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Accumulation of toxigenic *Fusarium* species and *Stenocarpella maydis* in maize grain grown under different cropping systems

Londiwe M. Mabuza · Belinda Janse van Rensburg · Bradley C. Flett · Lindy J. Rose

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Abstract Mycotoxigenic fungi such as *Fusarium graminearum*, *Fusarium verticillioides* and *Stenocarpella maydis* infecting maize grain can be detrimental to both humans and animals due to the toxins they produce. Disease management strategies include tillage practices and crop rotations, however, these have not been sufficiently evaluated in South Africa. The effect of cropping systems on ear rot accumulation and mycotoxin contamination in maize grain was investigated in two localities over a four and six-year period. Cropping systems evaluated were: 1) monoculture maize conventional tillage, 2) monoculture maize no-till, 3) two, and 4) three-year rotation systems consisting of maize/cowpea and maize/cowpea/babala (all no-till), respectively. In Buffelsvallei, two additional crop rotations, maize/sunflower and maize/sunflower/babala (all no-till) were included. Naturally infected trials were visually evaluated for disease severity or incidence while fungal and mycotoxin contamination of maize grain was quantified. Disease incidence and mycotoxin contamination were inconsistent throughout the study period due to seasonal and geographical differences. In Buffelsvallei, cropping system had a significant effect ($P < 0.05$) on the accumulation of fumonisins and *F. graminearum* for 2010/11, deoxynivalenol (2011/12) and *S. maydis* incidence (2013/14). *Fusarium graminearum* and fumonisin accumulation was significantly higher in the three-year maize/cowpea/babala rotation and two-year sunflower rotation in the 2010/11 season, respectively. Deoxynivalenol levels in monoculture maize, using conventional tillage (2011/12) was significantly higher when compared to all other cropping systems and *S. maydis* incidence was significantly higher in maize conventionally tilled, no-till and two-year maize/cowpea and maize/sunflower cropping systems in the 2013/14 season. Cropping systems had no significant effects on fungal infection or mycotoxin accumulation in maize grain obtained from trials conducted at Erfdeel. The results of this study indicate that Conservation Agriculture systems under the environments evaluated, did not increase the risk of maize ear rots and mycotoxin production.

Keywords Cropping systems · Ear rots · Maize · Mycotoxins

L. M. Mabuza · B. Janse van Rensburg (✉) · B. C. Flett
Agricultural Research Council- Grain Crops Institute, Private bag x1251, Potchefstroom 2520, South Africa
e-mail: BelindaJ@arc.agric.za

L. J. Rose
Department of Plant Pathology, Stellenbosch University, Matieland 7602, South Africa

Introduction

Maize (*Zea mays* L.) is an important staple, feed and energy crop in South Africa. It is also prone to a multitude of root, stalk, leaf and ear rot diseases (Fandohan et al. 2003). Predominant ear rots in the majority of maize producing areas include Fusarium ear rot (FER), Gibberella ear rot (GER) and Diplodia ear rot (DER) (Boutigny et al. 2012). Fusarium ear rot is mainly

caused by *Fusarium verticillioides* Sacc. Nirenberg (syn = *F. moniliforme* Sheldon), which is present in most maize-producing areas (Fandohan et al. 2003). It is responsible for substantial losses in grain yield and quality due to its ability to produce mycotoxins known as fumonisins (Fandohan et al. 2003). Fumonisins are the most predominant group of mycotoxins and have been classified as potentially carcinogenic, neurotoxic, mutagenic, immunosuppressive, and hepatotoxic (Gelderblom et al. 1992).

Fusarium graminearum (Schwabe) [Teleomorph *Gibberella zeae* (Schwein. Petch], which causes GER, produces pink to red mould that discolours infected maize kernels (Reid et al. 1999) and is responsible for the production of a wide range of toxic metabolites including zearalenone and trichothecenes such as deoxynivalenol and nivalenol. Zearalenone, which has structural similarity to oestrogen, is attributed to several reproduction disorders such as fecundity and stillbirths in some animal species (Zinedine et al. 2007). Deoxynivalenol and nivalenol are accountable for feed refusal, vomiting, gastric ulcers and decreased weight if ingested by animals (Youssef 2009).

Stenocarpella maydis (Berkeley) (Syn) (*Diplodia maydis*) (Berk.) (Sacc) is the causal agent of Diplodia ear rot (DER), a common maize ear rot found in most maize-producing areas. DER is responsible for massive yield losses; infected kernels are usually lighter and have decreased nutritional value (Flett and McLaren 1994). It has also been linked to mycotoxicoses of cattle and sheep commonly known as diplodiosis (Rabie et al. 1985). Symptoms of diplodiosis include paralysis, ataxia and still births (Odriozola et al. 2005). Apart from commonly being associated with southern African countries, there have been reports of occurrence in Brazil and Argentina (Odriozola et al. 2005; Masango et al. 2015).

Multitoxin contamination in agricultural commodities is of great significance due to impacts on productivity, the economy as well as human and animal health (Degraeve et al. 2016). Mycotoxigenic fungi are either classified as field or storage fungi (Placinta et al. 1999). In maize, the most important stage of ear rot infection and mycotoxin contamination is during pre-harvest production, where disease incidence and mycotoxin contamination is influenced by numerous factors ranging from climatic conditions, soil fertility, insect damage, susceptibility of plant variety and agricultural practices (Reid et al. 2001).

The use of Conservation Agriculture (CA) ensures the efficiency of a cropping system by enhancing the quality of the soil, thereby providing a cheaper, more productive and environmentally friendly crop production (Lawrance et al. 1999). The principal challenge in crop production is the need to sustainably produce high yielding crops, with minimal diseases and pests. Tillage influences both the physical and chemical properties of the soil, therefore a reduction in tillage practices may significantly influence pathogen species; this is however entirely dependent on the pathogen's life cycle and survival mechanisms (Govaerts et al. 2006). The effect of tillage practices on disease incidence is vaguely understood and sometimes contradictory (Lawrance et al. 1999). One of the reported setbacks involved with reduced tillage practices is the potential for increased disease incidence (Sumner et al. 1981) although Flett et al. (1998) found tillage practices to not have an influence on FER and GER accumulation in maize grain. Changes in cropping systems can have effects on factors that correlate to disease development such as soil structure, plant growth, closeness of crop to pathogens, residue availability, soil temperature and water content (Watkins and Boosalis 1994; Lawrance et al. 1999). Crop rotations have also been identified as a viable method for disease control in no till systems (Ward and Nowell 1998).

