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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1. SUMMARY

This report summarises the progress on this project for the initial first year. Delays were initially experienced when the project was started in January 2007. The reasons for the delay were the identification of the appropriate contact person at each mill and to obtain the necessary approval to take samples. This process eventually led to the inclusion of only 6 of the initially targeted 7 mills.

After the initial delay, work has progressed as planned. Sampling has been completed at 3 mills where 3 sets of samples have been taken at 3 different times of the year. Two sampling sets have been completed at the other 3 mills and it is anticipated that the outstanding sampling sets will be completed within a month. Mycotoxin analyses are currently done on these samples and will be, together with the fungal levels be statistically evaluated as soon as all data sets are available.

Confidentiality agreements have been signed with all the mills.

2. INTRODUCTION

The presence of fungi in maize poses a health risk to humans and animals due to the production of mycotoxins. These toxic substances can either be produced under conditions in the field during cultivation of the plants or during storage and processing. An indication whether the risk of mycotoxins exist is the presence of fungi. High levels of mycotoxigenic fungi also rises the risk of mycotoxins to be present, but it also indicates the way a food commodity has been handled or what the possible origin is. For example, South African maize has a higher risk to contain fumonisins compared to maize from the USA or Argentina, which are more likely to contain aflatoxins.

Considerable work has been done on the fungi associated with maize during cultivation and their possible role as plant pathogens. There is, however, a lack of information on the role of fungi and their mycotoxins during storage and processing, and how they influence the quality of the product. The ultimate questions are whether the milling industry contributes to the presence of these fungi and their mycotoxins and what role the industry can play in combating high levels of 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 without exposing their identity.

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 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.

4.1.5 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 and discussion

Moisture contents were done for all sampling sets including 3 sets for 3 of the 6 mills in the project. Three of the mills still need to be visited to obtain the final sampling sets. The final results are not given here due to the outstanding data of these three mills to complete the statistical analyses, but will be available in the final report.

4.3 Fungal enumeration and mycotoxin analyses

4.3.1 Background/introduction

To date completed sample sets from three mills were received. Two sample sets were received from the other 3 mills. There is thus only one sampling set outstanding for the latter three mills. The samples represented the milling process for each mill from the silo to the final products. Statistical analyses will be commenced when all sampling sets have been received.

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

Fungal enumeration has been completed in three of the six mills in the project. These data sets are ready for statistical analyses. Preliminary results from this study indicated that at least 45 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

