

**Maize Trust Project progress report:
Situational analysis of selected maize mills in order to track the
fungal mycotoxins and their distribution during processing**

by

GJ Marais, P Fakude, and C Erasmus

Biosciences
Agroprocessing and Chemical Technologies Group

CSIR/BIO/ACT/ER/2008/0068/B.....
GW DMS Pta no: 168783.....

Maize Trust Project progress report:
Situational analysis of selected maize mills in order to track the fungal
mycotoxins and their distribution during processing

by

GJ Marais, P Fakude, and C Erasmus

September 2008

Authorised by: _____ Date: _____

Client: Mr L du Plessis
The Maize Trust; c/o L & L Agricultural Services
PO Box 12203
Queenswood, 0121
(Tel) 012-333-3634
(Fax) 012-333-3634
l-lagric@mweb.co.za

CSIR contact: GJ Marais / C Erasmus
FABI University of Pretoria / CSIR Biosciences
012 420 5806 / 012 841 2163
gert.marais@up.ac.za / cerasmus@csir.co.za

1. SUMMARY

This report summarises the progress on this project for the past 18 months. In total six mills were included in the project where each mill was sampled at 3 different occasions. Sampling included all handling stages in the milling process, as well as the various fractions. Moisture contents and fungal enumeration were done on all samples and the statistical calculations are currently done to determine meaningful fluctuations in the levels of fungi and their mycotoxins in the milling system. It is envisaged that a final report will be presented to the Maize Trust by the end of 2008 as anticipated at the start of the project. It is expected that the project will have gathered information of each mill that will indicate the contribution of each milling system to the levels of fungi and their mycotoxins, but also indicate the overall role of milling systems in South Africa.

2. INTRODUCTION

The presence of fungi and their mycotoxins are regularly experienced and can influence the shelf life of food products such as maize. The formation of mycotoxins is not necessarily activated under the same environmental conditions where fungal growth occurs. However, the mycotoxins are only present when sufficient fungal development has taken place. The occurrence of mycotoxin-producing organisms does not indicate that any mycotoxins are present, but it can indicate a high risk scenario for the presence of mycotoxins.

There is a lack of information regarding the presence and migration of fungi and their mycotoxins in the milling process. This is especially true for milling systems in South Africa. There is also limited information on the levels of fungi in the products destined for human and animal consumption.

The occurrence of fungi and their mycotoxins is becoming more relevant for the South African maize milling industry. As more subsistence farmers are contributing to the production of maize, so are the types of practices changing in the cultivation, harvesting, transport, storage, and processing of maize. All these factors can influence the populations of fungi and their mycotoxins.

3. OBJECTIVES OF THE STUDY

The proposed project aims to achieve the following:

- Find evidence of the more important fungi and their mycotoxins in the post harvest processing phases of maize in South Africa
- Provide the industry with crucial data that can serve as a tool for proper risk management options in the milling industry

It is envisaged that the industry will be able to identify the high risk areas in the processing of maize kernels and through corrective action lower these risks. The data obtained in this project will indicate the development of mycotoxigenic fungi from the silo through to the end products in six mills in South Africa. Each mill will be able to compare itself with the tendencies in other maize mills.

4. SUMMARY OF PROJECT PROGRESS

The project consists of the following tasks:

- Identification of sampling procedures and statistical design
- Sampling
- Moisture content analyses
- Fungal enumeration and mycotoxin analyses
- Statistical manipulation

4.1 Identification of sampling procedures, statistical design and actual sampling

4.1.1 Background / introduction

Sampling plays a crucial part in the precision of the determination of the levels of mycotoxins in foodstuffs. The sampling method for cereals and cereal products adopted was that of the European Union (Commission Regulation (EC) No 401/2006 of 23 February 2006) as South Africa's Department of Agriculture does not have its own method.

4.1.2 Methods and materials

All six mills were visited and prior to sampling, critical sampling points were identified. The processing capacity of each mill was taken into consideration and the quantities of samples were calculated accordingly. To obtain a representative aggregate sample, a kilogram of incremental samples were taken for every ton per hour of processed maize. Sampling points were at the following unit operations; silo, screened/ cleaned maize kernels, conditioning stages, degermer (thrus and overs), first and last roller mills, and all final products produced. At each sampling point, three to eight incremental samples at 10 to 15 minutes intervals were taken. Samples were taken in labelled sealable plastic bags. To obtain representative aggregate samples, the incremental samples per unit operation were thoroughly mixed. Approximately 500 g of each aggregate sample was repackaged into plastic bags and sent for fungal enumeration and mycotoxin analyses. A fraction of each aggregate sample was placed in a jam jar which was sealed with insulation tape to trap the moisture inside the jar. Prior to moisture content analysis, the samples were stored at 4°C.

4.1.3 Observations

The use of sealable bags made sampling easier. Overall, it was difficult to obtain the accurate quantity of a sample as there were no weighing balances to use. Therefore sample quantities were either 1 to 2% less or more than the anticipated quantity.

4.1.4 Discussion and conclusions

Mixing of the incremental samples to obtain an aggregate sample resulted in the loss of sample moisture; by opening the sealed plastic bags, the moisture was released to the surrounding air.

4.2 Moisture content analyses

4.2.1 Methods and materials

Moisture content of samples was determined following the AACC method 44-15A (AACC, 1995). Whole maize kernels, overs and large maize grits were analysed using the two stage method and the remaining of the maize samples were analysed using the one stage method. Analyses were done in triplicates. One stage method was used for powdery samples that supposedly contained less than 13% moisture content. The two-stage method was used for whole maize kernels and large maize grits that contained 13% or more moisture. The latter involves the grinding of samples to increase surface area for better analyses of bound moisture. Mill 1, 3 and 6 add chlorine to the

first conditioning step, while mill 1 2, 4 and 5 do not add chlorine. Chlorinated water is used to decrease the microbial load conditioned maize (Dhillon et al. (2007). The conditioning step is the most important unit operation of the dry milling process, as it aids in toughening of the bran for easy removal and also softens the endosperm to make it easier to grind (Hoseney, 1994).

4.2.2 Results

Tables 1 to 6 summarise the moisture content levels determined for all the visited mills at the various sampling points.