With the lack of resistant cultivars and effective chemical control measures for maize ear rots, it is of fundamental importance that the effects of these agricultural practices be investigated to help limit disease incidence and mycotoxin contamination in maize grain. Therefore, the objective of this study was to investigate the effect of cropping practices on maize ear rots and mycotoxins and determine the potential role of crop rotations in CA systems with regards to maize ear rot infections and mycotoxin contamination. This knowledge will assist in identifying a suitable cropping system that improves grain quality by reducing ear rot infections and mycotoxin contamination while also providing more insight into the impact of CA on mycotoxigenic fungi and their metabolites.

Materials and methods

Conservation Agriculture field trials

Field trials were carried out for four (2011/12–2014/15) and six (2009/10–2014/15) seasons in two different localities, respectively. The localities, Buffelsvallei

(latitude −26.495; longitude 26.602, sandy loam soil) and Erfdeel (latitude −26.982 longitude 27.027, sandy textured soil) are based in the North-West and Free State provinces of South Africa, respectively. A randomized, complete block design with four replicates was implemented, which consisted of six cropping systems in Buffelsvallei and four cropping systems in Erfdeel. Maize cultivars in both localities were PAN6Q-521R in 2009/10, PAN5Q563R in 2010/11, PAN5Q649R in 2011/12 and 2012/13, PAN5Q649RR in 2013/14 and BG5685R in 2014/15 (Table 1). Treatments in Buffelsvallei included 1) maize monoculture, conventionally tilled (MM-CT), 2) maize monoculture, no-till (MM-NT), 3) no-till maize, two season rotation with sunflower (NT-SF), 4) no-till maize, two season rotation with cowpea (NT-CP), 5) no-till maize, three season rotation with babala and sunflower (NT-BA-SF) and 6) no-till maize three season rotation with babala and cowpea (NT-BA-CP) (Table 2). Treatments in Erfdeel included 1) maize monoculture, conventionally tilled (MM-CT), 2) maize monoculture, no-till (MM-NT), 3) no-till maize, two season rotation with cowpea (NT-CP) and 4) no-till maize, three season rotation with babala and cowpea (NT-BA-CP) (Table 3). The experiment was conducted for four years in Erfdeel due to highly acidic soil conditions, planting did not take place during the first two years of the study. Maize ears were naturally infected by ear rot causing fungi and each season maize ears were harvested from the two middle rows of each treatment.

Table 2 Analysis of variance indicating significant effects of cropping systems on target DNA of *F. graminearum* (2010/11), Diplodia ear rot incidence (DER, 2010/11),fumonisins (2010/11) and deoxynivalenol (2011/12) contamination in maize grain from Buffelsvallei

Source of variation	d.f.	s.s	m.s.	v.r.	F pr.
F. graminearum DNA (2010/11)					
Treatment	5	8610.0	1722.0	4.60	0.010
Residual	15	5617.0	374.5		
Total	23	17,289.4			
DER incidence (2010/11)					
Treatment	5	25.848	5.170	4.84	0.008
Residual	15	16.019	1.068		
Total	23	47.205			
Fumonisins (2010/11)					
Treatment	5	21.805	4.361	2.80	0.056
Residual	15	23.368	1.558		
Total	23	51.163			
Deoxynivalenol (2011/12)					
Treatment	5	12.3420	2.4684	3.37	0.031
Residual	15	10.9916	0.7328		
Total	23	26.9117			

Plots were fertilized with 600 mL/ha^{-1} N:P:K prior to planting (Table 1). Herbicides during planting included DUAL GOLD (960 g/L S-metalochlor, Syngenta, Basel, Switzerland) at a rate of 60 to 600 mL/ha, GRAMOXONE SL (250 g/L Paraquat, Syngenta,

Table 1 Cropping systems evaluated from 2009/10 to 2014/15 in Buffelsvallei and 2011/12 to 2014/15 in Erfdeel

Crop system	Cultivation	Season		
		1	2	3
Buffelsvallei				
1. Maize monoculture	CT*	Maize	Maize	Maize
2. Maize monoculture	NT#	Maize	Maize	Maize
3. Maize - Cowpea	NT#	Maize	Cowpea	Maize
4. Maize - Sunflower	NT#	Sunflower	Maize	Sunflower
5. Maize - Babala - Cowpea	NT#	Maize	Babala	Cowpea
6. Maize - Babala - Sunflower	NT#	Sunflower	Maize	Babala
Erfdeel				
1. Maize monoculture	CT*	Maize	Maize	Maize
2. Maize monoculture	NT#	Maize	Maize	Maize
3. Maize - Cowpea	NT#	Maize	Cowpea	Maize
4.1 Maize - Babala - Cowpea	NT#	Maize	Babala	Cowpea

*CT - Conventional till

#NT - No - till

Table 3 Mean *Fusarium graminearum* target DNA quantified during the 2010/11 season in maize grain grown under different tillage/rotation systems in Buffelsvallei

Crop system	Cultivation	*Fusarium graminearum* DNA (ng/uL)
1. Maize monoculture	CT*	4.63[a]
2. Maize monoculture	NT[#]	20.03[a]
3. Maize - Cowpea	NT[#]	2.62[a]
4. Maize - Sunflower	NT[#]	14.86[a]
5. Maize - Babala - Cowpea	NT[#]	59.07[b]
6. Maize - Babala - Sunflower	NT[#]	11.66[a]
CV%		69.3%

Different letters shown indicate significant differences ($P < 0.05$)

*CT - Conventional till

[#] NT - No - till

Basel, Switzerland) at a rate of 1 to 3 L/ha, ROUNDUP (540 g/L glyphosate, Monsanto, Missouri, USA) at a rate of 2 to 4 L/ha, KARATE (250 g/L Lambda-Cyhalothrin, Syngenta, Basel, Switzerland) at a rate of 70 mL/ha. Stalk borers were controlled using KOMBAT (25 g/L Carbaryl, Kombat, Greytown, South Africa) and BULLDOCK (25 g/L Beta-cyfluthrin, Bayer Crop Science, Leverkusen, Germany).