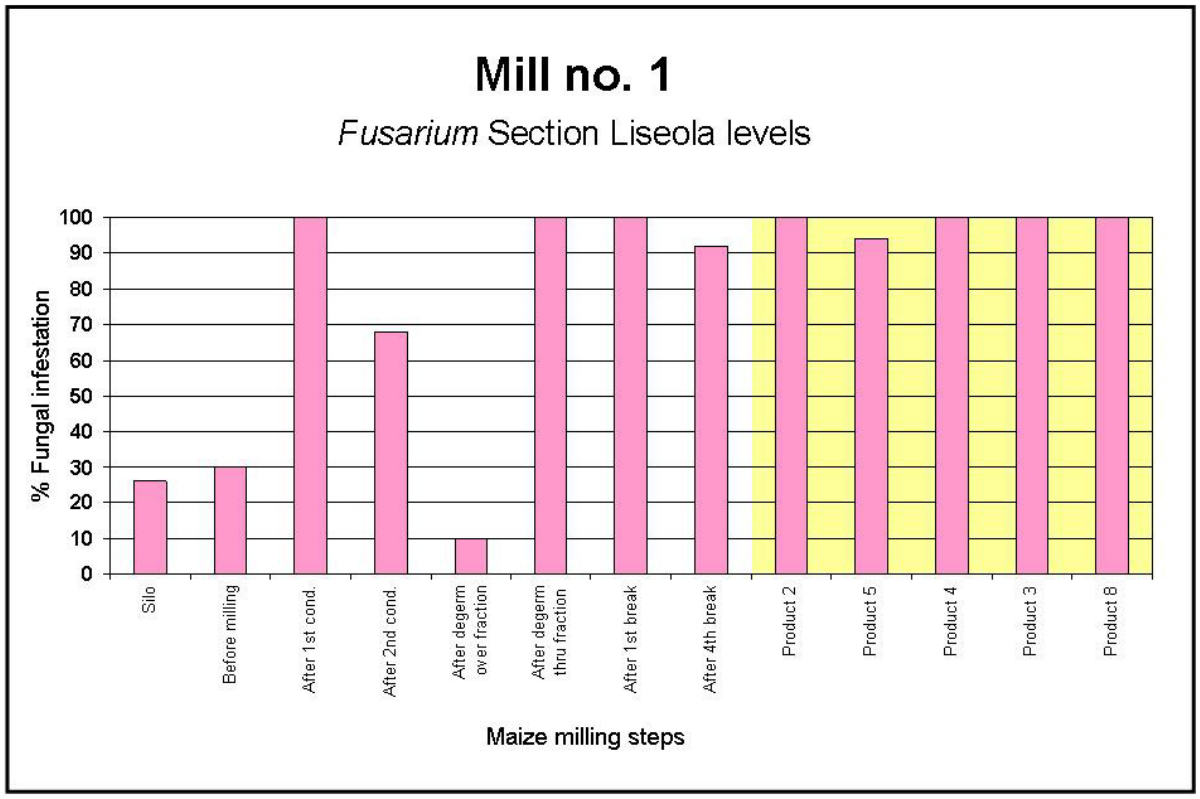
1	*Acremonium* spp.	16	*Eurotium chevalieri*	31	*Penicillium funiculosum*
2	*Alternaria alternata*	17	*Eurotium herbariorum*	32	*Penicillium griseofulvum*
3	*Aspergillus candidus*	18	*Eurotium repens*	33	*Penicillium implicatum*
4	*Aspergillus clavatus*	19	*Eurotium rubrum*	34	*Penicillium pinophilum*
5	*Aspergillus flavus*	20	*Fusarium* sp. (Liseola)	35	*Penicillium purpurogenum*
6	*Aspergillus fumigatus*	21	*Fusarium chlamydosporum*	36	*Penicllium oxalicum*
7	*Aspergillus niger*	22	*Fusarium equiseti*	37	*Penicillium variabile*
8	*Aspergillus ochraceus*	23	*Fusarium graminearum*	38	*Penicllium waksmanii*
9	*Aspergillus sydowii*	24	*Geotrichum* spp.	39	*Phoma sorghina*
10	*Aspergillus terreus*	25	*Mucor* spp.	40	*Pithomyces* spp.
11	*Aspergillus versicolor*	26	*Nigrospora* spp.	41	*Rhizopus oryzae*
12	*Aureobasidium pullulans*	27	*Penicillium aurantiogriseum*	42	*Rhizopus microsporus*
13	*Chaetomium* spp.	28	*Penicillium chrysogenum*	43	*Sordaria* spp.
14	*Cladosporium cladosporioides*	29	*Penicillium citrinum*	44	*Stenocarpella maydis*
15	*Eurotium amstellodami*	30	*Penicllium fellutanum*	45	*Trichoderma reesei*

The more important groups of fungi when it comes to the production of mycotoxins and quality purposes, include *Fusarium* species in the section Liseola, *Aspergillus flavus, Aspergillus clavatus. Eurotium* species, *Penicillium* species, *Cladosporium cladosporioides* and zygomycetous fungi. The importance of each of these groups of fungi has been discussed in the previous report (September 2007).

Individual fungal species have been tracked throughout the milling process and data generated indicate the distribution and prominence of these organisms. An example is the presence of *Fusarium* species that belong to the Liseola section. They include all the chain forming *Fusarium* species of which *Fusarium verticillioides* belong. The latter fungus is responsible for the production of fumonisins in maize.

The following graphs indicate the spread and prominence of these *Fusarium* species in all 6 mills sampled. Note that this represents only the first site visits and were not yet statistically analyzed. The graphs are only an example to indicate the tendencies experienced in these mills. A complete dataset will be available in the final report.

See graphs 1 to 6.

