FINAL PROGRESS REPORT
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PROJECT TITLE

Quantification of Fumonisin in South African Maize by means of Near Infrared Spectroscopy

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Introduction

Fumonisins are mycotoxins produced by *Fusarium verticillioides* in maize and were first isolated at PROMEC in 1988 (Gelderblom *et al.*, 1988). Considerable attention is presently focused worldwide on the human health implications of fumonisin ingestion (Thiel *et al.*, 1992; Marasas *et al.*, 1993).

A number of validated analytical methods for the detection of fumonisins have been developed, of which most are based on high-performance liquid chromatography (HPLC). Although commercial enzyme-linked immunosorbent assays (ELISAs) are available, many laboratories, especially in developing countries, still rely on thin-layer chromatography (TLC) as the primary methodology for mycotoxin determination (Shephard & Sewram, 2004). TLC, however, also requires lengthy sample extraction and clean-up, as well as requiring costly solvents. It is therefore evident that a need still exists for an easily applicable rapid screening method, which is cost effective and can provide quantitative data.

Near infrared spectroscopy (NIRS) has been used routinely during many years at elevators and mills for determination of protein and moisture in cereals. Recent research has focussed on investigating NIRS for the detection of the mycotoxin deoxynivalenol (Dowell *et al.*, 1999; Pettersson & Aberg, 2003; Berardo *et al.*, 2005) and fumonisins in maize kernels (Dowell *et al.*, 2002).

The objective of this project was to investigate the possibility of using near infrared spectroscopy (NIRS) for quantitative screening of ground maize for the detection fumonisin.

Materials and Methods

Samples and sample preparation

Ground maize samples (n = 73), with known fumonisin content (Crop Quality Survey Samples 2004/2005), were obtained from the Southern African Grain Laboratory (SAGL). Permission for this was granted by the Grain Silo Industry (Pty) Ltd and no results will by published before consultation with them and approval from the silo-owners. Unfortunately it was not possible to obtain whole grains of the same samples at the time. It was therefore decided to continue with this investigative study with only ground maize samples. The whole grain samples supplied by mills were not adequate in numbers for NIRS calibration development and will be combined into a data set in future work.

Reference methods for NIRS calibration model development

Quantification of fumonisin using the VICAM Fluorometer

Fumonisin content was determined at the SAGL by means of the VICAM Fluorometer test (n = 73) according to the VICAM Fumoni Test Instruction Manual (November 2002). Results of single analyses have been reported in ppm ($mg.kg^{-1}$).

Quantification of fumonisin using HPLC

Total fumonisin content was also determined using HPLC (n=22) for verification purposes, according to the method as described by Sydenham *et al.* (1996). Results of single analyses have been reported in ppm ($mg.kg^{-1}$).

Near infrared spectroscopy measurements

Collection of spectra

A Büchi NIRLab N-200 Fourier transform near infrared (FT-NIR) spectrophotometer with NIRLabWare (version 3.0) near infrared (NIR) measurement software was used to perform the NIRS measurements in diffuse reflectance mode. The ground maize samples were presented to the instrument in rotating glass petri-dishes and the NIR spectra collected from 1100-2500 nm at a resolution of 8 cm^{-1} resulting in 1557 data points as a data point was collected at every 3.86 cm^{-1}.

Spectral characterisation

Spectral characterisation was performed on the raw spectra (no pre-treatment) and FT-NIR spectra pre-treated with multiplicative scatter correction (MSC) were investigated by means of principal component analysis (PCA) using Unscrambler v9.2 (Camo Process, As, Norway) software. Loading plots of principal components (PCs) 1-3 were constructed and used to describe the physical and chemical influences, causing spectral variation.

NIRS calibration model development

Unscrambler v9.2 was used for calibration model development on spectra (n = 73) pre-treated with MSC using the VICAM Fluorometer test results as reference data. The calibration model was validated by means of full cross-validation (leave-one-out) and no outliers were removed. Calibration model development was also attempted on spectra (n = 22) pre-treated with MSC using the HPLC results as reference data. The model was validated by means of full cross-validation.

The accuracy of the calibration models was expressed by means of the standard error of calibration corrected for bias (RMSEC), standard error of cross-validation corrected for bias (RMSECV), the coefficient of determination (R^2) and the ratio of SEP to standard deviation of the cross-validation set (RPD), which is an indication of the efficiency of a calibration. The goal of model development is to obtain a calibration model with a low RMSECV, a high R^2, preferably above 0.91 and a RPD higher than 5.

Results and Discussion

Spectral characterisation

Typical raw (no-pretreatment) NIR spectra of ground maize samples are shown in **Figure 1**. Looking at the spectra is important as it is the first opportunity to identify obvious incorrect NIR measurements.

