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

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CSIR/BIO/ACT/ER/2007/0077B
GW DMS Pta no: 153211

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September 2007

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1. SUMMARY

This report summarises the progress on this project for the initial six months. Although the project started in January this year, initial delays were encountered in terms of contacting the appropriate person at each mill. Furthermore the initial number of mills has been reduced to six as one of the mills has chosen not to participate.

Despite the initial problems, the project is progressing as planned. To date, samples from three mills have been collected and are being analysed. Some of this data is presented in this report but no conclusions can be made to date.

As part of the confidentiality aspect of this project, non-disclosure agreements are being put in place and have already been forwarded to some of the appropriate contact people at each mill.

2. INTRODUCTION

High levels of fungi are a known phenomenon in South Africa and affect the shelf life of various food products as well as resulting in the presence undesirable mycotoxins. Mycotoxins are produced by fungi under certain conditions which may differ from the optimal conditions for growth. However, the toxin is typically not produced unless there are sufficiently high levels of the fungus. The occurrence of mycotoxin-producing organisms does not indicate that any mycotoxins are present, but it can indicate a high risk scenario which will then call for the need to analyse the mycotoxins.

Many fungi are associated with maize, especially after the milling process. However, not much is published on the presence of fungi during the storage or milling processes of maize. 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

The three 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 load and mycotoxin analysis. A fraction of each aggregate sample was placed in a jam jar which was sealed with insulation tape to trap the moisture inside 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.

4.1.5 Recommendations and further work still planned

It is recommended that the mixing of incremental samples be done at the mill, as soon as the triplicate samples have been taken. The distribution of samples for mycotoxin and moisture content analysis should also be done at the mill and the jam jars sealed with insulation tape. This should minimise the loss of moisture.

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, Product 1 and the degermer overs were analysed using the two stage method and the remainder of the maize samples were analysed using the one stage method. One stage method was used for powdery samples that supposedly contained less than 13% moisture and the two-stage method was used for whole maize kernels that contained 13% or more moisture. The latter reduces the possible loss of moisture during grinding of sample. Mill 1 and 3 added chlorine in the water for the first conditioning step. The maize kernel conditioning step is the most important unit operation of the dry milling process, it aids in toughing of the bran for easy removal and softens the endosperm and makes it easier to grind (Hoseney, 1994).

4.2.2 Results

Table 1 summarises the moisture content levels determined for each mill at the various sampling points.

Table 1: Moisture content results of maize and maize products from varying maize mills; note the product names have been omitted for confidentiality reasons

Sample	Mill 1 (%m/v)	Mill 2 (%m/v)	Mill 3 (%m/v)
Maize from silo *	11.2 ± 1.1	11.6 ± 0.3	10.7 ± 0.6
Cleaned maize *	11.9 ± 0.5	12.1 ± 1.1	9.8 ± 0.9
1st conditioning *	12.2 ± 0.2	12.8 ± 0.3	14.8 ± 0.5
2nd conditioning *	13.3 ± 0.4	15.3 ± 0.9	13.4 ± 0.2
3rd conditioning *	NU	NU	14.1 ± 1.7
Degermers – overs *	13.6 ± 0.5	13.3 ± 0.9	12.3 ± 0.0
Degermers – thrus #	13.2 ± 0.5	14.4 ± 0.2	20.9 ± 0.2
First break #	11.4 ± 0.2	13.4 ± 0.2	11.6 ± 0.8
Last break #	12.0 ± 0.0	13.2 ± 0.4	11.7 ± 0.4
Product 1*	NP	14.2 ± 0.5	13.3 ± 0.2
Product 2#	12.3 ± 0.3	13.2 ± 0.5	12.7 ± 0.4
Product 3#	12.9 ± 0.2	NP	13.2 ± 0.4
Product 4#	11.6 ± 0.2	14.3 ± 0.3	14.3 ± 0.4
Product 5#	11.5 ± 0.7	NP	12.6 ± 0.8
Product 6#	NP	NP	10.5 ± 0.4
Product 7#	NP	11.8 ± 0.2	NP
Product 8#	13.2 ± 0.5	14.1 ± 0.2	15.3 ± 0.0