Table 1: Moisture content results of maize and maize products sampled over a period of ten months at **MILL 1**; note the product names have been omitted for confidentiality reasons

Sample	1 st visit (% m/v)	2 nd visit (% m/v)	3 rd visit (% m/v)
Maize from silo *	11.2 ± 1.1	14.7 ± 0.1	11.7 ± 0.2
Cleaned maize *	11.9 ± 0.5	12.4 ± 0.0	12.6 ± 0.2
1 st conditioning *	12.2 ± 0.2	13.1 ± 0.0	12.9 ± 0.0
2 nd conditioning *	13.3 ± 0.4	14.8 ± 0.0	13.7 ± 0.2
Degermers – overs *	13.6 ± 0.5	14.6 ± 0.1	13.2 ± 0.0
Degermers – thrus #	13.2 ± 0.5	14.7 ± 0.0	12.4 ± 0.2
First brake #	11.4 ± 0.2	11.2 ± 0.4	11.4 ± 0.3
Last brake #	12.0 ± 0.0	12.0 ± 0.6	12.7 ± 0.2
Final product 1 #	12.3 ± 0.3	12.3 ± 0.4	12.5 ± 0.9
Final product 2#	11.6 ± 0.2	11.2 ± 0.7	12.5 ± 0.2
Final product 3 #	11.5 ± 0.7	12.5 ± 0.4	11.9 ± 0.2
Final product 4#	12.9 ± 0.2	12.4 ± 0.2	12.3 ± 0.0
Final product 5 #	13.2 ± 0.5	15.0 ± 0.3	12.4 ± 0.3

Moisture analyses were done in triplicate

applied one stage oven method

* applied two stage oven method

Table 2: Moisture content results of maize and maize products sampled over a period of ten months at **MILL 2**; note the product names have been omitted for confidentiality reasons

Sample	1 st visit (% m/v)	2 nd visit (% m/v)	3 rd visit (% m/v)
Maize from silo *	11.6 ± 0.3	12.0 ± 0.0	13.2 ± 0.6
Cleaned maize *	12.1 ± 1.1	13.1 ± 0.3	14.3 ± 0.2
1 st conditioning *	12.8 ± 0.3	13.5 ± 0.4	14.4 ± 0.2
2 nd conditioning *	15.3 ± 0.9	14.0 ± 0.5	15.2 ± 0.2
Degermers – overs *	13.3 ± 0.9	15.0 ± 0.6	14.7 ± 0.4
Degermers – thrus #	14.4 ± 0.2	13.4 ± 0.2	15.2 ± 0.2
First brake #	13.4 ± 0.2	12.0 ± 0.4	11.8 ± 0.2
Last brake #	13.2 ± 0.4	12.3 ± 0.4	12.8 ± 0.2
Final product 1 #	13.2 ± 0.5	12.0 ± 0.0	13.0 ± 0.0
Final product 2#	14.3 ± 0.3	12.2 ± 0.5	13.5 ± 0.4
Final product 3 *	14.2 ± 0.5	13.8 ± 0.4	13.5 ± 0.0
Final product 4#	11.8 ± 0.2	10.9 ± 0.2	13.4 ± 0.2
Final product 5 #	14.1 ± 0.2	13.1 ± 0.3	15.1 ± 0.2

Moisture analyses were done in triplicate

applied one stage oven method

* applied two stage oven method

Table 3: Moisture content results of maize and maize products sampled over a period of ten months at **MILL 3**; note the product names have been omitted for confidentiality reasons

Sample	1 st visit (% m/v)	2 nd visit (% m/v)	3 rd visit (% m/v)
Maize from silo *	10.7 ± 0.6	13.5 ± 0.6	12.8 ± 0.8
Cleaned maize *	9.8 ± 0.9	15.7 ± 0.2	12.3 ± 0.6
1 st conditioning *	14.8 ± 0.5	13.2 ± 0.6	13.4 ± 0.2
2 nd conditioning *	13.4 ± 0.2	14.7 ± 0.3	13.3 ± 0.5
3 rd conditioning *	14.1 ± 1.7	16.0 ± 0.2	14.2 ± 0.3
Degermers – overs *	12.3 ± 0.0	14.5 ± 0.5	13.7 ± 0.2
Degermers – thrus #	20.9 ± 0.2	19.3 ± 0.4	16.2 ± 0.4
First brake #	11.6 ± 0.8	11.3 ± 0.4	11.7 ± 0.1
Last brake #	11.7 ± 0.4	13.2 ± 0.2	12.0 ± 0.0
Final product 1 #	12.7 ± 0.4	12.5 ± 0.7	12.2 ± 0.5
Final product 2 #	14.3 ± 0.4	12.5 ± 0.7	11.9 ± 0.2
Final product 3 #	13.2 ± 0.4	12.9 ± 1.2	12.5 ± 0.7
Final product 4 #	12.6 ± 0.8	14.9 ± 0.3	13.4 ± 0.3
Final product 5 #	10.5 ± 0.4	12.7 ± 0.4	12.0 ± 0.0
Final product 6 *	13.3 ± 0.2	13.3 ± 0.7	12.0 ± 0.6
Final product 7 #	15.3 ± 0.0	13.2 ± 0.2	12.2 ± 0.3

Moisture analyses were done in triplicate

applied one stage oven method

* applied two stage oven method

Table 4: Moisture content results of maize and maize products sampled over a period of ten months at **MILL 4**; note the product names have been omitted for confidentiality reasons

Sample	1 st visit (% m/v)	2 nd visit (% m/v)	3 rd visit (% m/v)
Maize from silo *	12.3 ± 0.4	13.4 ± 0.3	12.6 ± 0.5
Cleaned maize *	12.2 ± 0.2	11.6 ± 0.2	12.6 ± 0.0
1 st conditioning *	14.1 ± 0.3	14.7 ± 0.2	14.5 ± 0.0
2 nd conditioning *	16.1 ± 0.3	15.7 ± 0.4	14.4 ± 0.8
Degermers – overs *	14.8 ± 0.5	14.2 ± 0.0	15.4 ± 0.0
Degermers – thrus #	14.7 ± 0.4	14.7 ± 0.6	17.0 ± 0.2
First brake #	13.2 ± 0.2	12.6 ± 0.5	12.4 ± 0.2
Last brake #	14.0 ± 0.0	13.2 ± 0.2	12.9 ± 0.2
1 st ground	14.7 ± 1.2	13.3 ± 0.4	14.4 ± 0.2
Final product 1 #	13.9 ± 0.3	11.9 ± 1.3	14.4 ± 0.2
Final product 2 #	13.6 ± 0.6	12.2 ± 0.4	13.4 ± 0.2
Final product 3 #	14.4 ± 0.2	11.0 ± 1.7	13.2 ± 0.2
Final product 4 #	16.0 ± 0.3	14.4 ± 0.2	17.1 ± 0.4

Moisture analyses were done in triplicate

applied one stage oven method

* applied two stage oven method

Table 5: Moisture content results of maize and maize products sampled over a period of ten months at **MILL 5**; note the product names have been omitted for confidentiality reasons

Sample	1 st visit (% m/v)	2 nd visit (% m/v)	3 rd visit (% m/v)
Maize from silo *	12.6 ± 0.8	11.2 ± 0.5	14.0 ± 0.4
1 st conditioning *	13.9 ± 0.1	13.8 ± 0.4	15.7 ± 0.4
2 nd conditioning *	16.7 ± 0.3	14.6 ± 0.5	16.6 ± 0.2
Degermers – overs *	15.8 ± 0.2	14.4 ± 0.1	15.9 ± 0.2
Degermers – thrus #	19.5 ± 0.0	14.6 ± 0.5	19.5 ± 0.4
First brake #	12.8 ± 0.2	11.9 ± 0.3	13.3 ± 0.3
Last brake #	14.0 ± 0.3	10.8 ± 0.9	13.1 ± 0.2
1 st ground	12.4 ± 0.2	12.2 ± 0.2	12.8 ± 0.2
Final product 1 #	14.1 ± 0.5	10.6 ± 0.2	12.7 ± 0.0
Final product 2 #	12.7 ± 0.4	11.1 ± 0.2	14.4 ± 0.5
Final Product 3 *	14.2 ± 0.5	13.6 ± 0.2	14.6 ± 0.2
Final product 4 #	12.3 ± 0.6	10.6 ± 0.2	13.1 ± 0.2
Final product 5 #	11.0 ± 0.4	10.9 ± 0.9	11.2 ± 0.4
Final product 6 #	11.0 ± 0.2	11.1 ± 0.9	14.0 ± 0.3
Final product 7 #	16.2 ± 0.0	12.7 ± 0.4	17.5 ± 0.3

