Fusarium spp. and levels of fumonisins in maize produced by subsistence farmers in South Africa

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Fusarium spp. produce fumonisins – mycotoxins that are of importance to maize production in South Africa. Fumonisins have been associated with human oesophageal cancer and cause various diseases in animals that are of concern to the animal feed industry. Maize samples, collected from subsistence farm fields in the Eastern Cape, KwaZulu-Natal, Limpopo and Mpumalanga provinces of South Africa during the 2006 and 2007 growing seasons, were analysed for *Fusarium* spp. and contamination with fumonisins. *Fusarium verticillioides* was the most common *Fusarium* species in maize followed by *F. subglutinans* and *F. proliferatum.* Levels of contamination with fumonisins ranged from 0 µg/g to 21.8 µg/g, depending on the region where samples were collected. Levels of fumonisins were highest in northern KwaZulu-Natal (Zululand) where 52% and 17% of samples collected in 2006 and 2007, respectively, exceeded 2 µg/g. Regression analyses showed a positive correlation between fumonisin-producing *Fusarium* spp. determined by real-time polymerase chain reaction and concentration of fumonisins (r = 0.93). Many samples from Zululand, and some from Mokopane (Limpopo) and Lusikisiki (Eastern Cape), contained fumonisins at levels well above the maximum levels of 2 µg/g set by the Food and Drug Administration (USA) and therefore also the limit of 1 µg/g set by the European Union for food intended for direct human consumption. Regulations governing contamination of grain with fumonisins are not yet implemented in South Africa. The high incidence of fumonisins in subsistence farming systems indicates the need for awareness programmes and further research.

Introduction

Maize (*Zea mays* L.) is grown in two production systems in South Africa: intensive commercial farming systems and resource-poor subsistence farming systems. Average production amounts to approximately 9.2 million tonnes per annum, of which 8 million tonnes is utilised in the country as food and fodder.[1] Depending on the surplus available, the remainder is exported to neighbouring countries.[2] Because maize quality and yield is important to commercial producers in South Africa, they implement strategies that reduce losses. Subsistence farmers, however, lack the required resources to ensure production of quality grain from field planting through to consumption.[3] With an insufficiency of hybrid seed, fertilisers and pesticides, their yield and product quality is often severely affected by poor soil fertility, fungal infections and pest damage.[4]

Over 600 000 households depend on subsistence farming in South Africa.[5] With maize being a staple food, the average intake per person per day may exceed 300 g.[6] The quality of maize consumed thus determines the quality of life. Maize produced in subsistence farming systems, however, is often affected by pre- and post-harvest damage, of which fungal infection can be considered one of the most significant problems. Most subsistence farmers plant their fields with seed retained from the previous harvest,[4] thereby increasing the risk of systemic infection by plant pathogens.[7] Late planting and practising maize monoculture also increases fungal inoculum and pest damage, thereby resulting in increased fungal infection of crops.[8] Agricultural practices, such as retaining crop residues on the soil surface, may further increase the severity of diseases such as stem, ear and root rot in the following season.[9]

One of the most important fungal pathogens affecting maize in South Africa is *Fusarium verticillioides* (Sacc.) Nirenberg (syn = *F. moniliforme* Sheldon), a ubiquitous facultative endophyte found in most maize-producing countries of the world.[10] *Fusarium verticillioides* can constitute up to 95% of all *Fusarium* strains recovered from maize fields in African countries.[11] This fungus produces fumonisins, toxigenic secondary metabolites that are well recognised for their cancer-promoting effects.[12] Other *Fusarium* spp. that produce mycotoxins in maize include *F. graminearum* Schwabe, that produces zearalenone and deoxynivalenol,[13] *F. proliferatum* (Matsushima) Nirenberg, that produces fumonisins[13] and *F. subglutinans* (Wollenw. and Reinking) P.E. Nelson, Toussoun and Marasas,[14] a producer of moniliformin.[15]

TABLE 1a: *Fusarium* spp. that produce fumonisins as determined by quantitative PCR, fumonisin contamination levels (µg/g) and climatic conditions present in rural maize-production areas of South Africa during 2006.