NU = no unit operation at particular mill
NP = particular product not manufactured at mill
# = 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 lower than expected. This can be attributed to moisture lost during the mixing of triplicate samples in order to obtain a representative sample. According to Duensing, (2003) the final moisture of maize kernels upon entering a degerming system should be in the 18 to 20% range. Moisture contents of milling process products were in the same range as reported in literature. Product 4 should be in the range of 13%, Products 7, 2 and 3 around 12%, and Product 1 at 13.8% (Duensing, 2003).

4.2.4 Recommendations and further work still planned

The remaining four maize mills will be visited for sampling. In future sampling sessions, it is recommended that samples taken during the conditioning stages should be mixed together, stored in buckets and the openings should be sealed with insulation tape to avoid loss of moisture.

4.3 Fungal enumeration and mycotoxin analyses

4.3.1 Background/introduction

To date samples from three mills were received for fungal enumeration. 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^{o}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 infested with a specific fungus.

4.3.3 Results

The levels of the fungi in two of the mills are available and the enumeration of the third mill will be completed at the end of September 2007. Preliminary results from this study indicated that at least 36 different fungal species can thus far be associated with South African maize and maize products (Table 2).

Table 2: Fungal species found to be present in analysed samples

1	*Acremonium* spp.	13	*Fusarium* sp. (Liseola)	25	*Penicillium implicatum*
2	*Alternaria alternata*	14	*Fusarium chlamydosporum*	26	*Penicillium pinophilum*
3	*Aspergillus clavatus*	15	*Fusarium equiseti*	27	*Penicillium purpurogenum*
4	*Aspergillus flavus*	16	*Fusarium graminearum*	28	*Penicllium oxalicum*
5	*Aspergillus niger*	17	*Mucor* spp.	29	*Penicillium variabile*
6	*Aspergillus terreus*	18	*Nigrospora* spp.	30	*Penicllium waksmanii*
7	*Aspergillus versicolor*	19	*Penicillium aurantiogriseum*	31	*Phoma sorghina*
8	*Cladosporium cladosporioides*	20	*Penicillium chrysogenum*	32	*Pithomyces* spp.
9	*Eurotium chevalieri*	21	*Penicillium citrinum*	33	*Rhizopus oryzae*
10	*Eurotium herbariorum*	22	*Penicllium fellutanum*	34	*Rhizopus microsporus*
11	*Eurotium repens*	23	*Penicillium funiculosum*	35	*Stenocarpella maydis*
12	*Eurotium rubrum*	24	*Penicillium griseofulvum*	36	*Trichoderma reesei*

Figures 1(a) to 1(g) and 2(a) to 2 (f) indicate the most prominent fungi in Mills 1 and 2 respectively, and also the fractions most affected by fungal contamination.

1(a)