Moisture analyses were done in triplicate

applied one stage oven method

* applied two stage oven method

Table 6: Moisture content results of maize and maize products sampled over a period of ten months at **MILL 6**; note the product names have been omitted for confidentiality reasons

Sample	1 st visit (% m/v)	2 nd visit (% m/v)	3 rd visit (% m/v)
Maize from silo *	12.1 ± 0.1	12.2 ± 0.0	12.8 ± 0.3
Cleaned maize *	12.6 ± 0.0	13.0 ± 0.5	11.0 ± 0.5
1 st conditioning *	13.9 ± 0.1	13.2 ± 0.1	14.2 ± 0.6
2 nd conditioning *	15.4 ± 0.2	15.9 ± 0.5	14.9 ± 0.4
Degermers – overs *	14.5 ± 0.0	15.3 ± 0.6	15.2 ± 0.2
Degermers – thrus #	16.2 ± 0.5	17.6 ± 0.4	16.0 ± 0.0
First brake #	13.8 ± 0.1	11.7 ± 0.5	14.5 ± 0.2
Last brake #	12.2 ± 0.2	13.0 ± 0.0	12.4 ± 0.2
Final product 1 #	13.0 ± 0.0	12.7 ± 0.5	13.3 ± 0.3
Final product 2 #	12.0 ± 0.0	14.0 ± 0.0	12.9 ± 0.2
Final product 3 #	12.5 ± 0.3	13.1 ± 0.7	14.1 ± 0.2
Final product 4 #	12.2 ± 0.2	13.0 ± 0.2	12.9 ± 0.2
Final product 5 *	14.1 ± 0.1	13.6 ± 0.3	14.7 ± 0.2
Final product 6 #	12.2 ± 0.8	12.8 ± 0.7	13.7 ± 0.0
Final product 7 #	13.7 ± 0.7	13.6 ± 0.4	16.0 ± 0.0

Moisture analyses were done in triplicate

applied one stage oven method

* applied two stage oven method

4.2.3 Discussion and conclusions

The final moisture content of the maize kernels after the conditioning stages was still lower than expected, even though the mixing and packaging of samples were done at the mills not at CSIR. Considering the high air temperature inside the mill, moisture was probably lost during the mixing of samples. Therefore the loss of moisture was attributed to high temperatures inside mill, a factor that was uncontrollable. According to Duensing, (2003) the final moisture of maize kernels upon entering a degerming system should be in the 18 to 20% range. In the current study the moisture content of kernels prior to degerming ranged between 13-16.7%. Moisture content values for milling products were found to be in the same range as reported in literature.

4.3 Fungal enumeration and mycotoxin analyses

4.3.1 Background/introduction

To date all samples from six mills at three different occasions were received for fungal enumeration and mycotoxin analyses. The samples represented the milling process for each mill from the silo to the final products.

4.3.2 Methods and materials

Fungal enumeration was done according to the method described by Rabie *et al.* (1997). Approximately 300 randomly selected maize kernels were sampled, surface sterilized by using 76% (v/v) ethanol, and rinsed twice by sterile distilled water. In cases where milled products were used, 300 knife points (the size of a maize kernel) were sub-sampled and not surface sterilized. The kernels and the sub-samples were placed on 3 different growth media including potato dextrose agar, penta-chloro-nitro-benzene agar and malt salt agar for analysis. In total, 10 petri plates of each medium were used, allowing 50 kernels on each medium. The plates were incubated for at least 10 days at approximately 25°C with 12 hour dark and light cycles. Fungi that grew from the kernels were morphologically investigated and identified to species level by using stereo and light microscopic equipment. In certain cases, it was not possible to do identifications to species level because of the lack of fruiting structures. In such cases the fungi were identified only to genus level. The results are expressed as a percentage of maize kernels or sub-samples infested with a specific fungus.

Mycotoxin analyses were done based on the ELIZA based technology with the VICAM testing system, using fluorometry. All samples are tested for the presence of fumonisins due to the high levels of the presence of *Fusarium verticillioides* in most samples. Levels of ochratoxin A and aflatoxins were also determined. Confirmatory tests on the samples that indicated high levels of the above mycotoxins are to be done based on LC-MS-MS technology.

4.3.3 Results

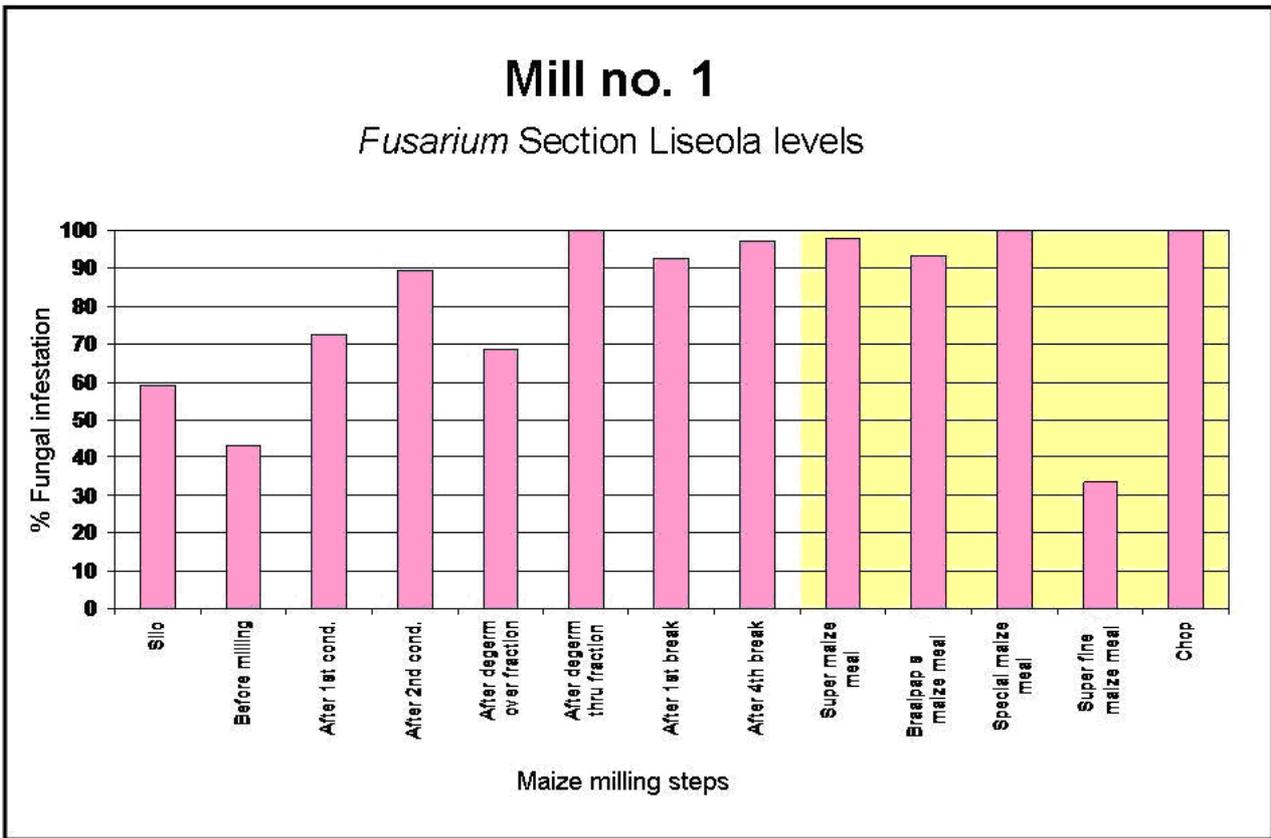
To date, the levels of the fungi in all six mills and three site visits are available. Results from this study indicated that at least 47 different fungi are associated with maize mills in South Africa (see Table 7).