Ground maize samples consist of particles of different sizes and there will be more light scatter with small particles than larger ones. This difference in light scatter in diffuse spectroscopy must be reduced to ensure that chemical differences are not overshadowed by physical differences when developing calibration models. The spectra after exclusion of particle size variation using MSC are shown in **Figure 2** from which it is clear that the variation in particle size has been effectively removed.

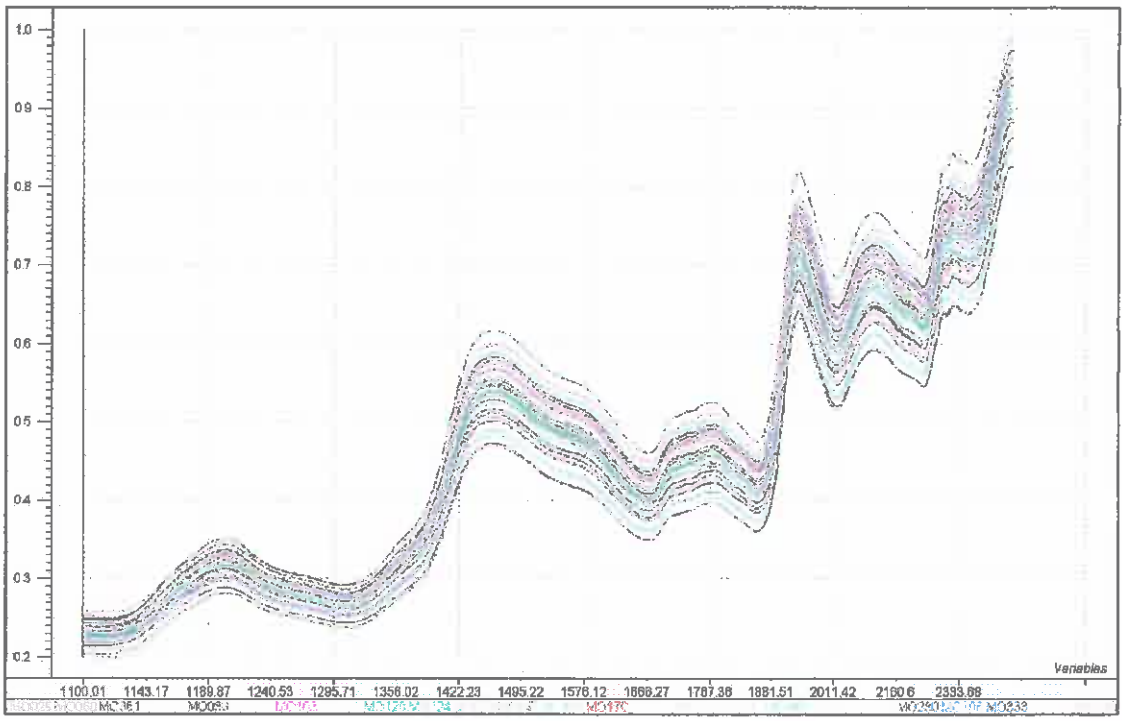

Figure 1 Typical NIR spectra of ground maize samples.

