodies recognise any particular mycotoxin and because of this, methods for their detection have been developed.

The use of ELISA was discussed in relation to the methods for mycotoxin determination and the mechanisms involved in the analysis was described. The participants had an opportunity to work with four different immunochemical methods in the laboratory. Three of the commercial kits worked properly for all students and one ELISA kit was not functional. This failure illustrated the difficulty with using immunochemical products in the developing countries. The use of the simple chemical tests was discussed in the lecture but no laboratory exercises were attempted. The students were encouraged to read the literature in the workshop manual in selecting methods for their own application. The important issues addressed were the theory and application of analytical methods for mycotoxins.

# A DISCUSSION SUMMARY FOR THE TWO WORKSHOPS ON DETECTION AND SURVEILLANCE OF MYCOTOXINS AND GRAIN STORAGE PROBLEMS AND UTILISATION

**Rapporteur - R Tanyongana**

The discussion was held on the last day of the workshops with participants and resource persons from both groups together. The objective was to summarise the activities of the week and get the participants' impressions and benefits gained from the workshops.

## MYCOTOXIN WORKSHOP SUMMARY BY PROFESSOR PARK

The main areas that were covered during the week were:

(a) Incidence and significance of mycotoxin with the purpose of creating an awareness among participants

(b) The importance of sampling techniques as a starting point for any surveillance programme was illustrated

(c) The rationale for control and regulatory programmes for mycotoxins was discussed as the major strategy for minimising exposure to mycotoxins

(d) The decontamination methods that can be used were discussed. These included physical, biological and chemical methods. The limitations that are associated with each method with reference to the region were highlighted

During the laboratory sessions (practicals) participants were trained to use the several methods of assessing the present of mycotoxins both qualitatively and quantitatively. These included TLC methods and ELISA methods.

It was, however, stressed that it is not possible to eliminate risk, but control is possible. There is need for simple equipment that can be used to screen commodities received for segregation purposes. There was a void in information and a lot of unknown areas. Therefore, it is important when dealing with mycotoxins in laboratories to minimise exposure to the laboratory staff.

For regulatory programmes, participants were advised that it was not practically possible to enforce a zero level as this was just as good as banning importation of produce. Therefore maximum tolerated or acceptance levels should be set to conform with the conditions or circumstances prevalent in each country.

## GRAIN STORAGE PROBLEMS WORKSHOP SUMMARY BY DR CHITSIKA

The areas that were discussed included:

(a) Nutritive aspects of cereals, their composition and technological processes that these commodities are subjected to during food processing. The effects of these processes on the nutritional quality.

(b) Grain quality, handling and grading control procedures. It was highlighted that in the region deliveries start in April and peak in July and August ending towards the end of November. All activities of grain handling were therefore concentrated in this time period.

The importance of meeting the requirement of the end user during the grading process was discussed with the ultimate goal of setting up an acceptance standard for the region. It was also evident that the main staple food in the region is white maize with yellow dominating the export market.

(c) Simple identification techniques for insects and the damage they cause. Methods of inspection and sampling for insect infestation detection were emphasised. The management and control of infestation and the methods used in both silos and bag stacks were discussed. The different pesticides used and their dosages were highlighted.

(d) The biological and physical changes during storage were shown to have a very significant impact on the quality of despatched commodities after months of storage. The storage structures found in the region and other parts of the world were discussed highlighting the advantages and disadvantages of each type and how they should be maintained.

During the laboratory sessions participants carried out microscopic identification of different species of grain spoilage insects highlighting the features that they used to reach their conclusion. Participants also learnt the ELISA technique for mycotoxin detection.

## COUNTRY REPORTS

### BOTSWANA AGRICULTURAL MARKETING BOARD

These are the major recipients of cereals, sorghum and maize both from local farmers and from imports. Wheat is imported and goes straight to the millers. Average local cereals production annually ranges between 30,000-60,000 tonnes. Therefore, there is a lot of importation. Storage is in warehouses and silos. Problems are with weevil and rodent infestation. Cost of treatment is very high.

The storagability of imported maize is very poor and by the time it is distributed it is not palatable. There is a lack of knowledge and expertise to inspect and test the grain when it is received. Pest control is done using Aluminium Phosphide and sometimes Methyl Bromide.