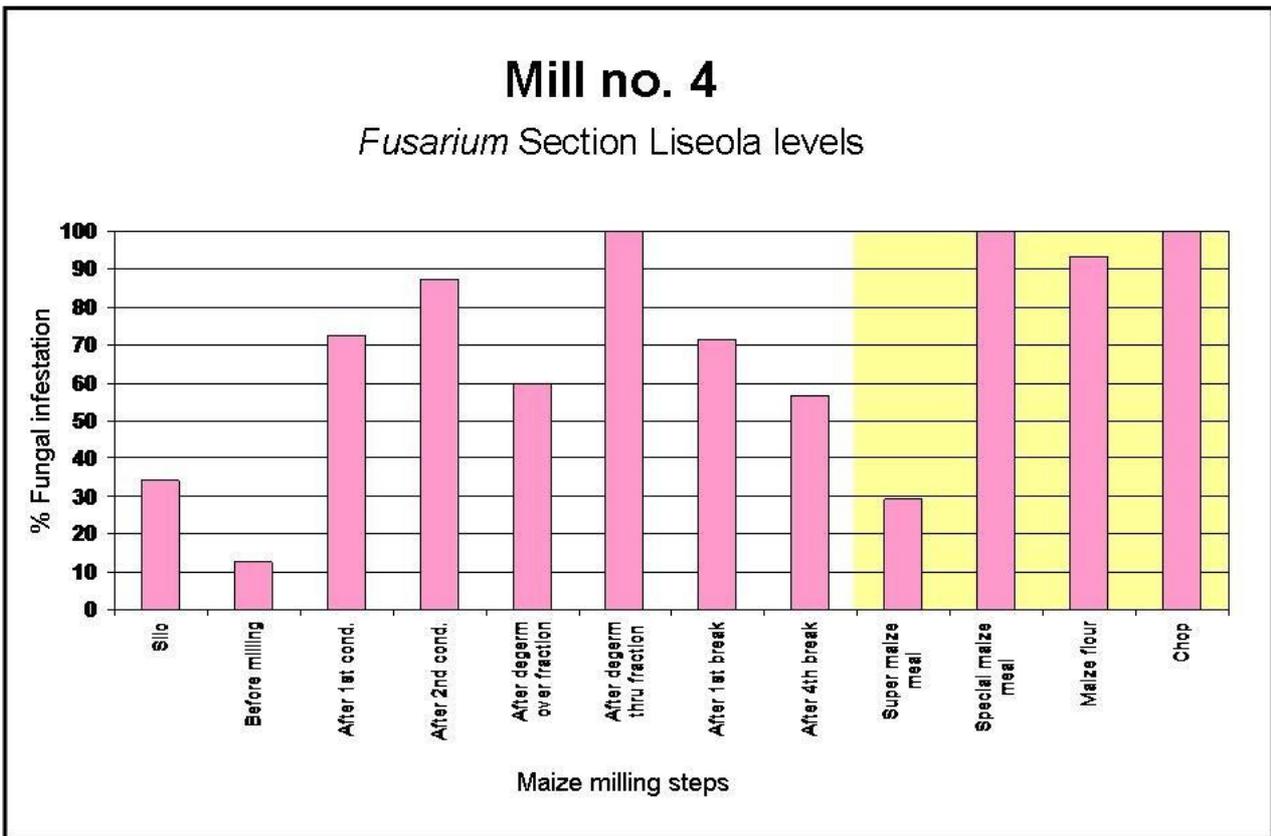
Table 7: Fungal species found to be present in analysed samples