Locality	No. of samples	Mean							Fumonisins present[c] (%)	Fumonisins >2 µg/g[d] (%)
		Rainfall[a] (mm)	Temperature[b] (°C)	*F. vert* (%)	*F. pro* (%)	*F. sub* (%)	qPCR (pg DNA/mg)	Fumonisin level (µg/g)		
Eastern Cape									**6**	**2**
Bizana	3	77.6	18.8	10.8	0.3	1.5	1.8	nd		
Butterworth	6	88.0	18.3	6.7	2.1	12.3	2.7	nd		
Cofimvaba	2	30.5	19.6	2.8	0.0	18.8	4.0	nd		
Elundini	5	55.3	16.8	0.5	0.2	29.0	0.8	nd		
Engcobo	4	87.3	21.9	14.1	0.6	18.9	4.1	nd		
Idutywa	8	46.5	18.8	5.1	0.5	15.3	4.5	nd		
Centane	6	58.2	19.9	9.8	0.2	11.1	0.8	nd		
Libode	2	79.0	16.3	0.0	0.5	9.0	5.4	nd		
Lusikisiki	1	75.4	19.2	12.5	0.5	5.0	7.3	2.2		
Maluti	2	63.3	11.9	4.8	0.0	10.3	0.1	0.5		
Mqanduli	2	66.8	17.3	0.5	0.0	2.4	0.0	nd		
Ngqeleni	2	71.4	18.1	8.4	0.0	7.0	0.5	nd		
Queenstown	1	72.7	18.2	1.0	0.0	69.5	62.4	nd		
Qunu	2	66.8	17.3	0.8	0.0	9.3	0.0	nd		
Whittlesea	4	44.4	18.3	5.5	0.5	9.5	0.6	nd		
KwaZulu-Natal Southern Region									**19**	**3**
Bergville	3	119.0	18.1	44.0	1.5	1.0	0.3	nd		
Highflats	4	51.0	22.6	38.4	0.3	3.5	4.4	nd		
Port Shepstone	9	53.0	21.7	28.6	0.4	10.8	3.6	nd		
Umzimkhulu	5	12.2	17.8	4.6	0.1	48.7	0.9	nd		
Underberg	5	157.0	15.4	2.5	0.2	37.3	0.5	nd		
Ndwedwe	6	114.0	18.9	31.3	0.3	7.9	6.7	0.5		
Zululand									**100**	**52**
Eshowe	3	66.5	23.5	28.5	20.1	19.5	68.9	0.8		
Jozini										
Ladysmith	3	142.0	20.0	76.7	0.3	11.5	101.0	1.3		
Manguzi	1	79.4	24.3	97.0	0.0	0.0	297.0	10.9		
Mbazwane	3	79.4	24.3	37.2	0.2	3.0	461.0	6.2		
Pongola	6	57.1	23.0	52.7	1.2	3.9	160.0	7.5		
Ulundi	2	28.3	24.1	5.8	1.0	21.3	112.0	3.9		
Vryheid	4	102.0	19.1	30.0	0.0	22.1	64.0	2.6		
Limpopo									**44**	**8**
Giyani	5	56.5	21.7	26.8	0.5	0.0	7.8	0.5		
Jane Furse	3	42.3	21.1	15.2	1.3	0.5	5.5	1.8		
Mokopane	3	35.1	25.1	36.2	0.5	2.3	15.8	2.4		
Polokwane	8	42.3	21.1	19.2	0.6	1.4	7.8	nd		
Venda	6	153.0	23.1	38.3	0.8	0.3	4.5	nd		
Mpumalanga									**50**	**0**
Balfour	1	124.0	17.7	3.0	0.0	74.5	0.0	nd		
Boshofontein	1	218.0	23.6	55.0	5.0	2.5	16.4	nd		
Daggaskraal	4	89.5	17.4	0.9	0.0	52.0	0.0	nd		
Driefontein	2	128.0	17.6	9.0	0.2	24.3	2.6	0.7		
Amersfoort	1	89.5	17.4	0.5	0.0	11.5	0.0	nd		
Ermelo	2	89.5	16.8	0.5	0.0	13.3	0.0	nd		
Ikhwezi	1	218.0	23.6	50.0	1.5	3.0	49.9	0.5		
KwaMhlanga	3	69.1	20.1	12.2	0.2	2.0	36.5	0.7		
Matibiti	3	N/A	N/A	9.8	0.2	1.7	78.3	0.7		
Mbuzini										