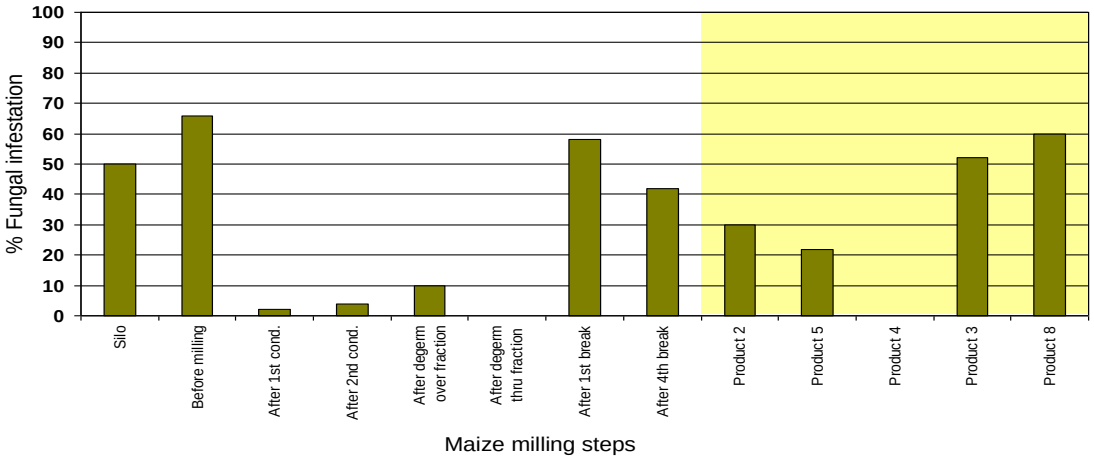
Mill no. 1

Fusarium Section Liseola levels

1(b)

Mill no. 1

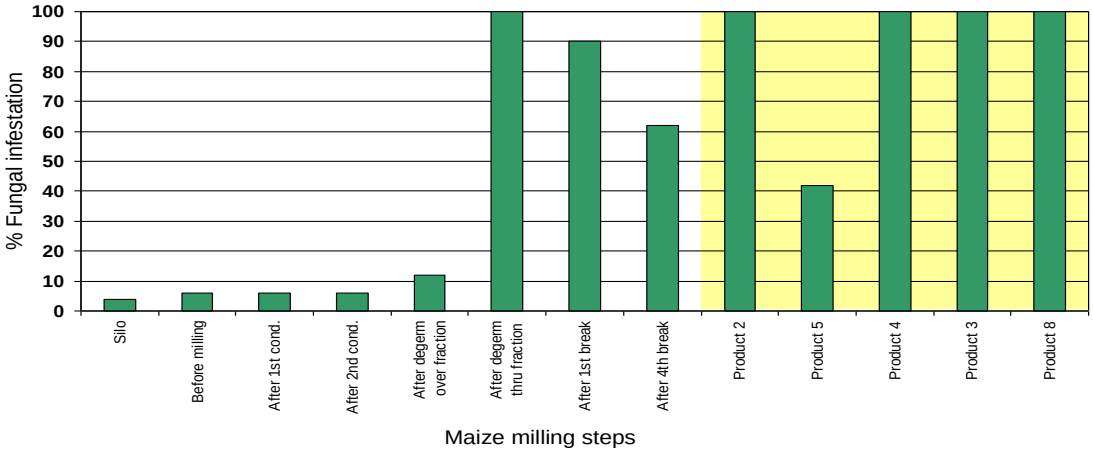
Cladosporium cladosporioides levels



1(c)

Mill no. 1

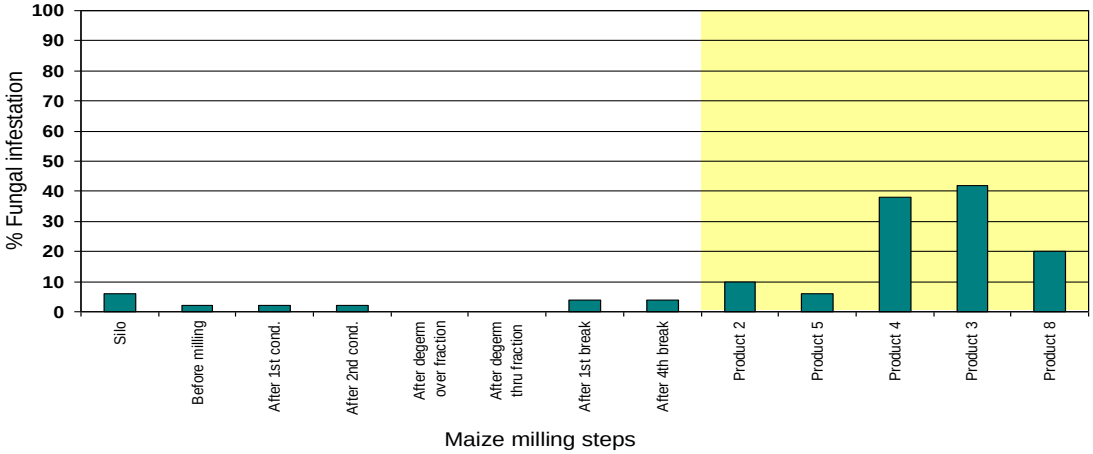
Penicillium levels



1(d)