### UNIVERSITY OF BOTSWANA STAFF: DR H K TALIGOOLA AND DR S MPUCHANE

The department carried out a preliminary survey to discover the incidence of toxigenic fungi and levels of mycotoxins in sorghum and maize. The results showed higher levels of mycotoxins in maize than in sorghum. These mycotoxin levels were higher in maize samples from the north of the country than in the south. All maize and sorghum samples were contamined by fungi though to varying degrees. Samples of imported grain from Australia and neighbouring countries also showed presence of mycotoxins. A sample of yellow maize

from China initially had very few fungi on it and mycotoxin levels were also low, but after some time in storage it is now showing a high degree of fungal contamination. Dr Mpuchane said hopefully a monitoring surveillance programme will be set up with the help of the Ministry of Agriculture. A mycotoxin laboratory is to be set up to analyse all the samples that will be collected from the programme. This is the beginning of a monitoring programme that will be maintained to ascertain the levels of mycotoxins in Botswana.

**DR OMO - FOOD TECHNOLOGY RESEARCH SERVICE**

She reiterated the impact the workshop had had and indicated reasons why there was so much concern about mycotoxins. However, pointed out that it is also important to know what symptoms to look for in mycotoxin affected animals. In addition what other methods can be used to validate figures obtained using screening methods.

It was suggested that there should be more communication and co-ordination of all mycotoxin activities so that all institutions with an interest are aware of what is going on.

**LESOTHO**

The participant was from the Ministry of Agriculture's Nutrition Department. Main produce is maize, sorghum and beans. Lesotho having limited resources has no aflatoxin labs to monitor. Food poisoning has been reported in institutions like boarding schools due to *Salmonella* infections. Extension work to improve family hygiene and proper handling or grain is going on. Bottling of fresh vegetables has been discouraged due to contamination. With the experience obtained from the workshop, the participant hopes to encourage strengthening of local research to include mycotoxins.

**NAMIBIA**

All samples for mycotoxins analysis in Namibia were previously sent to South Africa. With Independence there was need to establish their own analytical service. The objectives of the participants was to learn and then to go back and set up their own lab. From experience they hope to set up one in next few months. The laboratories are expected to start functioning in February 1994.

**SWAZILAND**

The mycotoxin laboratory was established in 1980. Mycotoxin analysis is carried out on maize and beans. Emphasis is on prevention through the extension service. There has been a low incidence of mycotoxins. However, recently a farmer lost some pigs and when his feed was analysed there were no aflatoxins. During the workshop it had become apparent that probably this feed had fumonisins rather than aflatoxins.

**ZAMBIA**

Have well established laboratories and have been monitoring mycotoxins on both local produce and imported maize. The imported maize had some high levels of mycotoxins and sampling on the ships that brought grain was difficult as equipment was limited. Ongoing programmes are in place to maintain awareness and prevention of mycotoxin with the help of extension services.

## ZIMBABWE

The country has several laboratories that do analyse in the private sector, parastatals, the University and Government. The participant came from Government Analyst Laboratories. Analyses were carried out on food and sample consumer products, etc. Both HPLC and TLC method are used. Zimbabwe's maximum limits is 20ppb for food intended for human consumption.

The participant had gained a lot by learning sampling methods and intends using the techniques learnt. He had also learnt that other mycotoxins other than aflatoxins were very important and intends to set up analysis of these. There was also need to increase range of commodities monitored.

Co-ordination of activities by networking was also encouraged for the country's laboratories.

## TANZANIA

Although there is no national programme on mycotoxin surveillance and control, most aspects of prevention are covered under Food Security Programme. In the mid '80s a survey on aflatoxin contamination on cereals in Dodoma region was conducted. The findings showed that aflatoxin contamination is a problem in that region. Since then, there is not much which has been done. However, tests for aflatoxins are carried out in three laboratories, at Tanzania Bureau of Standards, Government Chemical Laboratory and Tanzania Food and Nutrition Centre, not on a regular basis, but on request by customers who intend to look for the market of their products abroad. Therefore, there are three laboratories which can support surveillance programmes on aflatoxins if the programme is initiated. There are no regulations on mycotoxins which have been established.

## MALAWI

Though I am working for a Local Authority, under the Ministry of Local Government whereby my services reach one eighth of the population, Malawi as a nation, has a programme of detection and prevention of the presence of mycotoxins in foods and feeds.

There was a Workshop on Control and Surveillance of Mycotoxins in Foods and Feeds which drew Technologists from Ministry of Health, Ministry of Agriculture, Malawi Bureau of Standards and the Government Laboratory whereby the maximum accepted aflatoxin levels standard in Malawi in foods and feeds was set to be 5ppm.