Maize ear rot disease ratings

Maize ears were hand harvested and grain disease ratings were conducted according to Flett et al. (1998). DER incidence was determined based on discoloration, rot and mycelium, the percentage of visibly diseased grain samples was calculated by mass. Disease severity was rated as a percentage per ear surface showing visible symptoms, 'starbursts' and pink mold for FER and dark purple mold starting at the tip of the ear for GER.

Quantification of *F. verticillioides* and *F. graminearum*

DNA extraction Maize ears were hand harvested at ≤12% moisture, and threshed per treatment. A 250-g sub-sample was taken from each threshed sample, milled and passed through a 1-mm mesh using a Cyclotech sample mill (Foss Tecator, Hoganas, Sweden). These samples were stored at −20 °C for further analysis. DNA was extracted from 0.5-g milled flour using the DNeasy Plant Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's guidelines. The purity and the concentration of the DNA was measured using a Nanodrop® (2000c) Spectrophotometer (Thermo Scientific, Waltham, USA) at 260 nm (OD260). The DNA was diluted to 10,000 pg/μL and stored at −20 °C in 100 μL aliquots.

A high fumonisin-producing *F. verticillioides* isolate (MRC826) and *F. graminearum* isolate (MRC 394) were obtained from the Medical Research Council to generate standard curves. The respective fungi were plated out on potato dextrose agar (PDA) and DNA was extracted from mycelia after 1 week using the CTAB method adapted from Winnepenninckx et al. (1993).

Quantification of *F. verticillioides* and *F. graminearum* target DNA*:* A 10-fold dilution of *F. verticillioides* MRC826 DNA was used to generate a standard curve for quantification (Waalwijk et al. 2008; Janse van Rensburg et al. 2015). *Fusarium verticillioides* target DNA was determined as described by Janse van Rensburg et al. (2015). For *F. graminearum,* a 4-fold standard dilution was used to generate a standard curve for quantification (Nicolaisen et al. 2009). The primers FgramB379 and FgramB411 in combination with SYBRGreen were used as tested by Nicolaisen et al. (2009). A 96- well reaction plate was prepared consisting of a total volume of 25 μL of 12.5 μL of SYBR® green, 0.625 μL (250 mM) of FgramB379: CCA TTC CCT GGG CGT and 0.625 μL (250 nM) and FgramB411: CCT ATT GAC AGG TGG TTA GTG ACTGG, 9.25 μL of nuclease free water and 2 μL of DNA. Negative controls contained no template DNA but were treated similar to the reaction samples. A CFX96™ Real-Time PCR detection system (Bio-Rad, Hercules, USA) with a 96 well reaction plate was used for all qPCR assays. Cycling conditions for *F. graminearum* consisted of 5 min denaturation at 95 °C, 40 cycles at 95 °C for 10 s and 65 °C for 10 s, followed by a melt curve step of 95 °C, and a cooling step at 65 °C. After runs were completed, data was generated from the amplification curves. Regression equations of standard curves from runs were highly correlated ($R^2 > 0.99$). Slopes were within the accepted criterion (between −3.1 and − 3.6) and efficiencies were between 95 and 110%.

Mycotoxin quantification

Fumonisins Fumonisins were analysed using the HPLC-VICAM method (Anonymous 2002) according to Janse van Rensburg et al. (2015). Fumonisin

standards were obtained from Sigma-Aldrich Missouri, USA). To generate a standard curve, standards were evaporated and reconstituted with a calibration standard solution ranging from 2 to 20 mg/kg. Fluorescence was performed at excitation and emission wavelengths of 335 nm and 440 nm respectively using a Waters 2475 multi λ fluorescence detector equipped with a Symmetry C18 (5 μm 3.9 × 150 mm) analytical column (Waters, Milford, USA). The detection limit of the method used was 0.016 mg/kg and the recovery data were obtained in triplicate by spiking clean maize samples (VICAM) with 5 mg/kg fumonisin B_1 B_2 and B_3. The average recovery rates were 83% (FB_1), 81% (FB_2) and 83% (FB_3).

Zearalenone Zearalenone was analysed using the VICAM method adapted from Kruger et al. (1999). Milled sub samples (25 g) were mixed with sodium chloride (5 g) prior to extraction, and blended (Waring products division, Torrington, USA) in 100 mL of methanol: water (80:20 *v*/v) at high speed for two minutes. The extract was filtered through a 24-cm fluted filter paper, the filtrate (4 mL) was mixed with 96 mL HPLC grade water (18 MΩ.cm) and filtered again through a microfiber filter paper. The diluted extract (100 mL) was passed through the ZearaTest affinity column (VICAM) at a rate of approximately 3 drops per second and the column was washed with 25 mL HPLC grade water. Zearalenone was eluted with 0.75 mL methanol followed by 0.5 mL water. The eluate (50 μL) was injected into the HPLC system of which the mobile phase consisted of acetonitrile: methanol: water (46:46:8 v/v/v) set to a flow rate of 1 mL/min.

Zearalenone standards were obtained from Sigma-Aldrich Missouri, USA). To generate a standard curve, standards were evaporated and reconstituted with a calibration standard solution ranging from 0.25 to 2.5 mg/kg. Fluorescence was performed at excitation and emission wavelengths of 274 nm and 440 nm respectively using a Waters 474 multi λ scanning fluorescence detector and analytical column, Symmetry C18 3.9 × 150 mm (Waters, Massachusetts, USA). The detection limit of the method used was 0.0019 mg/kg and recovery data was obtained in triplicate by spiking clean maize samples (VICAM) with 5 mg/kg zearalenone. Average percentage recovery was 112%.