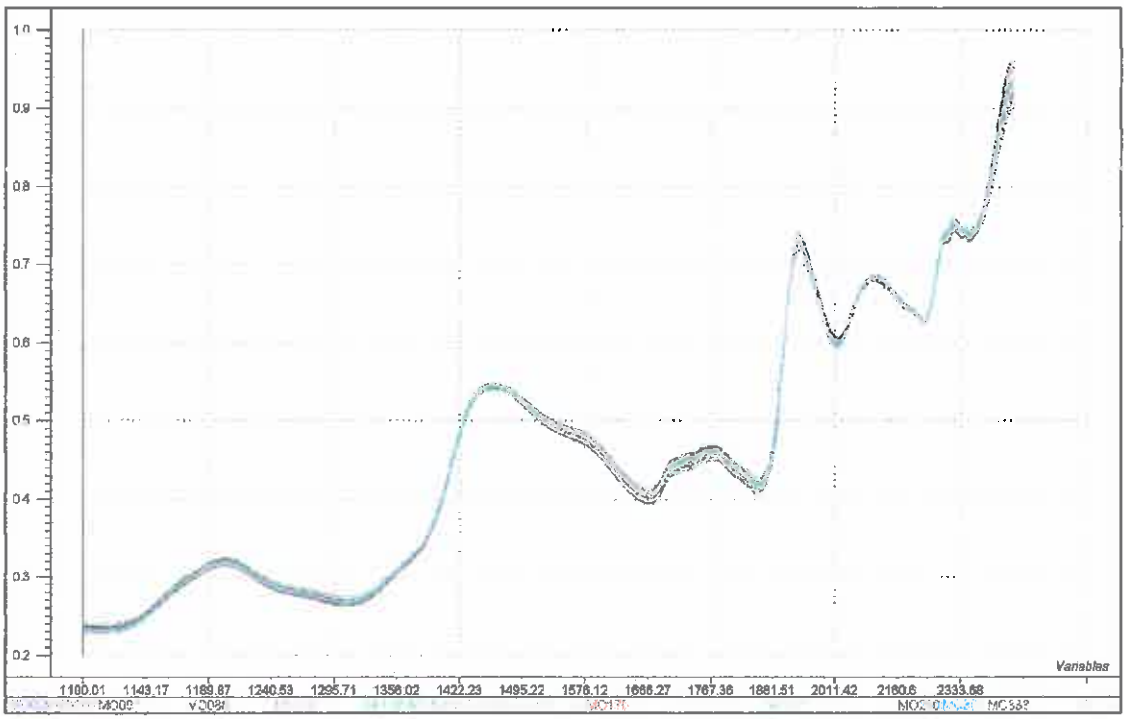

Figure 2 Typical NIR spectra of ground maize samples after removal of the effect of particle size using multiplicative scatter correction (MSC).

The loading plots of the first three principal components (PCs) for the ground maize samples before and after exclusion of particle size variation are shown in **Figures 3 and 4**, respectively. The PC loadings give useful insights into the structure of the NIR spectra, the most important wavelengths and subsequently the basis of the calibration.

The first two PCs of the raw data explain 99% of the variation in the data set (PC1 = 97%; PC2 = 2%). The shape of the loadings of PC1 is similar to that of the raw spectra indicating differences in particle size (**Figure 3**). The loadings of the second PC has the shape of an upside down spectrum also indicating particle size differences, with the loadings of PC3 having the shape of a typical moisture spectrum. This factor, however, does not contribute to the total variation in the data set. After removal of the effect of particle size differences, the shape of the loadings of the PCs changes to plots similar to that of a moisture spectrum indicating the effective removal of the effect of particle size variation (**Figure 4**). The first three PCs of the MSC corrected factors explain 94% of the variation in the data set (PC1 = 73%; PC2 = 13% and PC13 = 8%). The loadings of PC2 and PC3 show prominent peaks in the region of 1940 nm (O-H stretch + O-H deformation). This wavelength region corresponds most strongly to the absorption of water, indicating that most of the information about moisture content is contained in PC2 and PC3, but they only contribute 21% of the total variance. The influence of moisture is also noticeable in PC1, but to a lesser extent with the influence of starch at *ca.* 1900 nm (O-H stretch + 2 x C-O stretch) and 2100 nm (2 x O-H deformation + 2 x C-O stretch) evident. Absorption bands, previously linked to the presence of fumonisin in maize, by Berardo *et al.* (2005), are also present in PC1 at *ca.* 2242 nm (N-H stretch + NH_3^+ deformation), 2310 nm (C-H stretch + C-H deformation) and 2336 nm (C-H stretch + C-H deformation) (**Figure 4**).