Zomba Municipal Council has a programme on detection and prevention of the presence of pathogenic microbes in foods but nothing had been done on mycotoxins. Given equipment, I would like to use my experience from the workshop to start work on mycotoxin analysis in the municipality.

## GENERAL DISCUSSIONS

Each country was advised to set up strategic reserves of food for drought purposes and food security. Policy makers and operational staff should agree on reasonable and manageable quantities.

Communication and co-ordination of activities was seen as one of the achievements of the workshop and this was encouraged to continue so that a networking phenomenon could emerge.

Participants were encouraged to send their subordinates and colleagues to courses and workshops of this nature to raise awareness and levels of training in the countries. Selfishness was strongly discouraged.

Participants were encouraged to start off small with simple equipment and move onto more sophisticated equipment when the work load and standard results required is higher. FAO was mentioned as a source where participants can get help in setting up their laboratories and programmes.

## APPENDIX I

Full Addresses of Resource Persons

DR J M CHITSIKA
Chibuku Breweries
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DR L MAROVATSANGA
Department of Biological Sciences
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Harare
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Fax : 263-4-303292

DR F S MPUCHANE
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Gaborone
BOTSWANA
Fax : 267-356591

MR H NJAPAU
Mycotoxin Laboratory
National Council for Scientific Research
Private Bag 350049
Chilanga
ZAMBIA
Tel (work) : 260-01-278328

PROFESSOR DOUGLAS L PARK
Department of Nutritional Sciences
The University of Arizona
Tucson
Arizona 85721
USA
Fax : (602) 621-9446

MR E W SYDENHAM
Programme on Mycotoxins and Experimental Carcinogenesis (PROMEC)
Medical Research Council
P O Box 19070
Tygerberg 7505
SOUTH AFRICA
Fax : (021) 938-0260

DR H K TALIGOOLA
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University of Botswana
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Fax : 267-356591

MISS R TANYOGANA
Grain Marketing Board
P O Box 8014 Causeway
Harare
ZIMBABWE
Fax : 263-4-732038

DR J H VILJOEN
Maize Board
P O Box 669
Pretoria 0001
SOUTH AFRICA
Fax : (12) 324-3018/21-5570

PROFESSOR D M WILSON
College of Agriculture
The University of Georgia
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Tifton
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USA
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DR I M ZINZOMBE
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Fax : 267-356591

## APPENDIX II

## FINANCIAL STATEMENT

### 1a) Transport (Resource People)

| | | |
|---|---|---|
| 1 | Prof Park | P13,449-00 |
| 2 | Prof Wilson | P 9,844-00 |
| 3 | Dr Castle (British Council) | P---------- |
| 4 | Mr Sydenham | P 1,047-00 |
| 5 | Dr Chitsika | P 584-00 |
| 6 | Ms Tanyongana | P 584-00 |
| 7 | Mr Njapau | P 1,059-00 |
| 8 | Dr Viljoen | P 452-00 |
| 9 | Dr Marovatsanga | P 1,056-00 |
| | | P28,075-00 |

### 1b) Transport (Participants)

| | | |
|---|---|---|
| 1 | Dlamini | P 698-00 |
| 2 | Nsibande | P 698-00 |
| 3 | Jumbe | P 1,233-00 |
| 4 | Böckmann | P 938-00 |
| 5 | Musingarabwi | P 584-00 |
| 6 | Tsiame | P 777-00 |
| 7 | Urio | P 1,372-00 |
| 8 | Muzungaile | P 1,059-00 |
| | | P 7,360-00 |
| | | P35,435-00 |

### 2 Accommodation and Food

a) Resource People

| | | |
|---|---|---|
| 1 | Prof Park | P 1,574-00 |
| 2 | Prof Wilson | P 1,189-67 |
| 3 | Dr Castle | P 982-00 |
| 4 | Mr Sydenham | P 722-00 |
| 5 | Dr Chitsika | P 1,199-25 |
| 6 | Ms Tanyongana | P 1,215-90 |
| 7 | Mr Njapau | P 1,146-80 |
| 8 | Dr Viljoen | P 873-80 |
| 9 | Dr Marovatsanga | P --------* |
| | | P 8,904-75 |

* Accommodated by Dr Mpuchane

b) Participants (Student Hostels)

| | | |
|---|---|---|
| 1 | Dlamini | P 145-27+ |
| 2 | Nsibande | P 145-27 |
| 3 | Jumbe | P |
| 4 | Böckmann | P |
| 5 | Musingarabwi | P |
| 6 | Tsiame | P |
| 7 | Urio | P |
| 8 | Muzungaile | P 221-05+ |
| | | P 1,671-55 |