Deoxynivalenol and nivalenol Deoxynivalenol and nivalenol was extracted using the VICAM method (Anonymous 2012). Milled maize sub samples (50 g) were placed on a blender jar (Waring products division, Torrington, USA) with 200 mL of purified water. The sample was blended at high speed for three minutes. The blended extract was then filtered through a 24-cm fluted filter paper and the filtrate was collected. The filtrate (10 mL) was mixed with 40 mL phosphate-buffered saline (PBS) (8.0 g NaCI, 1.2 g Na_2HPO_4, 0.2 g KH_2PO_4, 0.2 g KCI, made to 1 L; pH 7.0) and filtered inside a funnel (11 cm). The filtered extract (5 mL) was passed through a deoxynivalenol−/nivalenol WB affinity column (VICAM) at a rate of approximately 1 drop per 2 s. The deoxynivalenol/nivalenol WB affinity column was washed with 10 mL PBS followed by 10 mL purified water at a rate of about 1 drop/s. Deoxynivalenol/nivalenol was eluted by 0.5 mL HPLC grade methanol and 1.5 mL HPLC acetonitrile.

Standards for deoxynivalenol and nivalenol were obtained from Sigma-Aldrich Missouri, USA). To generate a standard curve, standards were evaporated and reconstituted with a calibration standard solution ranging from 0.1 mg/kg, 0.5 mg/kg and 5 mg/kg for nivalenol and 0.1 mg/kg, 0.5 mg/kg and 5 mg/kg for deoxynivalenol. The level of detection for the method was 0.03 mg/kg for deoxynivalenol and 0.04 mg/kg for nivalenol. Average percentage recovery was 90% for both mycotoxins. Deoxynivalenol and nivalenol were separately quantified using liquid chromatography tandem mass spectrometry at the Central analytical facility (Dr M. Stander), Stellenbosch University, Stellenbosch, South Africa.

Stenocarpella maydis toxins To date, no method is available for the quantification of toxins produced by *S. maydis*.

Climatic data

Climatic data between 2009/10–2014/15 were collected in the Buffelsvallei and Erfdeel regions. Monthly maximum temperatures (°C), rainfall (mm) and relative humidity (%) were recorded between July of the planting year and June of the harvesting year.

Statistical analysis

Maize ear rot diseases, fungal target DNA and mycotoxin accumulation was analysed for each locality and season, respectively. The data was analysed using

Analysis of Variance (ANOVA) statistical models and skewed data were log transformed. To compare treatment effects, Fischer's protected least significant difference (LSD) was calculated at a 5% significance level (Gen Stat, version 15).

Results

Buffelsvallei

Disease ratings The mean FER severity was low and ranged from 0.10–15.30% throughout the six-year study period. Similarly, the mean GER severity ranged from 0 to 3.65% during the six years. No significant differences were observed between the cropping systems evaluated and FER of GER severity. Diplodia ear rot incidence ranged from 0.30% to 12.40% over the six-year period and was significantly affected by the cropping systems in the 2013/14 season of this study ($P = 0.008$, Table 2). Monoculture maize conventionally tilled (MM-CT; 3.89%), maize monoculture no-till (MM-NT; 3.00%), two-year maize/sunflower (NT-SF; 4.18%) and two-year maize/cowpea (NT-CP; 2.05%) had significantly higher DER incidence when compared to the three-year maize/sunflower/babala (NT-BA-SF; 0.87%) and three-year maize/babala/cowpea (NT-BA-CP; 0.94%) systems (data not shown).

Fungal target DNA Fusarium verticillioides target DNA accumulation in maize grain was moderate to high (311–2198 pg/μL) during the first two seasons (2009/10 and 2010/11), lower (2.10–223 pg/μL) during the 2011/12 and 2012/13 seasons and moderate (115–417 pg/μL) during the 2013/14 and 2014/15 seasons (data not shown). The cropping systems evaluated had no significant effects on *F. verticillioides* target DNA quantified during all seasons of the study (data not shown). The mean *F. graminearum* target DNA quantified in maize grain was not significantly influenced by the cropping systems evaluated; with the exception of the 2010/11 season of the study ($P = 0.010$; Table 4). The three-year maize/cowpea/babala (NT-CP-BA; 59.07 pg/μL) rotation system had significantly higher *F. graminearum* target DNA as opposed to the other cropping systems (Table 5). The lowest target DNA concentration was recorded in the two-year maize/sunflower rotation (NT-SF; 2.62 pg/μL) (Table 3). *Fusarium graminearum* target DNA was generally lower in the first three seasons of the study but increased as the study progressed (data not shown).

Table 4 Mean fumonisin contamination in maize grain samples quantified during the 2010/11 season in maize grain grown under different tillage/rotation systems in Buffelsvallei

Crop system	Cultivation	Fumonisins (mg/kg)
1. Maize monoculture	CT*	0.08 (−1.91)[a]
2. Maize monoculture	NT[#]	1.43 (−1.35)[a]
3. Maize - Cowpea	NT[#]	0.06 (−1.97)[a]
4. Maize - Sunflower	NT[#]	8.87 (0.81)[b]
5. Maize - Babala - Cowpea	NT[#]	0.56 (−0.51)[a]
6. Maize - Babala - Sunflower	NT[#]	1.27 (−0.74)[a]
CV%		23.9%

Values in brackets indicate log base 10 transformed data

Different letters shown indicate significant differences ($P < 0.05$)

*CT - Conventional till

[#] NT - No – till

Mycotoxins Fumonisin contamination in maize grain was found to be significantly affected by cropping systems in only one (2010/11) of the six seasons (Table 2). Fumonisin levels (8.87 mg/kg) were significantly ($P \leq 0.05$) higher in the two-year maize/sunflower (NT-SF) rotation for the 2010/11 season when compared to the other treatments (Table 5). The fumonisin levels in the NT-SF system, however, did not differ significantly from that of the maize monoculture no-till (MM-NT) treatment that had a mean fumonisin value of 1.43 mg/kg (Table 4). Trace amounts of zearalenone were quantified from some of the grain samples in only two of the six seasons (2009/10 and 2010/11) in Buffelsvallei (data

Table 5 Mean deoxynivalenol contamination in maize grain samples quantified during the 2011/12 season in maize grain grown under different tillage/rotation systems in Buffelsvallei