F. vert, Fusarium verticillioides; *F. pro, F. proliferatum*; *F. sub, F. subglutinans*; N/A, data not available; nd, not detected.
[a] mean rainfall of the daily mean from October to May.
[b] mean temperature of the daily mean from October to May.
[c] percentage of samples positive for fumonisins.
[d] percentage of samples contaminated with >2 µg/g fumonisins.

TABLE 1b: *Fusarium* spp. that produce fumonisins as determined by quantitative PCR, fumonisin contamination levels (µg/g) and climatic conditions present in rural maize-production areas of South Africa during 2007.

Locality	No. of samples	Mean							Fumonisins present[c] (%)	Fumonisins >2 µg/g[d] (%)
		Rainfall[a] (mm)	Temperature[b] (°C)	*F. vert* (%)	*F. pro* (%)	*F. sub* (%)	qPCR (pg DNA/mg)	Fumonisin level (µg/g)		
Eastern Cape									**34**	**6**
Bizana										
Butterworth	3	193.0	19.0	15.7	2.3	4.2	52.5	nd		
Cofimvaba	4	46.4	20.1	1.8	1.8	6.3	1.5	nd		
Elundini										
Engcobo	7	70.7	19.1	10.7	3.8	24.4	62.8	1.3		
Idutywa										
Centane	3	20.2	17.9	7.0	0.3	5.0	45.6	nd		
Libode	3	87.9	17.0	4.3	0.5	4.2	0.0	nd		
Lusikisiki	4	81.3	19.7	22.3	4.0	12.3	165.0	2.6		
Maluti										
Mqanduli	2	71.9	17.9	0.5	0.0	1.5	10.3	nd		
Ngqeleni	1	62.0	11.0	9.5	1.0	1.5	0.0	nd		
Queenstown	2	75.6	19.6	9.5	1.5	16.8	26.0	nd		
Qunu	1	71.9	17.9	9.5	0.0	1.5	0.0	nd		
Whittlesea	2	49.6	19.2	0.3	0.0	17.3	0.0	nd		
KwaZulu-Natal Southern Region									**0**	**0**
Bergville										
Highflats										
Port Shepstone	7	53.9	22.2	4.5	0.1	2.7	18.6	nd		
Umzimkhulu										
Underberg										
Ndwedwe										
Zululand									**56**	**17**
Eshowe										
Jozini	17	61.9	24.8	35.7	6.2	0.0	192.0	1.9		
Ladysmith	3	99.5	21.9	23.3	0.0	1.0	40.1	nd		
Manguzi	4	78.4	25.0	34.3	9.1	0.4	824.0	5.8		
Mbazwane	5	78.4	25.0	40.7	12.0	0.3	75.0	nd		
Pongola	6	86.6	23.6	22.3	0.0	2.0	43.7	nd		
Ulundi	3	57.4	24.3	13.8	0.0	1.8	22.0	nd		
Vryheid	1	107.0	19.6	21.5	0.0	1.5	140.0	4.7		
Limpopo									88	24
Giyani	4	40.0	25.1	11.9	0.9	0.1	7.8	0.6		
Jane Furse										
Mokopane	4	53.0	22.5	27.5	2.3	0.1	21.0	2.3		
Polokwane	5	35.9	22.1	12.1	1.5	0.4	0.0	1.5		
Venda	4	82.8	23.7	42.5	3.3	0.1	183.0	3.3		
Mpumalanga									**0**	**0**
Balfour										
Boshofontein										
Daggaskraal	7	59.8	20.2	0.5	0.1	9.2	0.0	nd		
Driefontein										
Amersfoort										
Ermelo	1	15.0	16.5	36.5	4.0	22.0	0.0	nd		
Ikhwezi										
KwaMhlanga										
Matibiti	7	130.0	17.2	3.8	1.1	2.9	3.5	nd		
Mbuzini	4	39.9	25.2	18.5	4.1	1.3	42.4	nd		