Mill no. 1

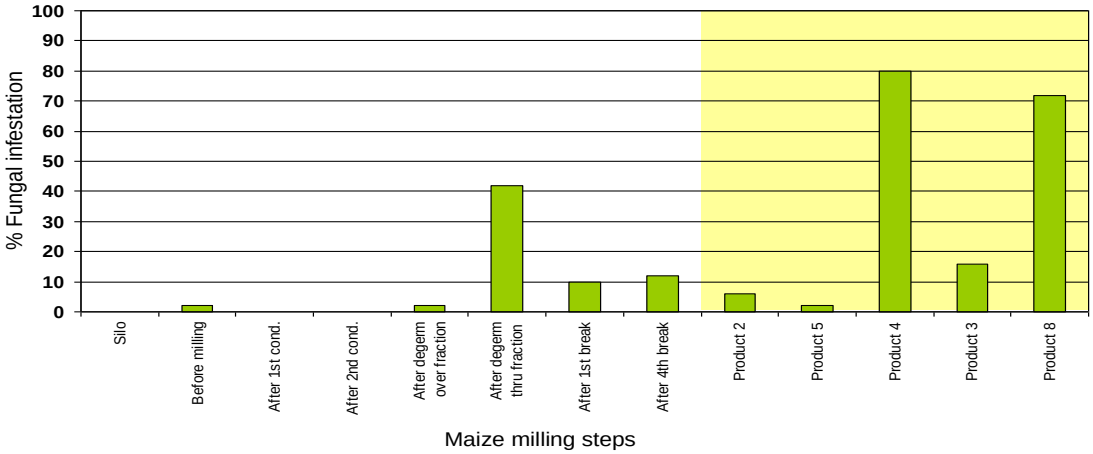
Eurotium spp. levels

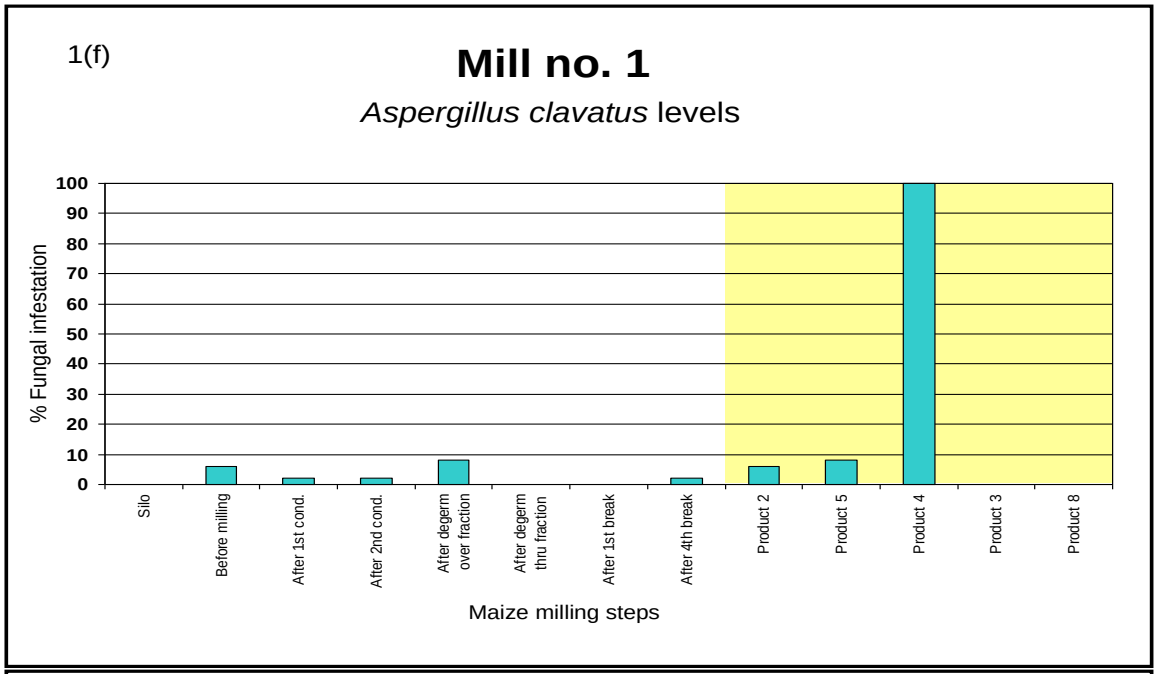


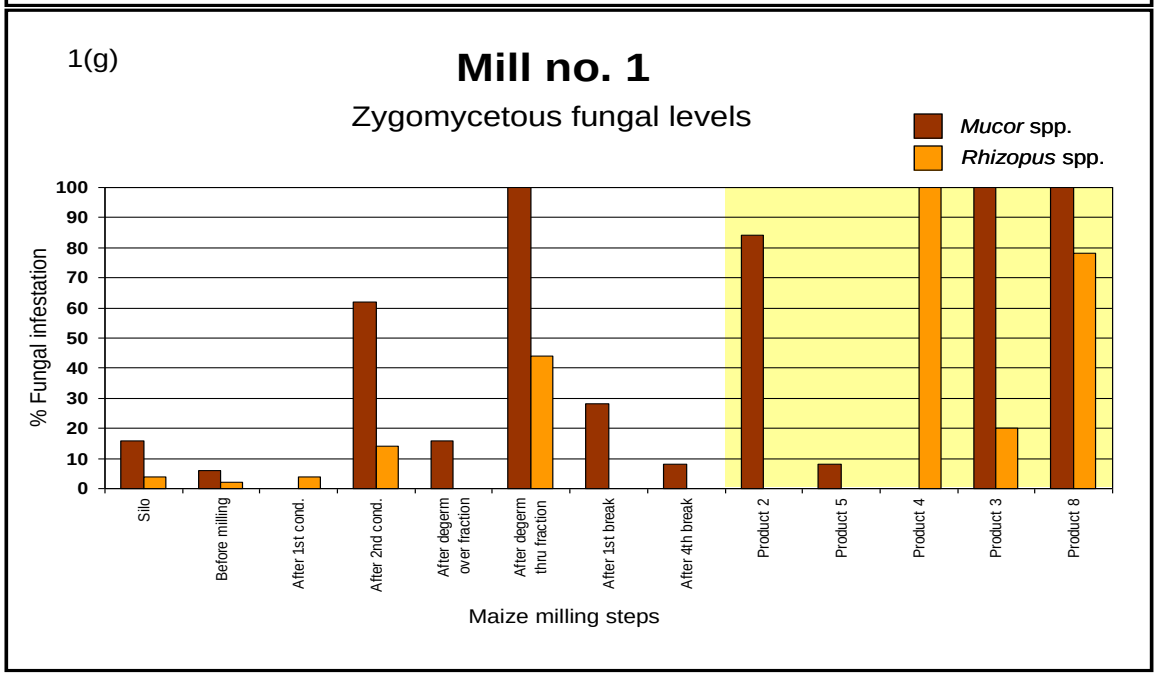
1(e)

Mill no. 1

Aspergillus flavus levels







Figures 1(a) to (g): Graphic representation of the most prominent fungi measure for Mill 1 at each sampling point

2(a)