Crop system	Cultivation	Deoxynivalenol (mg/kg)
1. Maize monoculture	CT*	0.516 (−0.73)[b]
2. Maize monoculture	NT[#]	0.175(−1.92)[ab]
3. Maize - Cowpea	NT[#]	0.025(−2.50)[a]
4. Maize - Sunflower	NT[#]	0.001(−3.00)[a]
5. Maize - Babala - Cowpea	NT[#]	0.019(−2.25)[a]
6. Maize - Babala - Sunflower	NT[#]	0.015(−2.56)[a]
CV%		23.9%

Values in brackets indicate log base 10 transformed data

Different letters shown indicate significant differences ($P < 0.05$)

*CT - Conventional till

[#] NT - No – till

not shown). Zearalenone accumulation in maize grain was not significantly influenced by any of the cropping systems in all seasons evaluated. Deoxynivalenol levels ranged from not detectable (ND) to 0.5 mg/kg throughout the six seasons in Buffelsvallei while no nivalenol was detected across all years (data not shown). Cropping systems only had a significant effect ($P = 0.03$; Table 2) on deoxynivalenol accumulation in one (2011/12) of the six seasons evaluated. The mean level of deoxynivalenol quantified in samples representing the maize monoculture conventionally tilled system (MM-CT; 0.52 mg/kg; Table 5) was significantly higher in comparison to the other treatments except for the maize monoculture no-till system (MM-NT; 0.18 mg/kg; Table 5).

Erfdeel

Disease ratings Cropping systems had no significant effect on FER disease severity throughout the study period. Mean FER severity ranged from 1.30–3.75% during the study period (data not shown). Mean GER severity ranged from 0 to 11.06% during the study period. GER severity in maize grain was not significantly affected by any of the cropping systems (data not shown). Diplodia ear rot incidence was low in Erfdeel throughout the study with the incidence percentages ranging from 0.40–5.90%. No significant differences were recorded in DER disease incidence in relation to cropping systems across all seasons (data not shown).

Fungal target DNA ANOVA indicated that cropping systems at Erfdeel had no significant effect on *F. verticillioides* target DNA accumulation in maize grain between 2011/12–2014/15. *Fusarium verticillioides* target DNA accumulation ranged from low to moderate (2.30–427 pg/μL) throughout the study period (data not shown). The *F. graminearum* target DNA accumulation was low in Erfdeel during the study, ranging from 5.90–214 pg/μL. There were no significant differences in *F. graminearum* accumulation in relation to cropping systems in Erfdeel between 2011/12–2014/15 (data not shown).

Mycotoxins Low to moderate levels of fumonisins ranging from non-detectable - 0.55 mg/kg were recorded during the study period in Erfdeel. No significant differences were found for fumonisin contamination in relation to cropping systems in Erfdeel between 2011/12–2014/15 (data not shown). Zearalenone was not detected during the study period in Erfdeel (data not shown). Deoxynivalenol frequency was low ranging from non-detectable - 0.47 mg/kg (data not shown). Nivalenol was not detected during the study period in Erfdeel. No significant differences were recorded in deoxynivalenol and nivalenol accumulation in relation to cropping systems across all seasons (data not shown) (Table 6).

Climatic data

In Buffelsvallei, the weather was characterised by dry and warm conditions. Mean maximum monthly temperatures steadily increased from 25.1 °C to 27 °C from the 2009/10 season to the 2014/15 season (Table 7). The observed rainfall pattern was generally higher during the planting and silking stages (November–March) and lower towards harvesting periods (April–August; Table 7). Seasons 2011/12–2012/13 recorded the lowest rainfall when compared to the other four seasons.

In Erfdeel, a similar pattern in the mean maximum monthly temperatures was observed compared to Buffelsvallei where the mean monthly temperatures increased from 26.1 °C to 27.1 °C from season 2009/10–2014/15 (Table 7). Mean maximum temperatures were slightly higher in Erfdeel when compared to Buffelsvallei during the six-year study period. Rainfall was generally higher during the planting and silking stages (November–March) and lower towards harvesting periods (April–August, Table 7), rainfall was how-

Table 6 Mean Diplodia ear rot (DER) incidence in maize grain samples quantified during the 2010/11 season in maize grain grown under different tillage/rotation systems in Buffelsvallei

Crop system	Cultivation	DER incidence (%)
1. Maize monoculture	CT*	0.4706[b]
2. Maize monoculture	NT[#]	0.3368[b]
3. Maize - Cowpea	NT[#]	0.5093[b]
4. Maize - Sunflower	NT[#]	0.2971[b]
5. Maize - Babala - Cowpea	NT[#]	2.1065[a]
6. Maize - Babala - Sunflower	NT[#]	1.3904[a]
CV%		33.1%

Different letters shown indicate significant differences ($P < 0.05$)

*CT - Conventional till

[#] NT - No – till

Table 7 Monthly accumulated rainfall and mean maximum temperatures at Buffelsvallei and Erfdeel