**3 Expenses (airport tax, en route expenses, airfare refunds)**

a) Foreign Resource People

| | | |
|---|---|---|
| 1 | Prof Park | P 904-05 |
| 2 | Prof Wilson | P 1,845-00 |
| 3 | Dr Castle | P ---------- |
| 4 | Mr Sydenham | P ---------- |
| 5 | Dr Chitsika | P 7-70 |
| 6 | Ms Tanyongana | P 7-70 |
| 7 | Mr Njapau | P ---------- |
| 8 | Dr Viljoen | P ---------- |
| 9 | Dr Marovatsanga | P 1,063-70 |
| | | P 2,928-15 |

b) Participants (including Airport Tax)

| | | |
|---|---|---|
| 1 | Dlamini | P --------- |
| 2 | Nsibande | P 160-00 |
| 3 | Jumbe | P 180-00 |
| 4 | Böckmann | P 150-00 |
| 5 | Musingarabwi | P 160-00 |
| 6 | Tsiame | P 170-00 |
| 7 | Urio (+ en route expenses) | P 450-00 |
| 8 | Muzungaile | P 170-00 |
| Partly sponsored Chakona (accommodation and food) | | P 150-00 |
| | | P 1,590-00 |

**4 Catering**

| | |
|---|---|
| Lunch for all participants & resource persons | P 3,900-00 |
| Breakfast for participants in Halls | P 949-00 |
| Evening meal | P 1,417-00 |
| Catering Supervisor (Ms Mokgethi) | P 400-00 |
| Tea | P 900-00 |
| | P 7,566-50 |

## 5 Reception

| | |
|---|---|
| Invitation cards and certificates | P 1,350-00 |
| Drinks | P 1,612-38 |
| Catering expenses | P 253-90 |
| Food | P 1,050-00 |
| Flowers | P 120-00 |
| | P 4,386-28 |

## 6 Stationery

| | |
|---|---|
| Brochures I & II | P 1,960-00 |
| Conference Bags | P 2,290-00 |
| Overhead paper/pens | P 200-00 |
| Stationery | P 643-85 |
| Stores | P 282-11 |
| Conference Badges | P 26-00 |
| | P 5,401-96 |

## 7 Honoraria

| | | |
|---|---|---|
| Resource People | - Foreign | P13,500-00 |
| | - Local | P 8,500-00 |
| | | P22,000-00 |

## 8 Reagents and Consumables

| | |
|---|---|
| Petri Dishes | P 1,300-00 |
| Cite Probe Kits | P 3,230-80 |
| Aflatest Kit (Rhone Poulenc) | P 2,461-60 |
| Neogen (1) | P 7,288-75 |
| Neogen (2) | P 845-25 |
| Plastic Gloves | P 22-50 |
| Pippettes and Tips | P 6,846-15 |
| Aflatoxin Kits (Weil) | P 5,940-00 |
| Culture Media | P 3,426-00 |
| | P31,361-05 |

## 9 Local Transport

| | |
|---|---|
| | P 350-00 |
| | P 1,000-00 |

## 10 Expenses (Local)

| | |
|---|---|
| Dr Zinzombe (food for participants, Day 1) | P 122-12 |

## 11 Photographs and Slides

P 604-50

| | | |
|---|---|---|
| **12** | **Secretarial (during Workshop)** | |
| | | P 114-00 |
| | | P 350-00 |
| | | P 404-00 |
| **13** | **Delivery (Freight charges)** | P 4,288-73 |
| **14** | **Equipment** | |
| | ELISA Reader (for Mycotoxin Kits) [UNICEF] | P25,753-46 |
| **15** | **Photocopying** | P 3,000-00 |
| **16** | **Proceedings** | |
| | Production | P 3,000-00 |
| | Typing | P 1,102-50 |
| | 30 Copies | P 954-00 |
| | Binding | P 500-00 |
| | | 5,556-50 |
| **TOTAL** | | P162,322-05 @ 2.59 |
| | | $ 62,672-61 |

**INCOME**

| | |
|---|---|
| UNIVERSITY OF BOTSWANA | (Facilities) |
| USAID | $ 49,589-60 |
| UNICEF (P25,000) | $ 9,652-51 |
| BOTSWANA GOVERNMENT (P5,000) | $ 1,930-50 |
| TWAS (Pledged) | $ 1,500-00 |
| | $ 62,672-61 |