Fusarium and fumonisin in GM maize grown by small-scale farmers in KwaZulu-Natal, South Africa

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The genetic modification (GM) of maize to contain proteins that act to control insects has become a widespread agricultural practice. Although the reduction of insect damage to maize ears could potentially increase crop yield, rural small-scale farmers might be reluctant to buy expensive GM seed every season even when the lower fungal infection of the GM maize might also result in health benefits. This study was conducted over 5 years in three districts of northern KwaZulu-Natal, South Africa, to study the *Fusarium verticillioides* and *F. proliferatum* infection and fumonisin contamination levels in *Bt* maize, Roundup-Ready® maize, conventional commercial maize and traditional landrace maize planted by rural farmers following their traditional agricultural practices. Mean *Fusarium* infection rates varied between 3.0% and 38.3% with large standard deviations. *Fusarium* infection was not significantly different ($p>0.05$) between the various genotypes, possibly due to the wide variation in results and low sample numbers. Although the fumonisin results also showed wide variation, the trend of contamination was lower in *Bt* maize compared to conventional commercial genotypes. The mean fumonisin levels in *Bt* hybrids were mostly <300 µg/kg, ensuring a safe maize supply in populations consuming maize as a dietary staple. The wide variations in *Fusarium* and fumonisin levels within each district point to the influence of local agricultural practices, local environmental conditions, and seasonal variations. Reducing exposure to fumonisins in these communities requires both further attention to the possible influence of these factors, as well as the use of appropriate post-harvest strategies.

Significance:

This study was the first in South Africa to follow rural farmers planting *Bt* and commercial maize hybrids as well as open-pollinated landrace maize, according to their traditional agricultural practices. The results show that in some instances the *Bt* maize had the lowest fumonisin levels.

Introduction

Bacillus thuringiensis (*Bt*) genes express an insecticidal crystal protein that has been shown to be a powerful deterrent of lepidopteran and coleopteran pests in maize, thereby reducing damage caused by these insect pests.[1-3] Insect damage in maize, particularly by stalk borers, is the main mechanism of fungal infection which can cause reduced crop yields. Hence, efficient insect control can have the additional benefit of a concomitant reduction in *Fusarium* infection rates, particularly that of the fumonisin mycotoxin producer, *Fusarium verticillioides*.[4,5]

South Africa has an estimated 50 000 large-scale conventional commercial farmers, 240 000 small-scale farmers and more than 1 million farmers who produce food on a subsistence level.[6,7] Insect-resistant, transgenic (*Bt*) maize has seen impressive adoption rates by conventional maize farmers since its introduction in the 1998/1999 crop season. A survey among maize farmers planting *Bt* maize in South Africa showed average maize crop yield improvements of 11% and reductions in pesticide use of between 50% and 80%.[8] At a subsistence level, the predominant practice remains the planting of open-pollinated landrace varieties using seed held over from the previous crop. Between these two groups, small-scale farmers plant either open-pollinated or conventional commercial varieties, but yields can be low.[6] However, the use of the improved *Bt* hybrids by small-scale farmers can be problematic. Both subsistence and small-scale farmers in South Africa may not be able to make use of the technology if it is not appropriate to their needs, if it is too expensive, or if it is not available to them because there is no government infrastructure or incentives for companies to sell genetically modified (GM) seed to small-scale farmers. Nonetheless, more efficient maize production methods suitable for small-scale farmers would bring economic benefits and add to the level of food security of the whole southern African region, where maize is the staple food, by increasing the availability of maize in the countryside.

In this study, we investigated the possible economic benefits that would accrue to smallholder farmers in South Africa through the adoption of *Bt* maize. The University of Pretoria, with funding from the Rockefeller Foundation, initiated this multi-year study of crop yields among farmers in three districts of the KwaZulu-Natal Province, South Africa.[6] For farmers adopting *Bt* technology, the data collected during the study years show economic benefits from reduced insecticide application and reduced harvest loss due to stalk borer in districts and/or years in which there was significant insect pressure.

Given the perceived benefits of GM maize, the aim of the multi-year study reported here, as an adjunct to the above economics study, was to measure the effect on *Fusarium* infection and fumonisin contamination of introducing new GM technologies in three rural maize growing districts in northern KwaZulu-Natal, South Africa. Given the improvement in economic parameters shown by the study, it was hoped that there would also be reduced fumonisin exposure and thus a health advantage to the adoption of these GM varieties by smallholder farmers, who also consume a portion of their crop in a subsistence scenario.