Month	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Total/ Mean
Buffelsvallei													
Rainfall (mm)													
2008/09	-	-	-	-	-	-	-	70	–	–	–	28	-
2009/10	6	23	20	36	60	109	109	88	122	76	14	0	663
2010/11	0	0	0	10	73	126	98	77	90	81	11	34	600
2011/12	0	0	0	46	37	141	47	26	87	4	0	13	401
2012/13	0	0	25	39	46	164	101	32	88	45	1	0	541
2013/14	0	0	0	27	41	120	101	209	103	4	2	3	609
2014/15	0	21	3	9	115	128	174	55	121	60	1	6	693
Mean maximum temperature (°C)													
2008/09	–	–	–	–	–	–	–	26.9	27.1	26.7	22.5	19.2	–
2009/10	17.3	21.3	27.3	27.4	27.1	30.6	26.9	29.6	28.3	25.5	20.5	19.2	25.1
2010/11	13.8	23.7	28.5	29.8	28.8	28.6	27.2	28.2	27.9	23.2	21.9	19.1	25.1
2011/12	17.8	22.2	27.5	28.9	30.0	29.0	31.0	29.7	28.8	25.0	24.9	20.0	26.2
2012/13	20.8	22.7	24.7	29.4	30.3	27.2	30.2	31.6	28.9	25.6	23.7	21.8	26.4
2013/14	21.4	22.1	27.6	29.0	31.3	27.0	30.8	28.7	25.9	24.7	24.7	21.0	26.2
2014/15	20.0	23.1	28.4	30.8	27.6	29.4	30.1	31.0	28.5	26.9	27.8	20.3	27.0
Erfdeel													
Rainfall (mm)													
2008/09	-	-	-	-	-	-	-	65	53	3	4	31	-
2009/10	2	0	28	49	74	156	161	74	87	26	33	0	690
2010/11	0	0	0	13	74	155	50	55	60	53	51	40	551
2011/12	8	0	4	24	24	135	71	84	30	9	1	13	403
2012/13	2	2	58	56	61	136	94	41	14	55	1	0	542
2013/14	0	0	0	42	63	150	111	62	139	8	13	4	594
2014/15	0	24	0	20	87	85	85	45	69	53	1	6	475
Mean maximum temperature (°C)													
2008/09	-	-	-	-	-	-	–	27.7	27.6	27.0	22.7	19.6	–
2009/10	18.2	22.5	28.2	28.0	27.4	31.1	27.7	30.4	29.2	25.7	24.2	20.9	26.1
2010/11	21.0	24.5	29.6	30.3	29.8	29.3	28.4	29.2	29.8	24.4	22.5	19.8	26.6
2011/12	18.6	22.8	27.7	29.0	30.2	29.1	31.6	29.8	29.8	26.2	26.2	20.0	26.8
2012/13	21.4	23.7	24.9	29.4	30.5	28.6	30.8	32.0	29.5	25.7	24.2	21.7	26.9
2013/14	21.8	22.4	27.6	29.5	30.8	27.9	31.2	29.6	26.6	25.5	24.9	21.2	26.6
2014/15	20.1	23.3	28.7	30.8	27.2	30.2	31.3	31.4	28.6	26.8	27.0	19.9	27.1

ever slightly lower in Erfdeel when compared to Buffelsvallei.

Discussion

Conservation cropping systems are based on three principles which are no-till, cover crop retention and crop rotations (Marocco et al. 2009). In this study, the effect of these cropping systems on ear rot diseases and mycotoxins were determined from 2009/10 to 2014/15. The cropping systems did not have a significant effect on *F. verticillioides* target DNA accumulation in both Buffelsvallei and Erfdeel in all evaluated seasons. The major setback of crop residue retention is the build-up of disease inoculum (Govaerts et al. 2006). Crop residue

retention is suspected to influence disease accumulation through the provision of suitable disease development conditions and harbouring inoculum for further infection (Watkins and Boosalis 1994). Almeida et al. (2000) found that *Fusarium* spp. isolated from buried soybean residues was higher than *Fusarium* spp. isolated from surface residues. The lack of significant effects in cropping systems involving crop residue surface retention over six and four years in this study, respectively, indicates that surface retention of crop residues did not lead to *F. verticillioides* inoculum build up. The results in this study are in agreement with findings by Flett and Wehner (1991) and Flett et al. (1998) where it was reported that cropping systems had no significant effects regarding *F. verticillioides* occurrence in maize grain. This is a noteworthy finding, indicating that conservation agricultural production systems can be used in the studied localities without the potential increase of *F. verticillioides* in maize grain.

Fusarium graminearum accumulation was significantly elevated in the three-year maize/cowpea/babala rotation system only for the 2010/11 season in Buffelsvallei. This result suggests that *F. graminearum* contamination is largely unaffected by cropping systems employing different tillage and cover crops. However, during years with high or average disease levels, cropping systems may affect disease severity. Crop rotations have been reported as effective in the control of *F. graminearum* in wheat but not as effective on maize (Flett et al. 2001). In wheat, crop rotations with soybean resulted in reduced levels of *F. graminearum* when compared to rotations with maize regardless of tillage practice (Dill-Macky and Jones 2000). This may be due to the fact that both soybean and wheat produce less crop residues when compared to maize (Champeil et al. 2004). Maize residues take longer to decompose when compared to other crops and are more likely to harbour *F. graminearum* inoculum much longer (Hooker and Schaafsma 2005). Cowpea and babala also produce minimal crop residues therefore the increase in *F. graminearum* target DNA may be due to its persistence in maize residues (Marburger et al. 2015).

Fumonisin contamination was observed to be above the 4 mg/kg allowable limit as per South African legislation (Anonymous 2016) in the two year maize/sunflower rotation during the 2010/11 growing season and corresponded with high *F. verticillioides* target DNA accumulation for the same period and cropping system. This may be attributed to changes in soil management and stresses on plants (Marocco et al. 2009). The high rainfall towards the harvesting period during this season could have caused the high fumonisin contamination (Ono et al. 1999). Fluctuations in rainfall patterns and relative humidity may influence fumonisin contamination in maize grain by inflicting physiological stresses on plants (Fandohan et al. 2003). Several other factors such as drought, presence of other diseases, high oxygen tension and low pH may have played a role in enhancing fumonisin contamination in maize grain (Parsons and Munkvold 2012). These conditions contribute to plant stress and predispose plants to infection by *F. verticillioides* and resultant mycotoxin contamination. It is evident from this six-year study that fumonisin contamination of maize grain is not a threat under local climatic and geographic conditions and it is not greatly influenced by cropping systems. The effect of crop rotations combined with tillage effects on fumonisin contamination in maize grain is not well documented in literature and requires more extensive research.

Deoxynivalenol was significantly lower in rotation systems as opposed to monoculture systems during the 2011/12 season, thus supporting findings by Bernhoft et al. (2012) where it was reported that a lack of crop rotation increased levels of deoxynivalenol in cereals. In wheat, rotation systems involving soyabean reduced deoxynivalenol concentration by 49% when compared to rotation systems involving maize (Champeil et al. 2004). This emphasises the importance of choosing the correct preceding crop to be used in a rotation system. The frequency of the rotation is also an important factor, as the longer the rotation, the higher the chance of reducing disease accumulation and potential mycotoxin contamination (Champeil et al. 2004). Results from this study suggest that when environmental conditions are favourable, a lack of crop rotations under no-till may have an impact on deoxynivalenol contamination (Lori et al. 2009). The absence of significant effect in zearalenone and nivalenol contamination can be attributed to their general low contamination throughout all seasons in both localities. Previous studies in South Africa have found nivalenol to be scarce in maize (Rheeder et al. 1995).