F. vert, Fusarium verticillioides; F. pro, F. proliferatum; F. sub, F. subglutinans; N/A, data not available; nd, not detected.
[a] mean rainfall of the daily mean from October to May.
[b] mean temperature of the daily mean from October to May.
[c] percentage of samples positive for fumonisins.
[d] percentage of samples contaminated with >2 µg/g fumonisins.

Fumonisins have been associated with high rates of human oesophageal cancer worldwide[16,17] and with increased incidences of neural tube defects in infants of mothers consuming maize-based products contaminated with fumonisins.[18] Fumonisins are also toxic to livestock.[19,20,21] Increased levels of fumonisins in mouldy maize kernels have been previously linked to the high incidence of human oesophageal cancer in several districts in the Transkei region of South Africa.[17] Little, however, is known about contamination of maize with *Fusarium* spp. and fumonisins in other rural areas of the country. The aim of this study, therefore, was to determine the *Fusarium* spp. and levels of fumonisins associated with maize in subsistence farming systems in South Africa.

Materials and methods

Field sampling

Maize samples were collected from randomly selected subsistence farming localities in the Eastern Cape, KwaZulu-Natal, Limpopo and Mpumalanga provinces of South Africa (Table 1). These post-harvest samples were taken during storage. Because of different climatic conditions in the northern (subtropical coastal) and southern (temperate coastal) parts of KwaZulu-Natal, localities in KwaZulu-Natal were split into the two regions: southern and northern KwaZulu-Natal (Zululand). In total, 147 and 114 maize samples, each approximately 1.5 kg, were collected from different farmers in the same district in the 2006 and 2007 growing seasons, respectively. These samples were in storage for less than two months. Shelled maize or maize ears were placed in cloth bags to prevent condensation that might promote fungal growth and were labelled with the source or locality of the sample and the cultivar planted. Global positioning system (GPS) co-ordinates were recorded at each sampling point. The collected samples were then stored in a cold room at 4 °C and 45% relative humidity until assayed.

Climatic data

Monthly rainfall and temperature data for weather stations closest to the sampling localities were obtained from the website of the Agricultural Research Council's Institute of Soil Climate and Water in Pretoria. Climatic data, stretching from October of the previous year to May in the year that maize samples were collected, were considered. These specific dates were chosen as they represent the growing season of maize in South Africa.

Isolation and enumeration of *Fusarium* spp.

Maize kernels were surface-sterilised by dipping them once in 70% ethanol, soaking them for 3 min in 1.6% NaOCl solution and rinsing them three times in sterile distilled water. The kernels were then plated out on Van Wyk agar, a *Fusarium* selective medium[22] in Petri dishes (90 mm in diameter). Each Petri dish contained 4 kernels, equidistant from each, and a total of 50 Petri dishes were used to plate 200 seeds. After 7 days of incubation at 25 °C, developing *Fusarium* colonies were identified morphologically to species level.[23]

Quantitative detection of fumonisin-producing *Fusarium* spp.