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Materials and methods

Study areas and sampling

This study was conducted over five successive crop seasons among dry land small-scale farmers in the districts of Simdlangentsha and Hlabisa, northern KwaZulu-Natal Province, South Africa. The study was extended to the Dumbe district for the last three seasons. Workshops were held, 2 years prior to the start of the trial, to introduce *Bt* maize to smallholder farmers during which they were provided with small bags of *Bt* maize seed for free. Thereafter, farmers who wanted to plant the new seed were required to purchase it. Further, for season 2, the Roundup-Ready® (RR) maize seed, genetically modified to enable the use of Roundup® herbicide, was also made available. In addition, farmers in the study areas planted conventional commercial varieties and some planted open-pollinated traditional maize.

Problems associated with maize sampling in rural subsistence farming areas have been previously noted.[9] Samples of all the above maize types were collected from farmers participating in the agricultural economics study of Gouse et al.[6] A minimum of 2 kg of shelled maize held by the farmer was obtained to be as representative of the harvest as possible. As the study did not involve organised field trials but rather was aimed at monitoring the maize harvested by farmers using their traditional practices and irrespective of their seed choice, sample numbers for traditional maize, conventional commercial maize, *Bt* maize and RR maize varied widely between districts and between years, depending on the maize type favoured in any one crop year, crop yield, farmer availability to field workers and farmer acceptance of participation in the economic study of the University of Pretoria.[6] Collected maize samples were stored in linen bags at 4 °C until analysed for fumonisins and *Fusarium* infection.

Fumonisin determination

Fumonisins B_1 (FB_1), B_2 (FB_2) and B_3 (FB_3) were determined by high-performance liquid chromatography as previously described.[10,11] Briefly, maize was ground in a laboratory mill to a fine meal and extracted with 100 mL methanol/water by homogenisation. A 10 mL aliquot was applied to a strong anion exchange solid phase extraction cartridge and the fumonisins were eluted with acetic acid in methanol. The purified extract was evaporated to dryness with nitrogen gas at 60 °C and the dried residue was stored at 4 °C whilst awaiting chromatographic analysis. The derivatised extracts were separated on a reversed-phase Luna 4 μm C18 (2) (150 x 4.6 mm I.D.) column (Phenomenex, Torrance, CA, USA) and the isocratic mobile phase of methanol/0.1 M sodium dihydrogen phosphate (pH 3.35) (77:23) was pumped at a flow rate of 1 mL/min. The chromatographic system consisted of a Rheodyne 7725i injector (Cotati, CA, USA), Waters Model 510 solvent delivery system (Milford, MA, USA), Borwin Chromatography Integration Software (Varian JBMS Developements, Le Fontanil, France) and Waters Fluorescence 474 detector (excitation – 335 nm and emission – 440 nm). Fumonisin data are indicated throughout the current study as total fumonisin levels (FB_1+FB_2+FB_3) and were not corrected for recovery.[10]

Fusarium determination

As *Fusarium verticillioides* and *F. proliferatum* are the main species contributing to fumonisin contamination in South African studies, they were identified by morphology and quantified as percent infected kernels as previously described.[7,12-15] Briefly, a subsample (approximately 150 g) from each well mixed sample was surface-disinfected for 1 min in a 3.5% sodium hypochlorite solution and rinsed twice in sterile water. One hundred kernels per subsample were plated (five kernels per petri dish) onto 1.5% malt extract agar (MEA) containing 150 mg/L novobiocin to minimise bacterial growth. The MEA plates were incubated in the dark at 25 °C for 5–7 days. *Fusarium* species that developed from the kernels were then identified according to their morphological characteristics.[14]

Statistical analyses

Statistical analysis was done on the mycology and fumonisin data using SPSS software (Chicago, IL, USA) to determine any significant differences between the maize categories/groups (i.e. traditional, conventional, *Bt*, and Roundup-Ready). Locations were not compared with each other, neither were seasons. Analyses were conducted on the '*F. verticillioides* + *F. proliferatum*' FvFp and total fumonisin variables only, with natural log transformation of the latter variable. Pearson's correlation coefficients, between the FvFp fungal and ln-transformed total fumonisin levels, were calculated for each location.