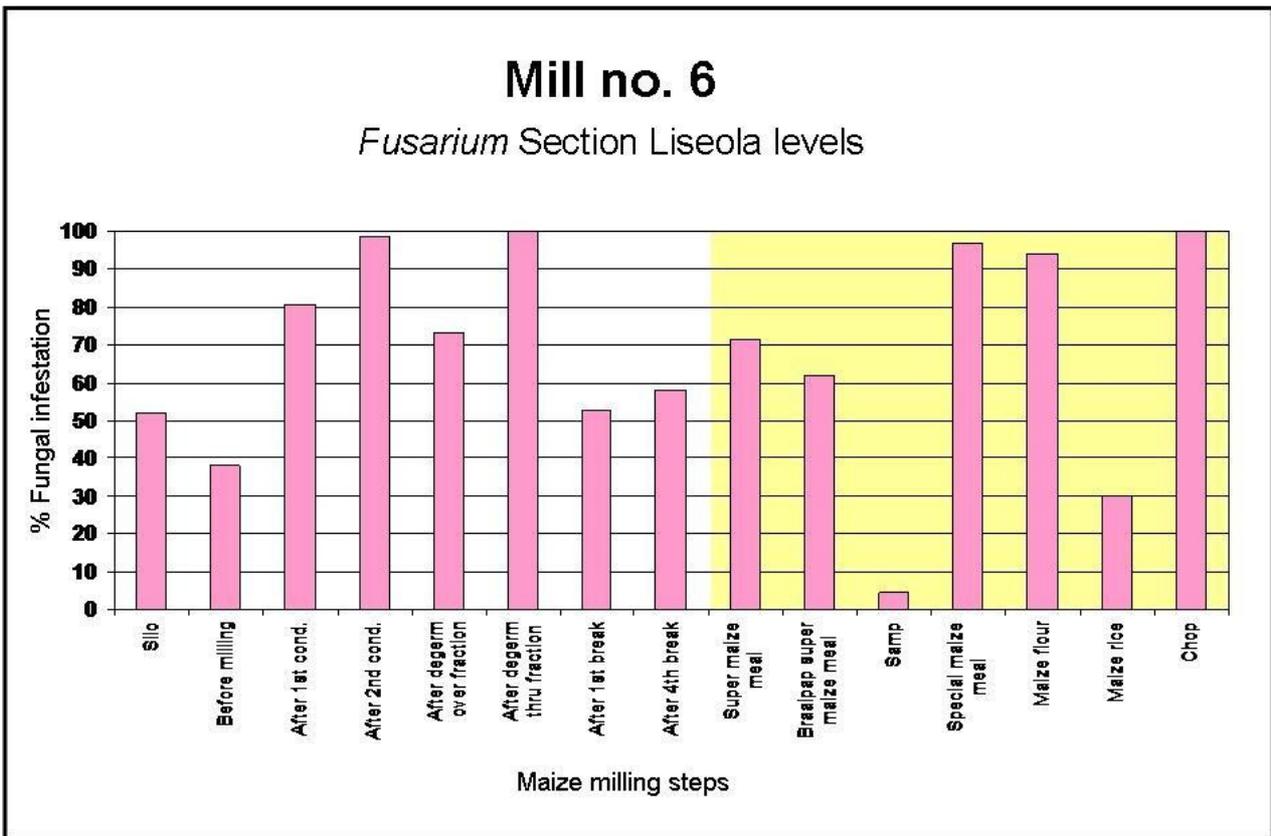
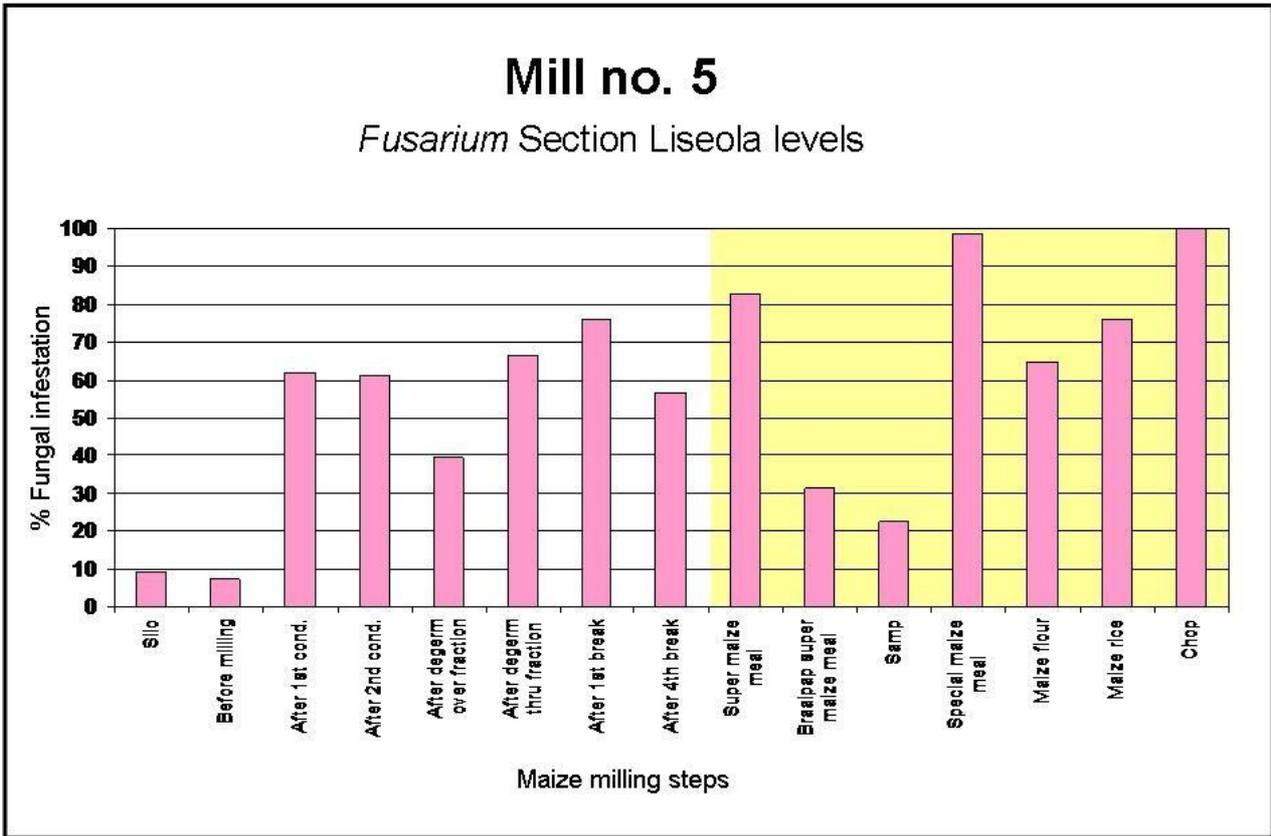
No	Taxon	No	Taxon	No	Taxon
1	<i>Acremonium</i> spp.	17	<i>Eurotium herbariorum</i>	33	<i>Penicillium griseofulvum</i>
2	<i>Alternaria alternata</i>	18	<i>Eurotium repens</i>	34	<i>Penicillium implicatum</i>
3	<i>Aspergillus candidus</i>	19	<i>Eurotium rubrum</i>	35	<i>Penicillium pinophilum</i>
4	<i>Aspergillus clavatus</i>	20	<i>Fusarium</i> sp. (Liseola)	36	<i>Penicillium purpurogenum</i>
5	<i>Aspergillus flavus</i>	21	<i>Fusarium chlamydosporum</i>	37	<i>Penicillium oxalicum</i>
6	<i>Aspergillus fumigatus</i>	22	<i>Fusarium equiseti</i>	38	<i>Penicillium variabile</i>
7	<i>Aspergillus niger</i>	23	<i>Fusarium graminearum</i>	39	<i>Penicillium waksmanii</i>
8	<i>Aspergillus ochraceus</i>	24	<i>Fusarium oxysporum</i>	40	<i>Phoma sorghina</i>
9	<i>Aspergillus sydowia</i>	25	<i>Geotrichum</i> spp.	41	<i>Pithomyces</i> spp.
10	<i>Aspergillus terreus</i>	26	<i>Mucor</i> spp.	42	<i>Rhizopus oryzae</i>
11	<i>Aspergillus versicolor</i>	27	<i>Nigrospora</i> spp.	43	<i>Rhizopus microsporus</i>
12	<i>Aureobasidium pullulans</i>	28	<i>Penicillium aurantiogriseum</i>	44	<i>Sordaria</i> sp.
13	<i>Chaetomium</i> spp.	29	<i>Penicillium chrysogenum</i>	45	<i>Stenocarpella maydis</i>
14	<i>Cladosporium cladosporioides</i>	30	<i>Penicillium citrinum</i>	46	<i>Trichoderma reesei</i>
15	<i>Eurotium amstelodami</i>	31	<i>Penicillium fellutanum</i>	47	<i>Trichoderma</i> spp.
16	<i>Eurotium chevalieri</i>	32	<i>Penicillium funiculosum</i>		

It is envisaged that all the major fungi in the maize mills will be discussed in detail in the final report. But for the purpose of this report *Fusarium verticillioides* is used to demonstrate the kind of results that can be extracted from the datasets obtained by fungal enumeration. Figures 1 to 6 indicate the average levels of *F. verticillioides* of each sampling point in each of the 6 milling systems included in this project. Figures 7 to 12 compare the various products produced by the different milling systems. Certain products are not produced by all mills included in this study and, therefore, some histograms contain blank areas where information is not available.