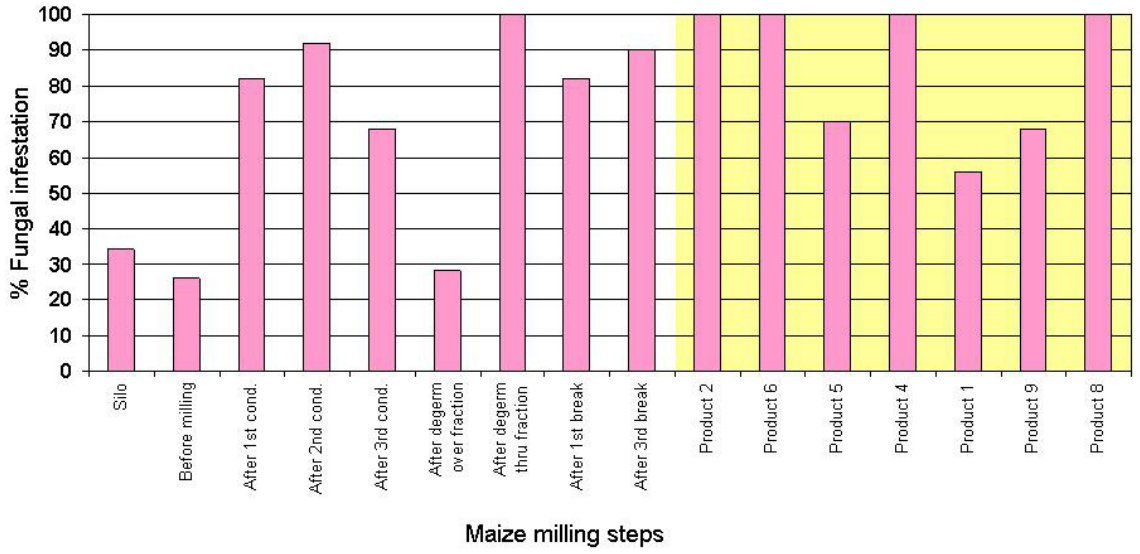

Mill no. 1
Fusarium Section Liseola levels
% Fungal infestation
100
90
80
70
60
50
40
30
20
10
0
Silo
Before milling
After 1st cond.
After 2nd cond.
After degerm over fraction
After degerm thru fraction
After 1st break
After 4th break
Product 2
Product 5
Product 4
Product 3
Product 8
Maize milling steps