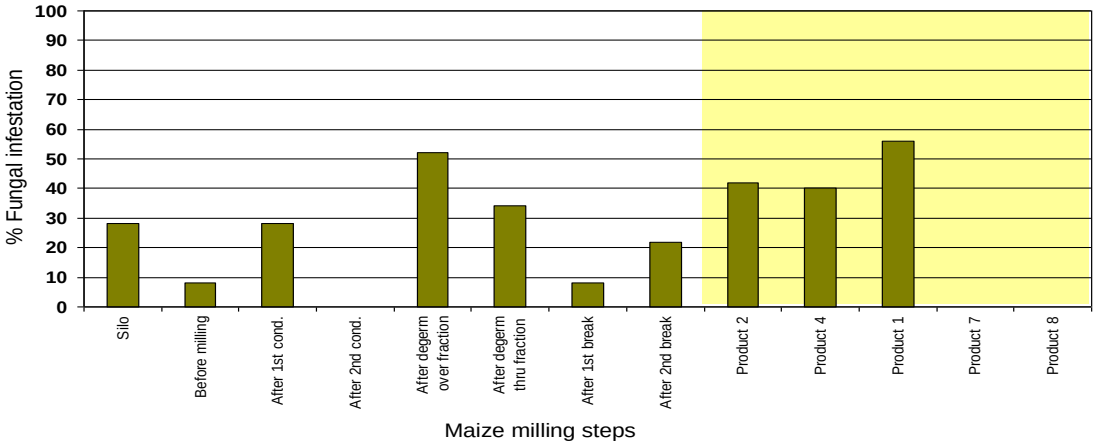
Mill no. 2

Fusarium Section Liseola levels

2(b)