Results and discussion

This study was intended to monitor the *Fusarium* and fumonisin contamination of the maize harvest of small-scale farmers planting a variety of maize genotypes, where the farmers themselves selected the varieties to plant and conducted their own normal agricultural practices.[16] A summary of total fumonisins is given in Table 1. Detailed data (number of samples, number of positive samples, mean, standard deviation and range) of *F. verticillioides, F. proliferatum*, their sum, FB_1, FB_2, FB_3 and their sum as total fumonisins are shown in Supplementary table 1. Consequently, sample numbers varied greatly depending on cooperation of the farmers and the actual uptake of the new GM maize varieties. As the results show, standard deviations of the mean data for *Fusarium* infection and total fumonisins were large, and consequently, only a limited number of results were statistically significantly different at the 5% level.

Of the two fumonisin-producing *Fusarium* species analysed, *F. verticillioides* predominated by far and in many cases *F. proliferatum* was not found in the samples. Mean infection rates for *F. verticillioides* ranged from 3.0% (Simdlangentsha *Bt* maize season 3) to 38.3% (Dumbe Roundup-Ready® season 3). The low sample numbers obtained in these results precluded statistical analysis. The infection rates varied widely within years, and, in general, no consistent trend was observed between the different maize genotypes. In only one instance was there a significant difference in mean *F. verticillioides* and *F. proliferatum* infection rates, namely in Simdlangentsha in season 5. In this instance, *Bt* maize (mean 11.4 $\pm$ 12.3%) and RR maize (mean 10.7 $\pm$ 9.9%) differed significantly ($p<0.05$) from conventional varieties (mean 30.4 $\pm$ 24.5%). The low incidence of *F. proliferatum* is similar to mycological results from the Centane and Mbizana areas of the former Transkei in a study conducted over 3 years.[14] In that study of good and mouldy maize harvested by rural farmers, no *F. proliferatum* was detected using the same analytical method. The traditional practice by the subsistence farmers of keeping the best cobs of the harvest for use as seed the following season might have acted as a natural breeding programme, selecting out the hardiest plant material.

Total fumonisin levels varied widely with high standard deviations (Table 1) and no correlation was found with *Fusarium* infection rates. Significant statistical differences between hybrids within individual areas were limited due to the high standard deviations. Nevertheless, Table 1 shows the five cases in which statistical differences were obtained where the *Bt* hybrid was statistically ($p<0.05$) less contaminated than conventional varieties. Overall, *Bt* maize performed well with low fumonisin levels, apart from a few notable exceptions, such as Hlabisa in season 1. In this case, it was reported that the *Bt* hybrid planted produced a poor ear morphology, with sheath leaves that did not cover the tip of the ear. In addition, these ears did not droop at maturity, so were exposed to the unseasonal rainfall at Hlabisa in 2004. This explains the high fumonisin levels in the Bt hybrid in that particular year.[6] This poor ear morphology was corrected by the relevant seed company in the following years. The maximum level set for fumonisin mycotoxins for raw maize (4000 μg/kg) by the Codex Alimentarius Commission have been incorporated into the South African health regulations.[17] Although none of the mean total fumonisin levels in the Supplementary table 1 exceeded 4000 μg/kg, there were several of the highest levels of the ranges of the total fumonisin levels exceeding this set maximum level for fumonisin mycotoxins for raw maize. In general, the *Bt* hybrid achieved mean contamination levels of the order of or less than 300 μg/kg – a level that has been suggested to achieve a safe maize supply in rural areas, where a large daily consumption of maize is part of the typical diet.[18] Consequently, these lower fumonisin levels carry health benefits in that they reduce exposure to these carcinogenic mycotoxins in rural areas.[9,19]

Table 1: Mean values of all samples (± standard deviation) of total fumonisin (sum of FB_1, FB_2 and FB_3) contamination (µg/kg) of maize varieties collected in three districts (Simdlangentsha, Hlabisa and Dumbe) of KwaZulu-Natal, South Africa over 5 years