Mill no. 2

Fusarium Section Liseola levels

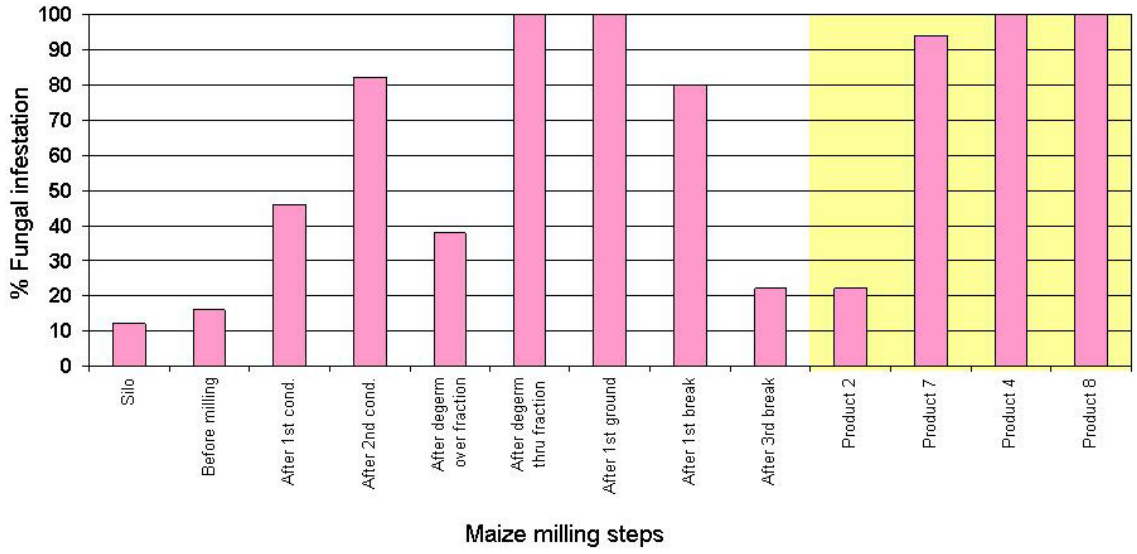
Mill no. 3

Fusarium Section Liseola levels



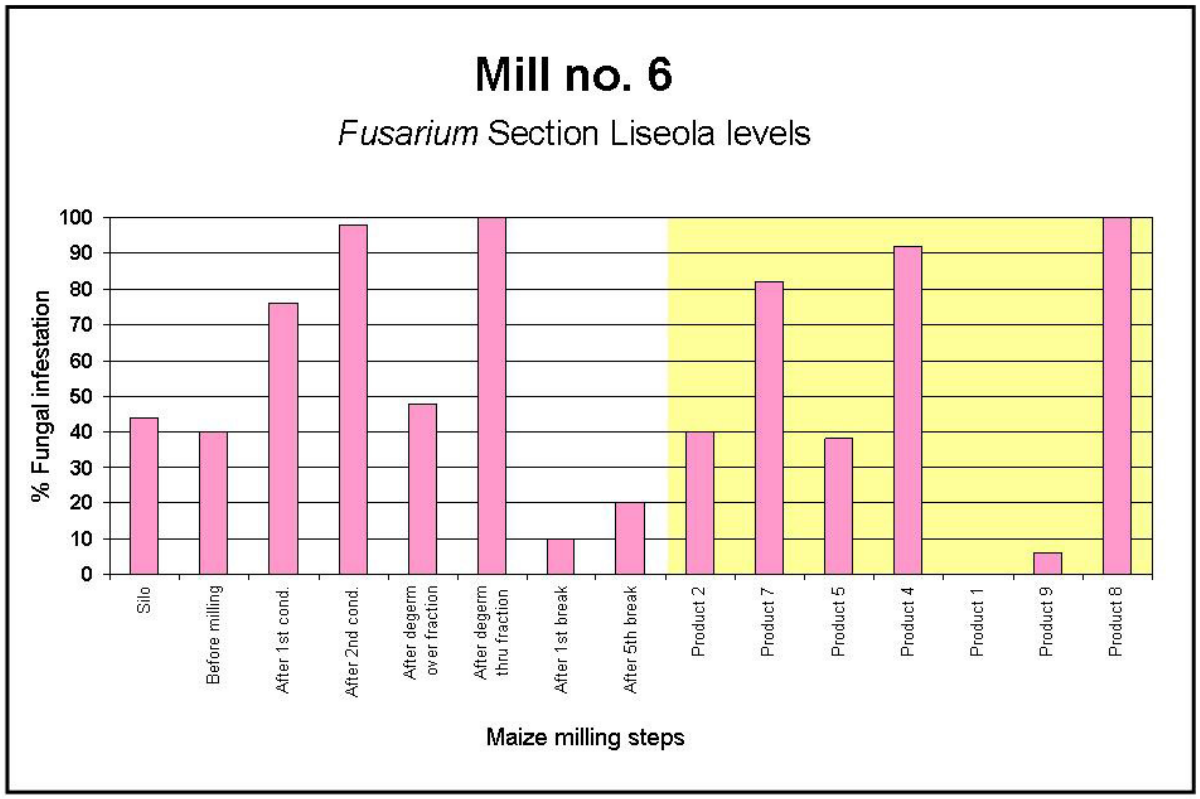
Mill no. 4

Fusarium Section Liseola levels



Mill no. 5

Fusarium Section Liseola levels



Graphs indicating the distribution and prominence of other fungi were purposefully not included in this report as the final statistical analyses have not yet been done and the results are preliminary at this stage. A numbering system is currently used for the various products indicated in the graphs to ensure confidentiality.

Mycotoxin analyses are currently done by using ELIZA based technology e.g. the VICAM test system. Confirmation still needs to be done and therefore results are not available for reporting yet.

4.3.4 Discussion and conclusions

The identification of fungi was done via morphological characteristics. It became evident that the identification to species level could be inaccurate only considering morphology. An additional MSc project is conducted where identifications are now done by using DNA based characteristics. The most predominant seems to be *Fusarium verticillioides*, or a *Fusarium* species closely resembling this fungus. In all 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 was evident that the milling process in general seems to contribute to the higher levels of fungi such as *Penicillium* species and *Aspergillus flavus*. In total, 11 different species of *Penicillium* are associated with the maize mills in this study.

4.3.5 Recommendations and further work still planned

Almost all sampling sets at all 6 mills have been completed. The initial findings as discussed in the previous report still seem to be the same. This indicates 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 have a high risk of introducing mycotoxins into the maize products.

The findings to date are only indications and the statistical analyses still need to be done to confirm these initial observations.

4.4 Statistical manipulation

Statistical manipulation of data will be carried out when the final sampling sets of the 3 outstanding maize mills were analyzed.

5. REFERENCES

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