Maize samples were analysed for *Fusarium* spp. that produce fumonisins using quantitative real-time polymerase chain reaction (qPCR). A Cyclotec sample mill (Foss Tecator, Hoganas, Sweden) was used for grinding maize samples into a fine powder such that more than 75% of the ground material passed through a 20-mesh sieve. DNA was then isolated from 20 mg of each sample using Qiagen DNeasy Plant Mini Kits (Cat 69106, Qiagen, Hilden, Germany). The TaqMan method was used to detect *Fusarium* spp. that produce fumonisins using primers and probes designed for the polyketide synthase gene *fum1*.[24] qPCR was performed using a MicroAmp Optical 96-well reaction plate and MicroAmp Optical Caps (Applied Biosystems, Foster City, USA). An ABI Prism 7700 Sequence Detection System (Applied Biosystems) was used to perform the PCR and assess fluorescence. Each amplification reaction consisted of 2 µl of DNA preparations, 1 x real-time PCR buffer (Applied Biosystems), 5 mM $MgCl_2$, 83 nM of the FAM-labelled FUM-probe, 1.5 U of Hot Goldstar DNA polymerase (Eurogentec, Seraing, Belgium) and 333 nM of forward and reverse primers for the target DNA (Taqfum-2F in combination with Vpgen-3R, VertFum-3R or ProFum-3R). As an internal control, 100 pg of potato leaf roll virus (PLRV) DNA, forward primer PLRV-F and reverse primer PLRV-R (both at 333 nM) were included in the reaction along with 83 nM of the VIC-labelled PLRV probe.[24]

Analysis for fumonisins

Levels of fumonisins were quantified using the Veratox enzyme-linked immunosorbent assay (ELISA) quantitative fumonisin 5/10 test kit (Neogen Corp, Lansing, MI, USA) according to the manufacturer's instructions. Results above 6 µg/g were extrapolated from the standard curve (r = 0.99) using Veratox® software.[25] An inter-laboratory comparison was done with the Division of Toxicology, Onderstepoort Veterinary Institute, South Africa to validate the method. Each analysis was repeated three times to determine reproducibility of the results.

Statistical analyses

Simple linear regression on Statgraphics 5 Plus[26] (Manugistics Inc, Rockville, MD, USA) was used to determine the relationship between levels of fumonisins quantified by ELISA and target DNA of *Fusarium* spp. that produce fumonisins for the 2006 and 2007 seasons, and for both seasons combined. The correlation was done to determine whether the qPCR technique was a reliable method in detecting *Fusarium* spp. that produce fumonisins in maize as compared to morphological identification. Temperature and rainfall data were also correlated to the production fumonisins using simple linear regression analyses on Statgraphics 5 Plus.[26]

Results

Isolation and enumeration of *Fusarium* spp.

Three *Fusarium* spp. (Section: Liseola) were isolated from maize kernels collected from subsistence farmers' fields

in South Africa. *Fusarium verticillioides* was the dominant *Fusarium* species in maize collected in Limpopo and Zululand and *F. subglutinans* was the dominant species in the Eastern Cape and Mpumalanga provinces (Table 1). Less than 5% of all maize kernels were infected by *F. proliferatum*, except those collected from Eshowe in Zululand, which yielded a mean infection level of 20.1% in 2006 (Table 1).

Quantitative determination of fumonisin-producing *Fusarium* spp.

Quantitative PCR results supported the seed isolation data, with *Fusarium* spp. that produce fumonisins being found in maize produced in Zululand at far greater levels than in any other province in both 2006 and 2007 (Table 1). The average amount of fungal DNA found in maize from Zululand was more than three times greater than that found in any other province (Table 1). *Fusarium* spp. that produce fumonisins were absent in maize kernels from Mqanduli (Eastern Cape) and several localities in Mpumalanga in 2006; Libode, Ngqeleni and Whittlesea in 2007 and Qunu (Eastern Cape) in both seasons (Table 1).

Analysis for fumonisins

Samples from Zululand and, to a lesser extent, those from Limpopo were contaminated with higher levels of fumonisins than those collected in the other provinces (Table 1). Levels of fumonisins in samples collected at Manguzi, Mbazwane, Pongola, Ulundi and Vryheid were generally in excess of 2 µg/g in 2006 (Table 1a). Maize samples from Manguzi had consistently high levels of fumonisins in both seasons. In Limpopo, it was only the samples collected in Mokopane and Venda that contained fumonisins at levels above 2 µg/g (Table 1). None of the samples collected in Mpumalanga were contaminated with more than 2 µg/g fumonisins. In the Eastern Cape, where contamination of maize with fumonisins has been reported previously,[6,10,17] only samples collected from Lusikisiki had contamination levels above 2 µg/g in both 2006 and 2007 (Table 1).