Maize type	Total fumonisins ± standard deviation (µg/kg)		
	Simdlangentsha	Hlabisa	Dumbe
Season 1			
Traditional	**753 ± 814 (5)a***	159 ± 91 **(4)**	No sample
Conventional	**623 ± 917 (8)a***	450 ± 627 **(11)**	No sample
Bt	**239 ± 411 (7)b***	1150 ± 1430 **(8)**	No sample
Season 2			
Traditional	271 ± 352 **(6)**	250 ± 353 **(2)**	No sample
Conventional	815 ± 1150 **(12)**	**472 ± 506 (15)a****	No sample
Bt	396 ± 395 **(7)**	**22 ± 25 (11)b****	No sample
Season 3			
Traditional	996 ± 1290 **(5)**	2070 ± 3230 (9)	**426 ± 606 (6)a***
Conventional	No sample	1380 ± 2820 (8)	No sample
Bt	152 ± 64 **(2)**	804 ± 989 (13)	**110 ± 139 (3)a***
Roundup-Ready®	1200 ± 949 **(6)**	870 ± 1600 (12)	**2600 ± 4140** (3)**b***
Season 4			
Traditional	No sample	No sample	3390 ± 5240 (6)
Conventional	**1580 ± 2590 (9)a***	66 ± 103 (5)	932 ± 1510 (16)
Bt	**51 ± 70 (6)b***	129 ± 138 (10)	501 ± 927 (4)
Roundup-Ready®	**2230 ± 4490 (5)a***	466 ± 1370 (12)	179 ± 223 (2)
Season 5			
Traditional	No sample	No sample	No sample
Conventional	**500 ± 962 (10)a***	16 ± 26 (10)	1690 ± 4170 (8)
Bt	**55 ± 169 (11)b***	45 ± 117 (13)	1140 ± 2130 (15)
Roundup-Ready®	**2 ± 3 (11)b***	101 ± 245 (10)	674 ± 1440 (12)

Numbers in brackets indicate the number of samples collected; significant differences between maize types within a year within a location are indicated by bold type; values with different lowercase letters are significantly different, while those with the same lowercase letters are not significantly different:

*$^{*}p < 0.05$

$^{**}p < 0.001$*

Studies conducted in the former Transkei over a number of years have also shown that fumonisin contamination levels can vary widely within a district.[14,15] Thus, improvements in food safety by reducing fumonisin levels may lie in targeting individual agronomic practices and understanding seasonal environmental factors.[9,19] Studies conducted in Argentina and the Philippines to model the effects of environment, insect damage and maize genotype on fumonisin contamination concluded that most of the variability in fumonisin levels could be ascribed to location and weather, rather than to the maize genotype.[1]

The results achieved with RR maize suggest that this hybrid type carries no advantage over *Bt* maize planted in these districts. Indeed, in seasons 3 and 4, RR maize was significantly (p<0.05) more contaminated than the *Bt* hybrids planted, resulting in some of the highest mean contamination levels over all 5 years of the study period. Only a limited number of samples of traditional maize were obtained. The results in Table 1 show that the mean fumonisin contamination levels of traditional maize compare mostly favourably with those of conventional commercial hybrids grown in the same district in the same year. The traditional practice of keeping the best cobs of the harvest for use as seed the following season might have acted as an adaptation mechanism in the form of a natural breeding programme. The variation in fumonisin levels and their reduction may best be approached through studies of individual agronomic practices.

Over the past 20 years several field trials have indicated that, together with reduced *Fusarium* infection rates, a reduction in the levels of the fumonisin mycotoxins was observed.[20-26] Meta-analyses of data contained in 21 publications have confirmed the positive effect of *Bt* genetic modification on fumonisin levels, with decreases of 14–67%, although the degree of reduction reported can depend on the type of statistical approach followed.[27] Thus, the use of GM maize seed that expresses insecticidal proteins has become a common maize production practice.[28]

Conclusions

This study was the first in South Africa to follow rural farmers who planted various maize hybrids as well as open-pollinated landrace maize according to their traditional agricultural practices. The maize harvested by these smallholder/subsistence farmers, irrespective of their seed choice of traditional, commercial, *Bt* or Roundup-Ready® maize, showed lower fumonisin contamination in general and the *Bt* maize in some instances showed the lowest fumonisin levels. However, the sustainability of planting *Bt* maize, which is a technology aimed at reducing insect damage and not directly at harvest yield, has been questioned[29], as it depends on the perceptions of the rural farmers as to whether they are prepared to pay the additional cost of this technology.

The large variations in fumonisin levels observed within each area for each maize variety planted indicate the impact of local farming practices, plus the immense role that environmental and climatic conditions played in the outcome of this study. Thus, more controlled studies by researchers (rather than smallholder farmers) with larger sample sizes would be required to determine if GM maize would be less susceptible to *Fusarium* infection and fumonisin contamination under South African conditions. It should also be kept in mind that traditionally the smallholder/subsistence farmers select their best seed visually when they harvest their crops for planting in the following season. Thus, to ensure that fumonisin levels are kept as low as possible, both improved agricultural practices as well as appropriate post-harvest interventions are required to achieve a safe rural maize supply.[30]

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Competing interests

We have no competing interests to declare.

Authors' contributions

J.P.R.: Conceptualisation; methodology; data collection; data analysis; writing – the initial draft. L.v.d.W.: Methodology; data analysis; writing – revisions.

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