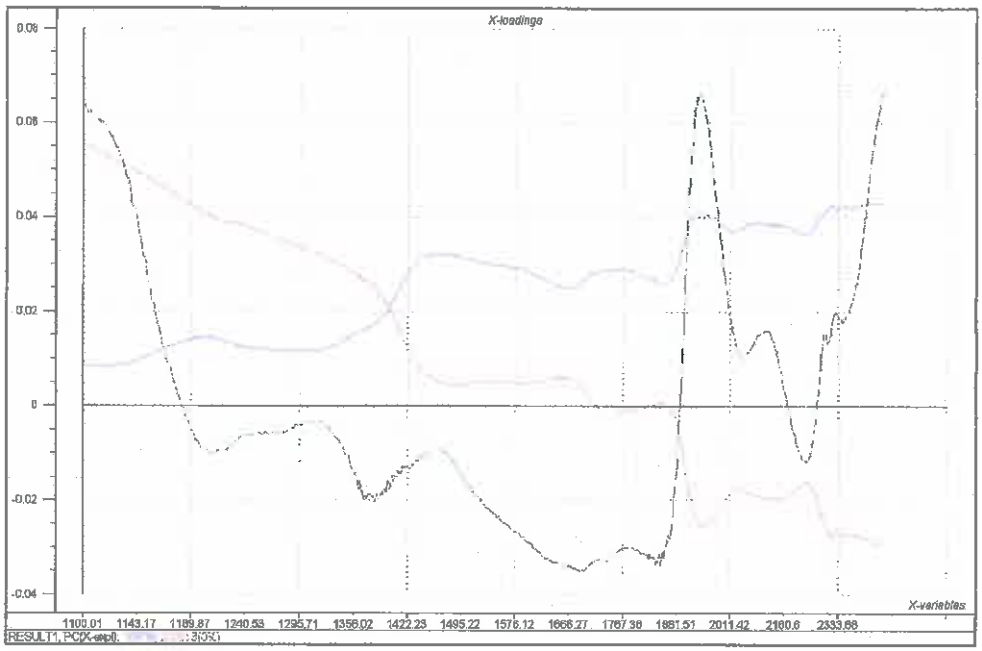

Figure 3 PCA loading plots of the first three principal components of the raw ground maize spectra.

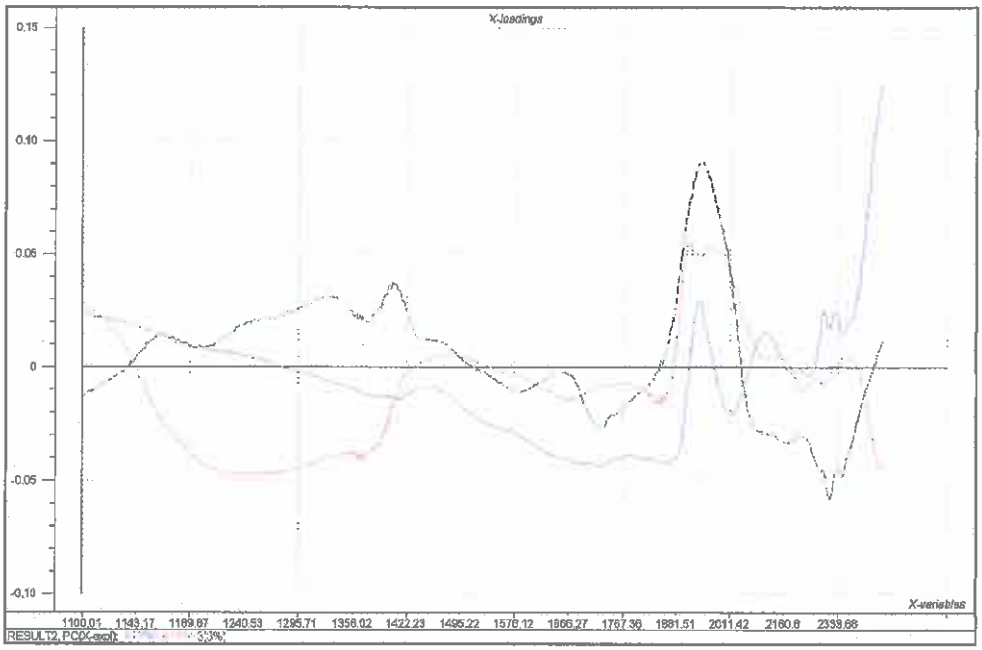

Figure 4 PCA loading plots of the first three principal components of the MSC corrected ground maize spectra.

NIRS calibration model development

A summary of the reference data for fumonisin content is given in **Table 1** and a summary of the NIRS calibration model and full cross-validation results for the prediction of fumonisin in ground maize in **Table 2**. It is clear from the results in **Table 2** that it is not possible to determine fumonisin in ground maize by NIRS with the calibration model developed with the data currently available. One of the most important factors to be taken into account during the development of NIRS calibrations is the accuracy of the reference methods. It was therefore decided to verify the VICAM Fluorometer test results with results obtained by HPLC analysis. The lack of a good correlation between the results, of these two methods as determined on 22 of the samples, is shown in Figure 5. Attempting to develop a calibration model with these 22 samples and HPLC as reference method, shows improvement, however, the calibration model is still not useable. These results indicate that the VICAM Fluorometer test might not be adequately accurate to be used as a reference method for NIRS calibration development to quantify fumonisin in ground maize.

Table 1 Summary of reference data for prediction of fumonisin content.

	Fumonisin VICAM Fluorometer	**Fumonisin** VICAM Fluorometer	**Fumonisin** HPLC
n	73	22	22
Range (ppm)	0 – 6.600	0 – 3.126	0 – 5.300
Mean (ppm)	1.162	0.948	0.372
Standard deviation (SD)	1.268	1.267	0.753

It is also important to increase the number of samples. The total number of samples currently in the data set (n = 73) is not adequate for effective calibration development and should be increased. It is, however, important that the sample set is not only increased in number but also that the range of fumonisin contamination be increased.