Climatic data

The lower average daily temperatures were recorded in the mountainous district of Ngqeleni in the Eastern Cape while the higher average temperatures were recorded in the inland areas of Limpopo and Zululand (Table 1). Rainfall data in KwaZulu-Natal, Limpopo and Mpumalanga provinces varied notably among regions (Table 1).

Correlation between *Fusarium* spp., levels of fumonisins and climatic data

A significant positive correlation was obtained between target DNA of *Fusarium* spp. that produce fumonisins and levels of fumonisins for the 2006 ($r = 0.74$, $p \leq 0.05$) and 2007 ($r = 0.93$, $p \leq 0.05$) growing season (Figure 1) and for the combined seasons ($r = 0.80$, $p \leq 0.05$). A poor correlation, however, was obtained between *F. verticillioides* quantified by plating and target DNA of *Fusarium* spp. determined by qPCR ($r = 0.14$, $p \leq 0.05$). There was a significant positive correlation between the combined occurrence of *F. verticillioides* and *F. proliferatum* and fumonisins ($r = 0.39$, $p \geq 0.05$).

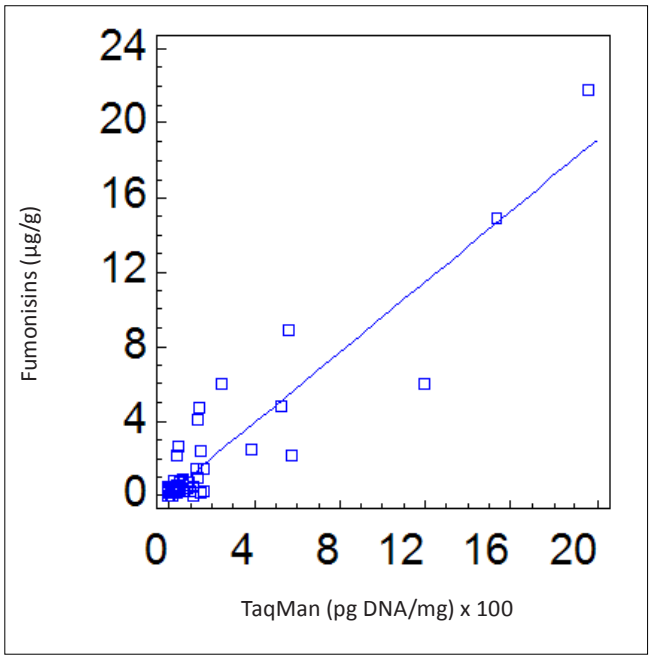

FIGURE 1: Correlation ($r = 0.93$, $p \leq 0.05$, $n = 150$) between quantitative real-time polymerase chain reaction analysis of *Fusarium* spp. that produce fumonisins (pg DNA/mg) and levels of fumonisins (µg/g) determined by enzyme-linked immunosorbent assays) in maize in subsistence farming systems in South Africa in 2007.

TABLE 2: Regression analyses for the interaction between climatic conditions and production of fumonisins and *Fusarium* spp. infection during both seasons.

Climatic condition		2006 growing season		2007 growing season	
		r	*p*-value	*r*	*p*-value
Rainfall (mm)	Fumonisins	-0.100	0.53	0.120	0.54
	qPCR	-0.004	0.98	0.120	0.54
	F. verticillioides	0.370	0.01*	0.040	0.85
	F. subglutinans	-0.006	0.96	-0.180	0.38
	F. proliferatum	0.080	0.63	0.004	0.98
Temperature (°C)	Fumonisins	0.500	0.00*	0.300	0.12
	qPCR	0.480	0.00*	0.360	0.07*
	F. verticillioides	0.640	0.00*	0.480	0.01*
	F. subglutinans	-0.370	0.02*	-0.370	0.05*
	F. proliferatum	0.300	0.05*	0.410	0.03*

* statistically significant at $p \leq 0.05$.

There was no correlation between temperature and level of fumonisins ($r = 0.3$, $p \geq 0.1$) in 2007. But there was a significant correlation between temperature and target DNA of *Fusarium* spp. determined by qPCR as well as *Fusarium* spp. quantified by plating in 2006 and in 2007 (Table 2). There was no correlation between rainfall data and the level of fumonisins in either season (Table 2).