Mill no. 2

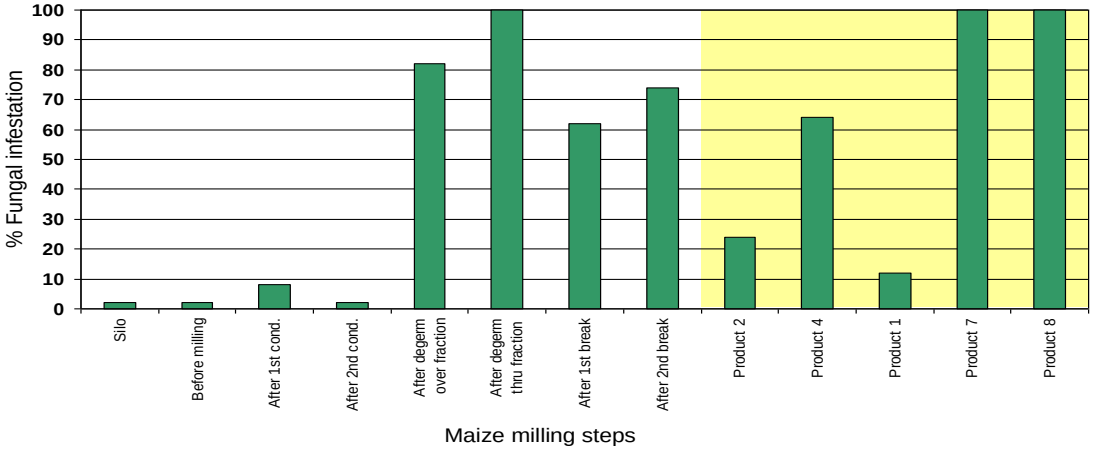
Cladosporium cladosporioides levels

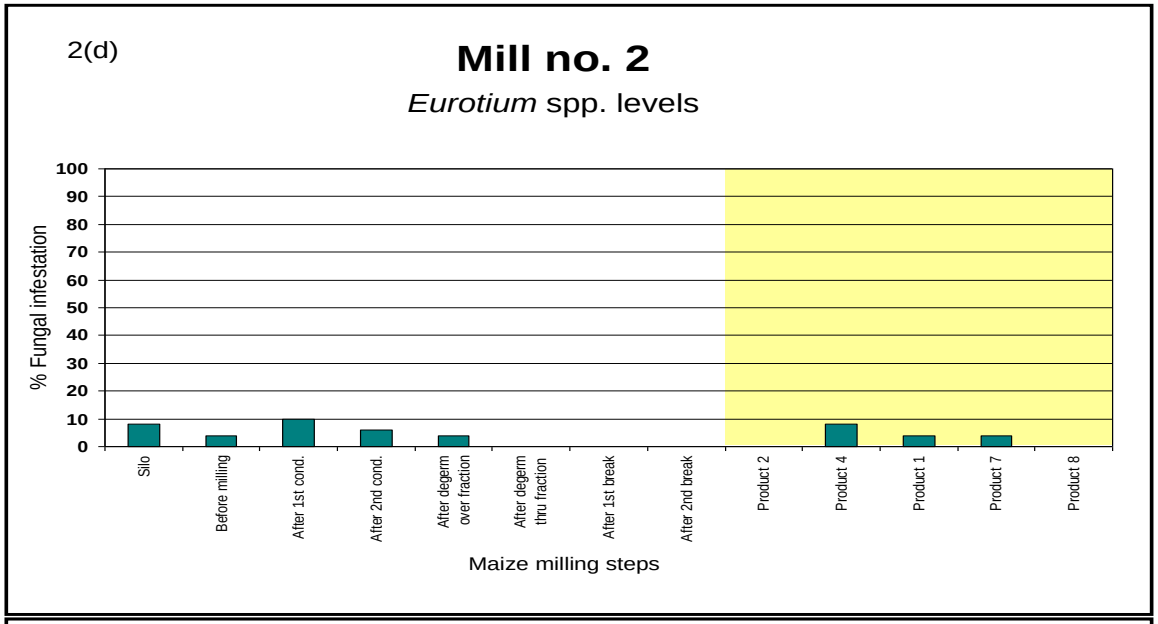


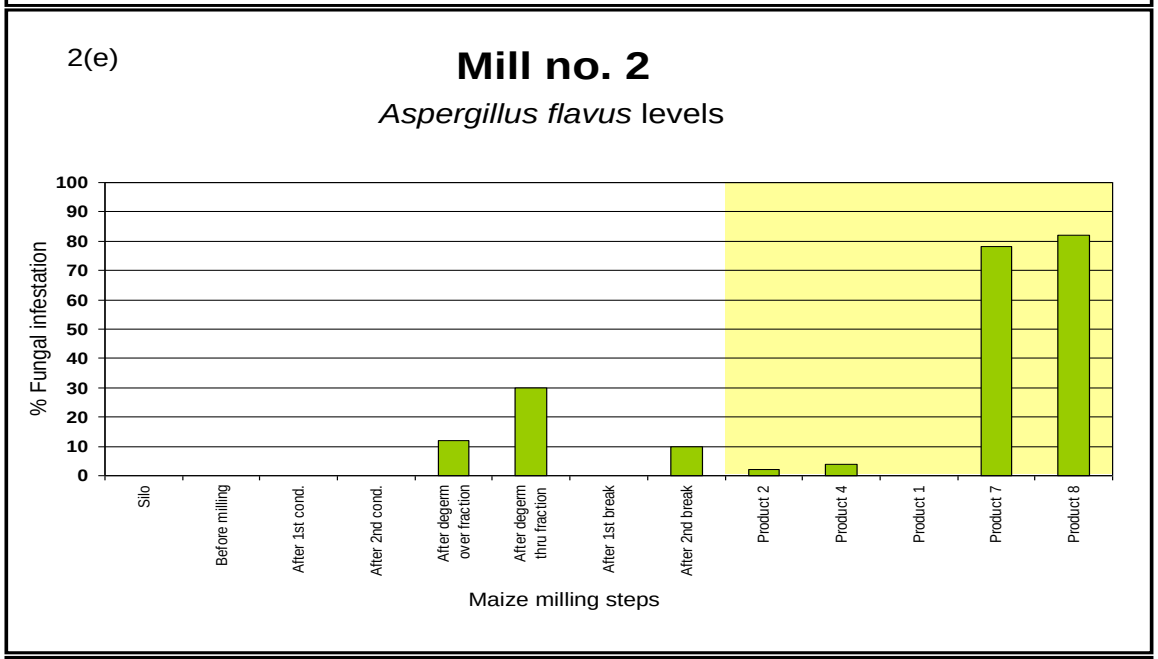
2(c)