Table 2 Summary of the NIRS calibration and full cross-validation results for the prediction of fumonisin content in ground maize.

	Fumonisin VICAM Fluorometer	**Fumonisin** VICAM Fluorometer	**Fumonisin** HPLC
SEC (ppm)	1.249	1.184	0.658
R^2	0.020	0.080	0.200
Bias	0.000	0.000	0.000
RMSECV (ppm)	1.290	1.370	0.746
R^2	0.010	0.010	0.040
Bias	-0.002	-0.025	0.000
PLS factors[a]	1	1	1
RPD	1.017	1.080	0.991

[a] Number of PLS factors used.

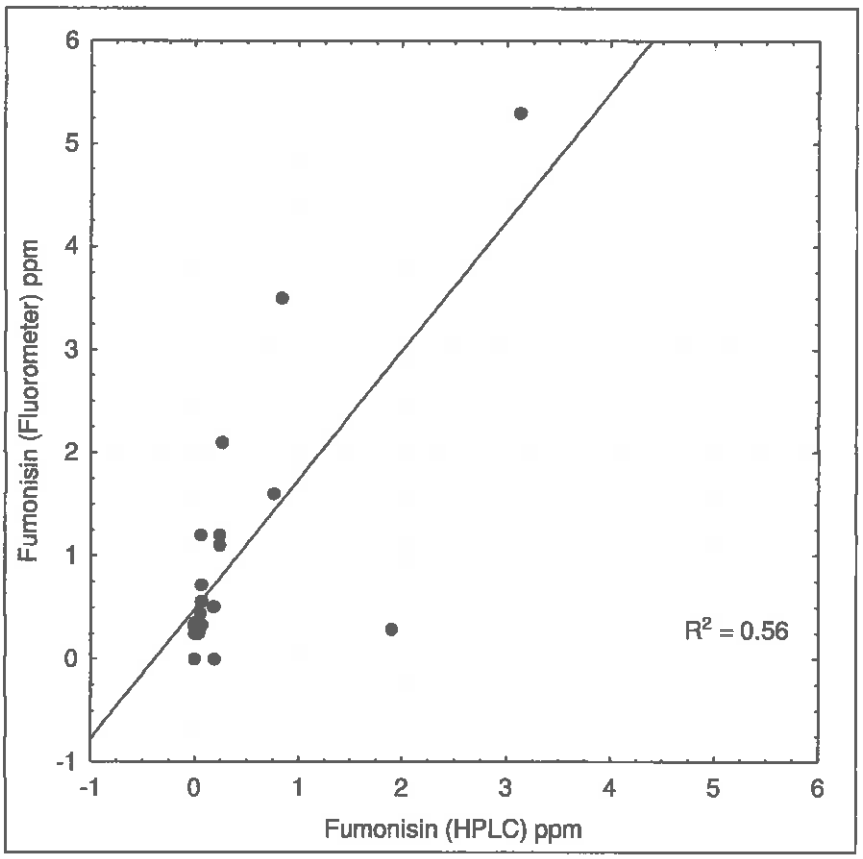

Figure 5 Correlation plot between fumonisin data as determined by HPLC and Fluorometer test.

Conclusions

Although the current results do not look promising, it was possible to identify factors that could have been the cause of the poor results. These factors should be taken in consideration in future work which, if appropriately addressed, is expected to result in improved calibration models. Apart from increasing the number of samples in the data set and using HPLC as reference method it is also crucial that the range of the results be increased. It should be attempted to obtain more samples with much higher levels of contamination. Replacing the glass petri-dishes, currently being used as sample holders, with a sample holder with a quartz bottom (became available recently) could also possibly contribute to improved results.

Constraints

Constraints that affected the outcomes of the current project were firstly the resignation of Evan Springfield who originally submitted the funding application for the project; it took longer than anticipated to obtain samples. Pumza Gatyeni, who was responsible together with Evan Springfield for the HPLC analyses, also resigned. Both posts are still vacant and it resulted in not all the HPLC analyses being completed to date. We are, however, continuing with the HPLC analysis after which new calibration models will be developed and the results forwarded to the Maize Trust immediately.

Budget and expenditure statement

To be submitted.

Dissemination of results

Once a suitable calibration has been obtained the results will be submitted for publication in a peer reviewed scientific journal as well as in a popular magazine such as Farmer's Weekly.

Utilisation of project results

The results obtained so far are not suitable for utilisation in the South African maize industry. Implementing the identified factors should result in an improved calibration model. Once a suitable calibration model has been established it would lead to tremendous savings, knowing the cost of current mycotoxin determinations.

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