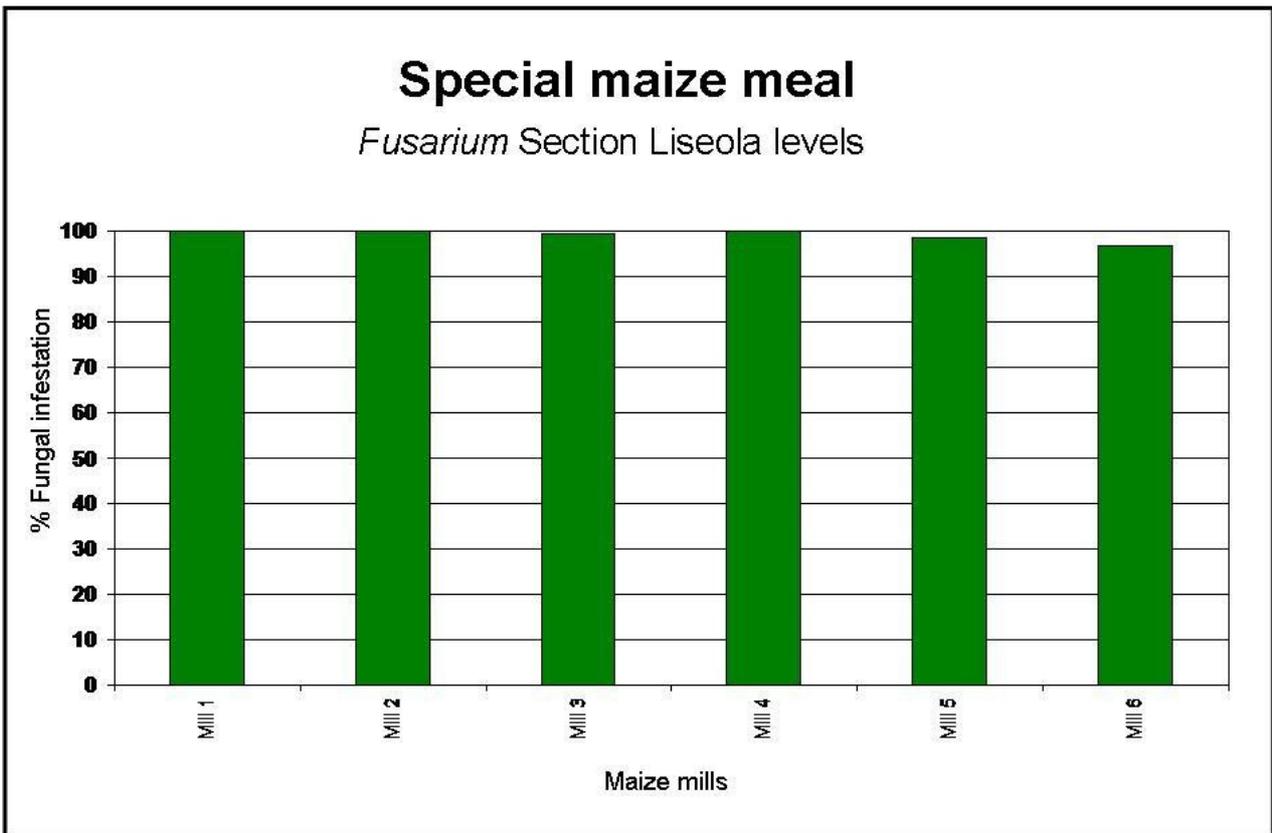
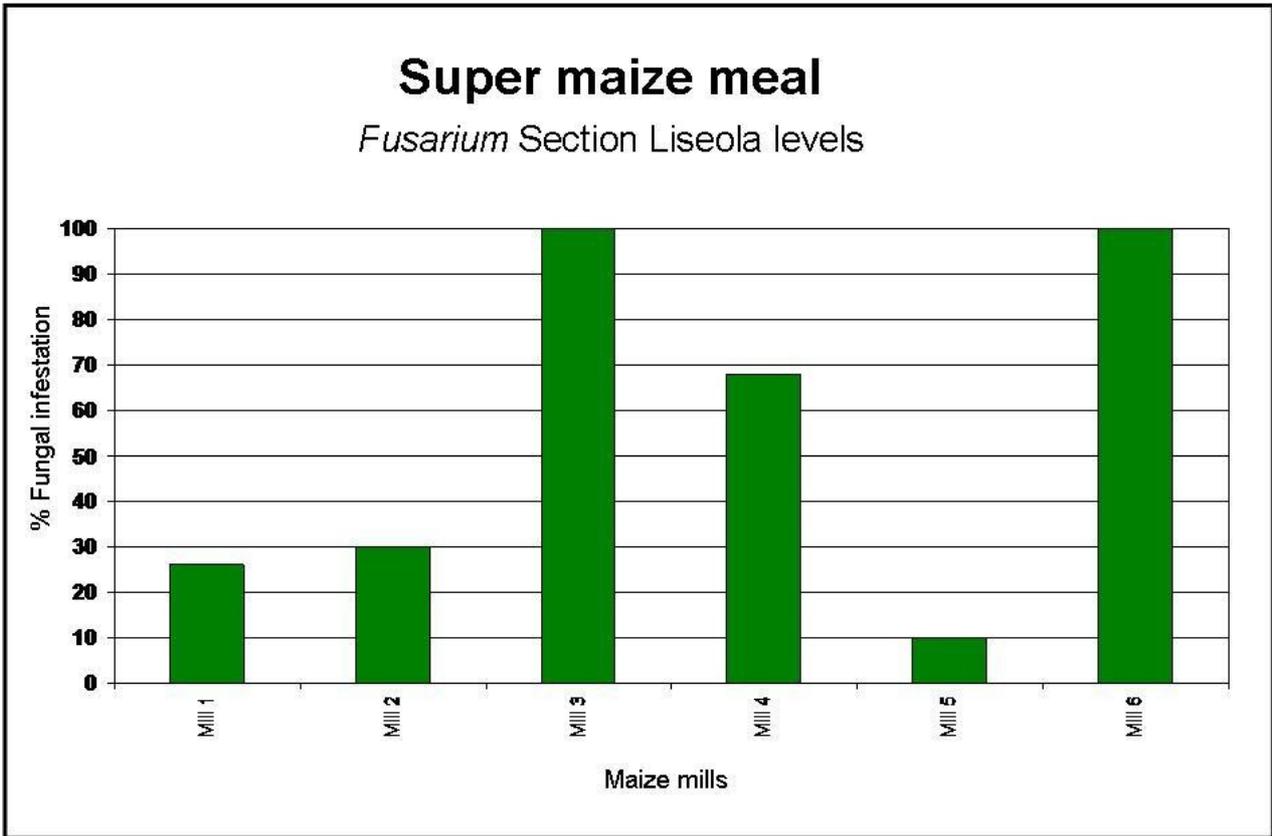
See figures below:

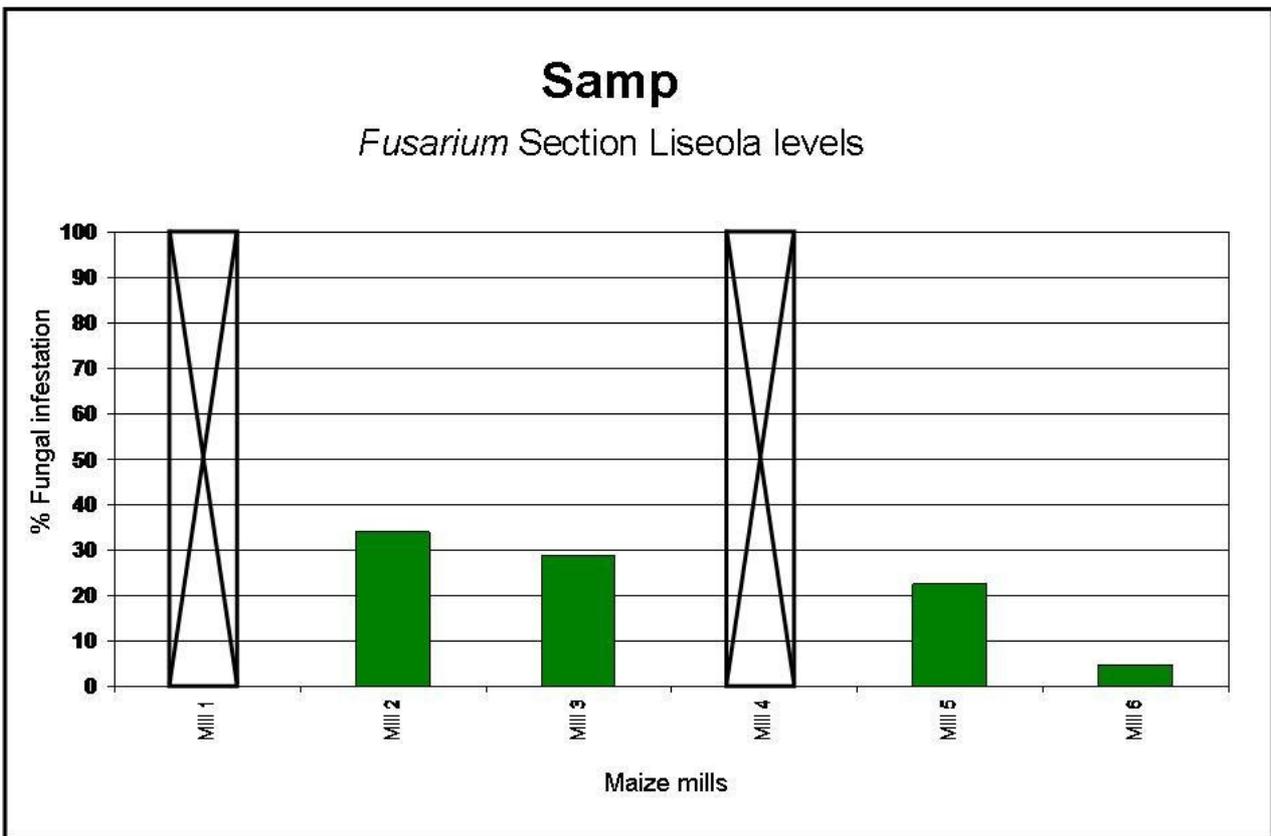
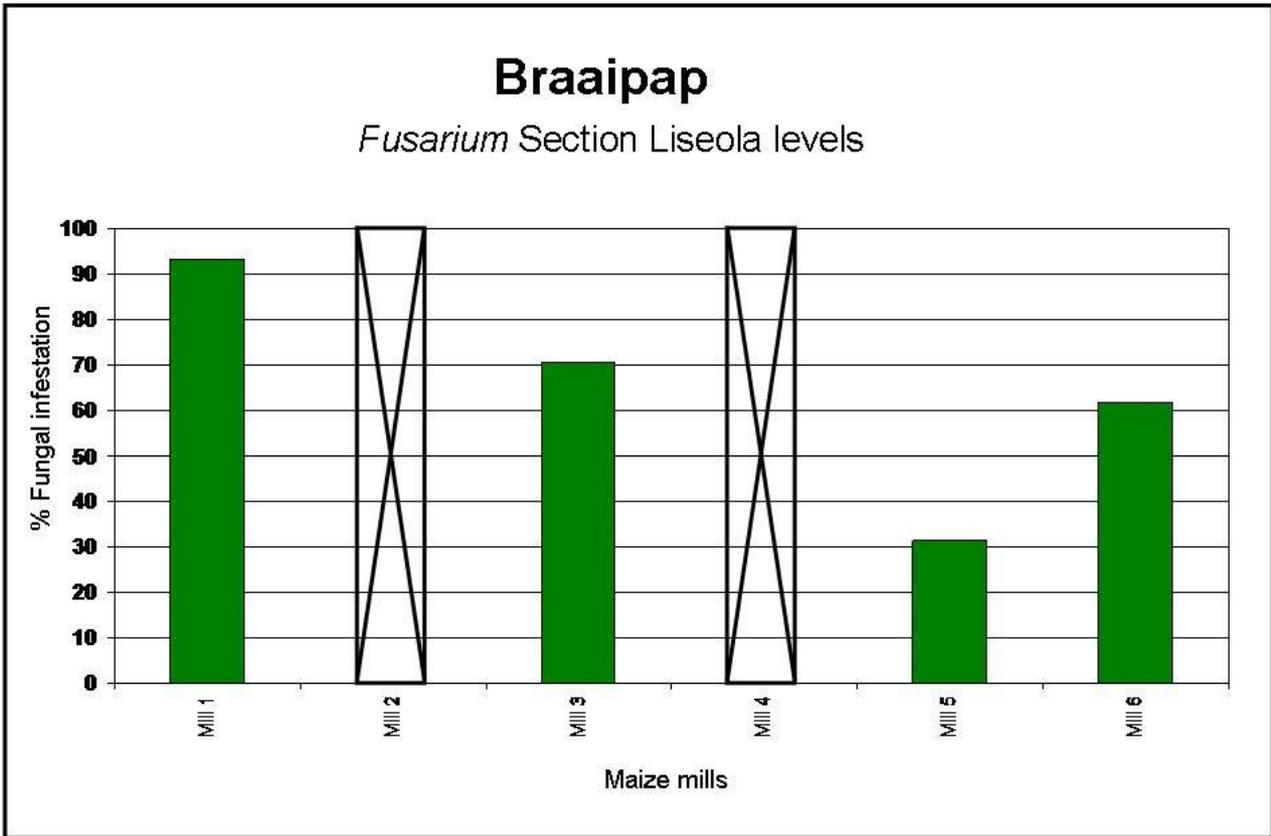