In this study, the three-year rotations resulted in reduced levels of DER incidences when compared to other treatments, but only in the 2013/14 season. These results do support findings by Flett and Wehner (1991), Baliukoniene et al. (2011) and Kheyrodin (2011) that maize monoculture (till and no-till) over a period of years leads to a build-up of *S. maydis* inoculum. This may well be due to the extended periods of *S. maydis*

inoculum persistence on maize residues as they take longer to decompose (Glenn 2007). Maize is the only known commercial host for *S. maydis* and this would explain its persistence when maize is grown under monoculture (Masango et al. 2015). Flett et al. (2001) reported that wheat, soybean and peanut are better suited in reducing DER incidences as opposed to sunflower. It is therefore recommended to optimize tillage systems to control fungal infection in crop production by introducing efficient rotation systems (Oldenburg and Ellner 2015).

Previous reports in South Africa have indicated that tillage practices have no effect on *F. verticillioides* and *F. graminearum* accumulation in maize grain (Flett and Wehner 1991; Flett et al. 1998). It was evident from this study that *F. graminearum* target DNA accumulation, DER incidence, fumonisin and deoxynivalenol contamination in maize grain may be affected by tillage and rotation systems in seasons with average or high disease. This difference can be attributed to their use of outdated plating out methods for fungal biomass quantification. Morphological characteristics are not enough to correctly identify fungal isolates at species level (Gong et al. 2014). The real-time polymerase chain reaction (qPCR), method of quantification used in this study offers rapid, accurate, specific and sensitive target DNA detection and quantification (Nicolaisen et al. 2009).

Primary inoculum and weather conditions are suspected to play a critical role in the inconsistency observed between seasons in this study. Rotation systems restricted to cereal crops, in combination with no-till, are more probable to enhance Fusarium infection than longer rotations including legumes or catch crops (Baliukoniene et al. 2011). This study simultaneously examined the combined effects of tillage and rotation practices while previous studies only focused on tillage systems. No tillage paired with rotations and residue retention enhance plant growth and generally decrease disease incidence (Govaerts et al. 2006) while crop residue retention in no till systems increase microbial diversity in the soil and further enhance biological control potential. Balanced crop rotations in this system further assist in the regulation of pathogenic species (Govaerts et al. 2006), however, crop rotations have also been found to be less effective to control diseases caused by *Fusarium* spp. due to their wide host range and long term survival abilities (Krupinsky et al. 2002). *Fusarium* spp. are able to colonise and survive on tissue of plants not necessarily considered as hosts (Munkvold 2003) and this may limit the effectiveness of crop rotation systems in conservation agriculture.

The absence of significant effects during most of the study period indicates that conservation agriculture can be used without the possibility of drastically increasing the risk for disease and mycotoxin contamination in South African maize grain. However, the effect of CA on disease and mycotoxin contamination should be periodically surveyed especially during years where prevailing environmental conditions differ significantly to previous years. It is evident that many factors individually play a critical role in disease development and mycotoxin production. Disease accumulation, incidence and mycotoxin contamination varied between seasons as well as geographical location. It is therefore important to consider factors such as environmental conditions and geographical location that might play a role in disease development and mycotoxin contamination. Predictive models may assist farmers in making informed decisions regarding the potential of disease accumulation and mycotoxin infection as cropping systems were observed to not have a major effect in this study.

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References

Almeida, A., Saraiva, O., Farais, J., Gaudencio, C., & Torres, E. (2000). Survival of pathogens on soybean debris under no-tillage and conventional tillage systems. *Pesquisa Agropecuária Brasileira, 36*, 1231–1238.

Anonymous. (2002). Vicam fumonitest instruction manual. Vicam L.P. 313 Pleasant Street, Water town, MA 02472, United States of America.

Anonymous. (2012). Vicam DON and NIV WB instruction manual. Vicam L.P 34 Maple street, Milford, MA, United States of America.

Anonymous. (2016). Cosmetics & Disinfectants Act, 1972 (Act No. 54 of 1972). Regulations governing tolerances for fungus–produced toxins in foodstuffs: amendment. Government Gazette, Government notices, Department of Health, No 40250, South Africa.

Baliukoniene, V., Bakutis, B., Januškevičienė, G., & Mišeikienė, R. (2011). Fungal contamination and Fusarium mycotoxins in cereals grown in different tillage systems. *Journal of Animal and Food Sciences, 20*, 637–647.

Bernhoft, A., Torp, M., Clasen, P., Loes, A., & Kristoffersen, A. (2012). Influence of agronomic and climatic factors on fusarium infestation and mycotoxins contamination of cereals in Norway. *Food additives and contaminants, Part A, chemistry, analysis, control exposure and risk assessment, 29*, 1129–1140.

Boutigny, A., Beukes, I., Small, I., Zulhke, S., Spiteller, M., Janse van Rensburg, B., Flett, B., & Viljoen, A. (2012). Quantitative detection of Fusarium pathogens and their mycotoxins in South African maize. *Plant Pathology Journal, 61*, 522–531.

Champeil, A., Dore, T., & Fourbert, J. (2004). Fusarium head blight: epidemiological origin of the effects of cultural practices on head blight attacks and the production of mycotoxins by fusarium in wheat grains. *Plant Science, 166*, 1389–1415.

Degraeve, S., Madege, R., Audenaert, K., Kamala, A., Ortiz, J., Kimanya, M., Tiisekwa, B., Meulenauer, B., & Haesaert, G. (2016). Impact of local pre-harvest management practices in maize on the occurrence of *Fusarium* species and associated mycotoxins in two agro-ecosystems in Tanzania. *Food Control, 59*, 225–233.

Dill-Macky, R., & Jones, R. (2000). The effect of previous crop residues and tillage on Fusarium head blight of wheat. *Plant Disease, 84*, 71–76.