Discussion

Levels of fumonisins in some samples found in Venda and Mokopane (Limpopo), Lusikisiki (Eastern Cape) and Mbazwane, Jozini, Pongola and Manguzi (Zululand) far

exceeded the maximum levels of 2 µg/g set by the US Food and Drug Administration in the USA[27] and the 1 µg/g set by the European Union[28] for food intended for direct human consumption. This was possibly as a result of local agricultural practices, such as lack of fungus disease control, planting dates, harvesting dates, storage, crop residue disposal, land tillage methods, crop rotation, seed sources and maize stalk borer control, that might have promoted the growth of *Fusarium* spp. that produce fumonisins. In this study, levels of fumonisins in Butterworth and Centani and other areas in the Eastern Cape were found to be below 0.5 µg/g, possibly because of the provision of hybrid seed in the former Transkei by the Provincial Department of Agriculture.[4] Because subsistence farmers produce maize for their own consumption, they could be at higher risk of exposure to fumonisins and concomitant mycotoxicoses in their diet than people living in urban areas of South Africa.

The high incidence of *F. verticillioides* and *F. subglutinans* in 2006 and 2007 can partly be explained by existing environmental conditions in the local rural maize production areas. *F. verticillioides* is known to proliferate in warm, humid regions,[9] similar to those reported for Zululand, Venda in Limpopo and Lusikisiki and Engcobo districts in the Eastern Cape. *F. subglutinans*, however, is known to multiply more rapidly in temperate climates[29] and was the dominant species found in the cooler districts of Mpumalanga and the Eastern Cape, as well as some mountainous districts in southern KwaZulu-Natal. Subsistence farmers in these areas might be at risk to moniliformin, which is the primary mycotoxin produced by *F. subglutinans*.[15] As was reported in related studies on *Fusarium* spp. in maize,[11] *F. proliferatum* proved to be a minor coloniser of maize kernels in subsistence farmer fields, except those collected from Eshowe in Zululand, possibly as a result of cross-over infections from sugarcane grown in and around Eshowe.[30] High levels of co-infections of maize with *F. verticillioides* and *F. subglutinans* also occurred in all provinces, and subsistence farmers in these areas could be simultaneously exposed to both fumonisins and moniliformin.

More pronounced infections of maize in Zululand by *F. verticillioides* might have been caused by farmers planting seed of open pollinated maize varieties retained from the previous harvest,[4] as such seed could lead to increased systemic infections and concomitant production of fumonisins.[7] These infections could also have been caused by crop residues left on the lands, late harvesting and by practising maize monoculture.[4] These practices are more predominant in Zululand than anywhere else in South Africa. The low levels of *F. verticillioides* found in the Eastern Cape and southern KwaZulu-Natal could be a result of cooler temperatures prevailing in those regions. Colonisation of maize kernels with *Fusarium* spp. that produce fumonisins sometimes differed considerably within and between districts in the same region, which could be attributed to different farming practices that were followed by individual farmers.

A positive correlation was found between the target DNA of *Fusarium* spp. that produce fumonisins and levels of fumonisins. However, there was a poor correlation between *Fusarium* spp. determined by qPCR and *F. verticillioides* quantified by plating on *Fusarium*-selective medium. This study, therefore, shows that fungal biomass, as measured by qPCR, shows a significant correlation with contamination of maize with fumonisins. This is in agreement with several studies, such as that of Waalwijk et al.[31]

The occurrence of fumonisins in subsistence farmer crops has previously been reported for maize produced in the Eastern Cape.[6,17] This study, however, is the first to show that fumonisins are produced in maize in all the subsistence production regions of South Africa. Implementation of disease-management practices, such as planting of regionally adapted maize varieties, reducing insect damage, early harvesting and discarding mouldy kernels and farmer education in rural areas can result in the reduction of mycotoxin contamination. This is particularly important because subsistence farmers rely on their maize produce as the primary source of food and income, irrespective of its quality.

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