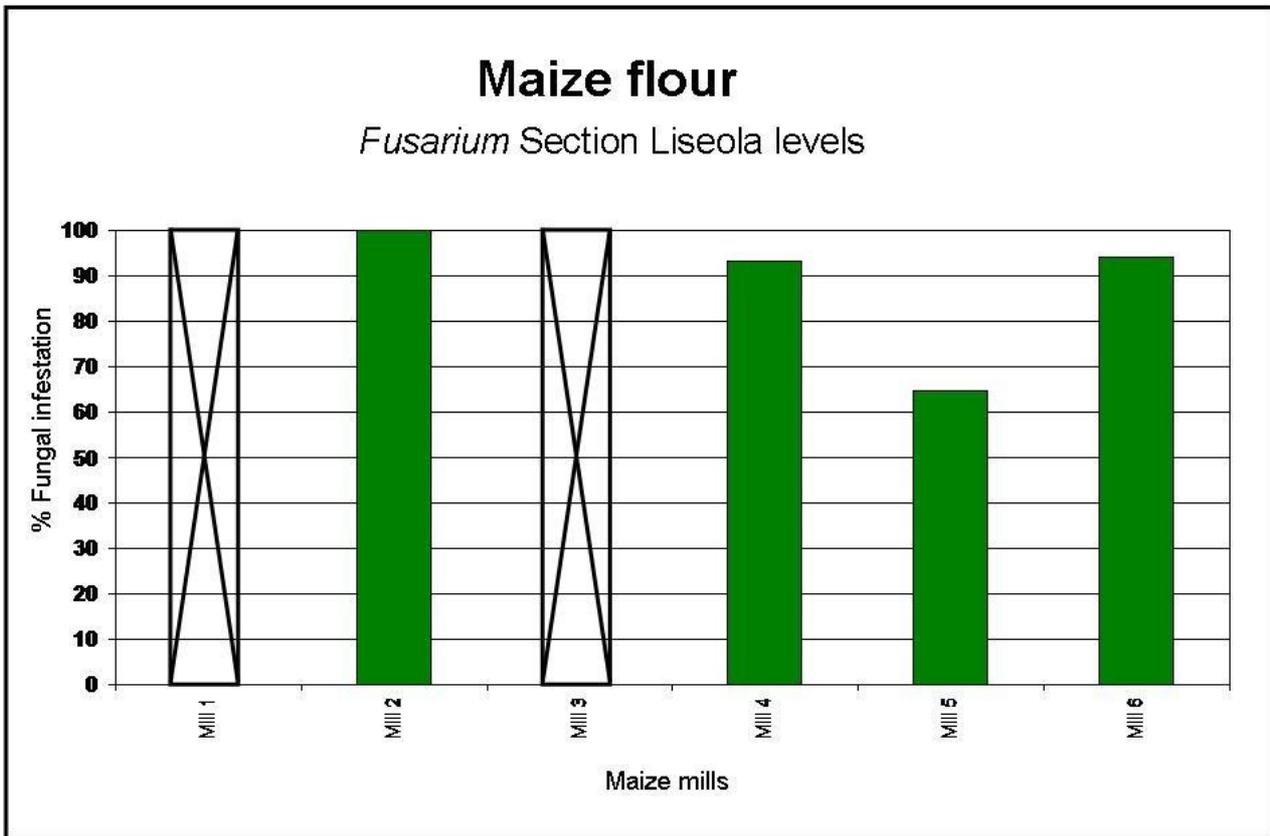




Comparison of the average levels of *Fusarium verticillioides* between the various mills is illustrated in the figures below.







The most prominent fungi associated with maize during the milling process are mentioned below:

- *Fusarium* Section Liseola
Fusarium species placed under this section have conidial spores in chains like *Fusarium verticillioides*. However, certain characteristics are atypical, making an accurate identification difficult on the basis of morphology. Fungi in this section are known fumonisin producers.

- *Aspergillus flavus*
This fungus is known to produce aflatoxins that cause liver cancer in humans and animals. Legislation in South Africa states that no commodity destined for human consumption may contain more than 10 ppb total aflatoxins of which only 5 ppb may be aflatoxin B₁.
- *Aspergillus clavatus*
This fungus is known to produce the mycotoxin, cytochalasin E, which is associated with necrosis of the liver, kidney, spleen, pancreas, and small intestine. It is also associated with brain edema, pulmonary hemorrhages, and injury to vascular walls. It was surprising to observe this fungus in one of the mills due to the fact that it is not seen as a major problem in South African maize.
- *Eurotium species*
These fungi are able to grow at moisture contents between 14 and 17%, making them the first colonisers of a commodity when moisture contents rise above 13%. Some isolates have shown to be able to produce the mycotoxin, sterigmatocystin, a precursor of aflatoxins.
- *Penicillium species*
Penicillium islandicum and *P. oxalicum* are becoming more prominent in maize. Although these fungi are normally associated with poor storage conditions, they are now starting to develop on maize kernels before harvesting. Mycotoxins associated with *P. islandicum* include luteoskyrin that can be acutely toxic. *Penicillium oxalicum* produces secalonin D, a mycotoxin that is also acutely toxic to test animals.
- *Cladosporium cladosporioides*
This fungus tends to produce blackish discoloured kernels that cause problems regarding the aesthetic appearance of cereal corn flakes. No mycotoxins are known to be produced from this fungus, but it has been associated with allergic reactions when high levels of spores are inhaled by patients.
- *Zygomycetous fungi*
These fungi can either be associated with poor field or storage conditions and have shown to be toxic to test animals. The mycotoxins produced by these fungi are still unknown, but it is known that some *Rhizopus* isolates can produce rhizoxin A that can be acutely toxic.

The above fungi will be discussed in detail in the final report at the completion of the project.

4.3.4 Discussion and conclusions

Fungi were morphologically identified and it became clear that the *Fusarium* species were not typical in their characteristics. The most predominant fungus was a *Fusarium* species closely resembling *Fusarium verticillioides*. In all mills included in the study levels of *Fusarium verticillioides* were moderate, which eventually increased as the milling process is done. This raises the issue that it is likely that mycotoxin levels could also be elevated during the milling process. It also became evident that the milling process in general seems to contribute to the higher levels of fungi such as *Penicillium* species and *Aspergillus flavus*.

Results also indicated that *Cladosporium cladosporioides* is not significantly affected by the milling process and that the levels do not increase. This is important for the milling industry as this fungus has been implicated in the dark discolouration of broken maize kernels destined for the production of corn flakes.

It was also indicated that the levels of fungi such as *Fusarium verticillioides* in the final products differ between different maize milling systems. Results are not consistent between mills and in some cases certain mills seem to produce better quality fractions of a certain product, but not in others. This clearly indicates that different milling systems have different areas of high risks that are unique to each mill. Although some aspects within South African mills can be generalized, high risk areas should be addressed on an individual basis.

4.3.5 Recommendations and further work still planned

Although only a limited amount of data has been captured thus far it seems that fungi such as *Fusarium* species, *Aspergillus flavus* and *Penicillium* species could have the ability to substantially increase in numbers during the milling process. It is also this group of fungi that could be responsible for mycotoxin production.

The project is come towards the final completion of the work with the only outstanding part to be the completion of the mycotoxin analyses and statistical manipulation of the data. It is envisaged that this work will be completed at the end of 2008 as planned in the original proposal.

5. REFERENCES

American Association of Cereal Chemists, 1995. Method 44-15A Moisture – Air Oven Method. *Approved methods of American Association of Cereal Chemists*, 9th Ed. The Association: St. Paul, MN

Dhillon, B., Sandhu, H., Wiesenborn, D., Manthey, F. and Wolf-Hall, C., 2007. A comparison between chlorinated water and ozonated water as antimicrobial treatment during tempering of wheat. ASAE Annual Meeting 076169.

Duensing, W.J., Roskens, A.B., and Alexander, R.J., 2003. Corn drying milling: processes, products, and applications. *In: White, P.J., Johnson, L.A., (Eds.), Corn: Chemistry and Technology*. St Paul: American Association of Cereal Chemists, Inc. pp407-447

Hoseney, R.C., 1994. Dry milling of cereals. *In: Hoseney, R.C (Ed.), Principles of Cereal Science and Technology*. 2nd ed. St Paul: American Association of Cereal Chemists, Inc. pp125-145