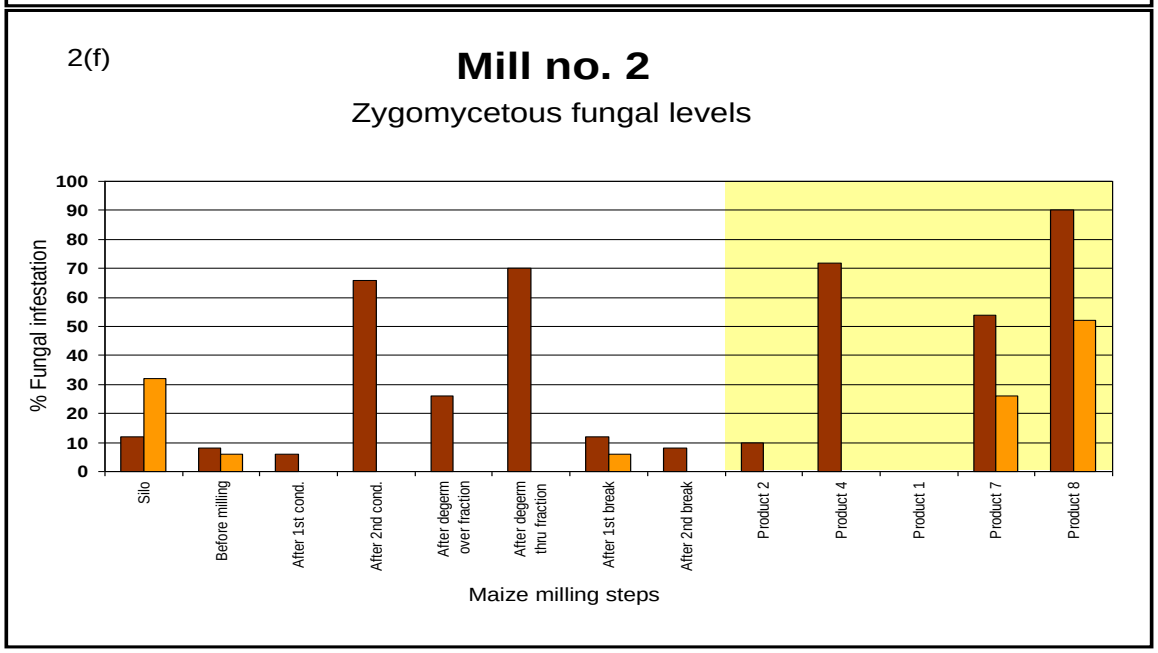
Mill no. 2

Penicillium levels









Figures 2(a) to (f): Graphic representation of the most prominent fungi measure for Mill 2 at each sampling point

The importance of above-mentioned fungi are as follows:

- *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 that 10 ppb total aflatoxins of which only 5 ppb may be aflatoxin B_1.
- *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 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 acute toxic. *Penicillium oxalicum* produces secalonic acid D, a mycotoxin that is also acute 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 rhizonin A that can be acutely toxic.

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 both mills tested the levels of this *Fusarium* species were moderate in the silos, but as soon as the maize was processed levels increased substantially. It also became evident that the milling process in general seems to contribute to the higher levels of fungi such as *Penicillium* species (see table above) 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.

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 findings to date are only indications and a substantial amount of the data, especially from the outstanding mills, is still needed in order to make any conclusions.

4.4 Statistical manipulation

Statistical manipulation of data will only be carried out at the end of the first mill visitations.

5. REFERENCES

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PROJECT NO. V5GPP11

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7	End date	22 Feb 2006
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