Fandohan, P., Hell, K., Marasas, W., & Wingfield, M. (2003). Infection of maize by *Fusarium* species and contamination with fumonisin in Africa. *African Journal of Biotechnology, 2*, 570–579.

Flett, B., & Wehner, F. (1991). Incidence of Stenocarpella and Fusarium cob rots in monoculture maize under different tillage systems. *Journal of Phytopathology, 133*, 327–333.

Flett, B., & McLaren, N. (1994). Optimum disease potential for evaluating resistance to *Stenocarpella maydis* ear rot in corn hybrids. *Plant Disease, 78*, 587–589.

Flett, B., McLaren, N., & Wehner, F. (1998). Incidence of ear rot pathogens under alternating corn tillage practices. *Plant Disease, 82*, 781–784.

Flett, B., McLaren, N., & Wehner, F. (2001). Incidence of *Stenocarpella maydis* ear rot of corn under crop rotation systems. *Plant Disease, 85*, 92–94.

Gelderblom, W., Marasas, W., Vleggar, R., Thiel, P., & Cawood, M. (1992). Fumonisins: Isolation, chemical characterization and biological effects. *Mycopathologia, 117*, 11.

Glenn, A. (2007). Mycotoxigenic *Fusarium* species in animal feed. *Animal Feed Science and Technology, 137*, 213–240.

Gong, L., Jiang, Y., & Chen, F. (2014). Molecular strategies for detection and quantification of mycotoxin-producing *Fusarium* species: a review. *Science of Food and Agriculture, 95*, 1767–1776.

Govaerts, B., Mezzalama, M., Sayre, K., Crossa, J., Nicol, J., & Deckers, J. (2006). Long term consequences of tillage, residue management and crop rotation on maize/wheat root rot and nematode populations in Sub tropical highlands. *Applied Soil Ecology, 32*, 305–315.

Hooker, D., & Schaafsma, A. (2005). Agronomic and environmental considerations of deoxynivalenol and fumonisin B_1 in corn across Ontario. *Epidemiology, 27*, 347–356.

Janse van Rensburg, B., McLaren, N., Flett, B., & Schoeman, A. (2015). Fumonisin producing *Fusarium* spp. and fumonisin contamination in commercial South African maize. *European Journal of Plant Pathology, 141*, 491–504.

Kheyrodin, H. (2011). Crop rotations for managing soil borne plant diseases. *African Journal of Food Science and Technology, 2*, 1–9.

Kruger, S., Kohn, B., Ramsey, C., & Prioli, R. (1999). Rapid immunoaffinity- based method for determination of zearalenone in corn by fluorometry and liquid chromatography. *Journal of AOAC International, 82*, 1364–1368.

Krupinsky, J., Bailey, K., McMullen, M., Gossen, B., & Turkington, K. (2002). Managing plant disease risk in diversified cropping systems. *Agronomy Journal, 94*, 198–209.

Lawrance, K., Prinsloo, M., & Berry, W. (1999). Long and short term effects of tillage systems on grain yield, the incidence of diseases and production costs for maize. *South African Journal of Plant and Soil, 16*, 85–91.

Lori, G., Sisterna, M., Sarandon, S., Rizzo, I., & Chidichimo, H. (2009). Fusarium head blight in wheat: Impact of tillage and agronomic practices under natural infection. *Crop Protection, 28*, 495–502.

Marburger, D., Venkateshwaran, M., Conley, S., Esker, P., Lauer, J., & Ane, J. (2015). Crop rotation and management on *Fusarium* spp. populations. *Crop Science, 55*, 1–12.

Marocco, A., Tabaglio, V., Pietri, A., & Gavazzi, C. (2009). Monoculture maize (*Zea Mays L.*) cropped under conventional tillage, no-tillage and N- fertilization: (II) fumonisin incidence on kernels. *Italian Journal of Agronomy, 3*, 69–75.

Masango, M., Flett, B., Ellis, C., & Botha, C. (2015). *Stenocarpella maydis* and its toxic metabolites: A South African perspective on diplodiosis. *World Mycotoxin Journal, 8*, 341–350.

Munkvold, G. (2003). Epidemiology of *Fusarium* diseases and their mycotoxins in maize ears. *European Plant Pathology Journal, 109*, 705–713.

Nicolaisen, M., Suproniene, S., Nielsen, L., Lazzaro, I., Spliid, N., & Justesen, A. (2009). Real-time PCR for quantification of eleven individual Fusarium species in cereals. *Journal of Microbiological Methods, 76*, 234–240.

Odriozola, E., Odeon, A., Canton, G., Clemente, G., & Escande, A. (2005). *Diplodia maydis*: a cause of death of cattle in Argentina. *New Zealand Veterinary Journal, 53*, 160–161.

Oldenburg, E., & Ellner, F. (2015). Distribution of disease symptoms and mycotoxins in maize ears infected by *Fusarium culmorum* and *Fusarium graminearum*. *Mycotoxin Research, 31*, 117–126.

Ono, E., Suguira, Y., Homechin, M., Kamogac, M., Vizzoni, E., Ueno, Y., & Hirooka, E. (1999). Effect of climatic conditions on natural mycoflora and fumonisins in freshly harvest corn of the state of Parana, Brazil. *Mycopathologia, 147*, 139–148.

Parsons, M., & Munkvold, G. (2012). Effects of planting date and environmental factors on fusarium ear rot symptoms and fumonisin B_1 accumulation in maize grown in six North American locations. *Plant Pathology, 61*, 1130–1142.

Placinta, C., D'Mello, J., & Macdonald, A. (1999). A review of worldwide contamination of cereal grains and animal feed with *Fusarium* mycotoxins. *Animal Feed Science and Technology, 78*, 21–37.

Rabie, C., Kellerman, T., Kriek, N., Van Der Westhuizen, G., & De Wet, P. (1985). Toxicity of *Diplodia maydis* in farm and laboratory animals. *Food and Chemical Toxicology, 23*, 349–353.

Reid, L., Nicol, R., Ouellet, T., Savard, M., Miller, J., Young, J., Stewart, W., & Schaafsma, A. (1999). Interaction of

Fusarium graminearum and *Fusarium moliforme* in maize ears: Disease progress, fungal biomass, and mycotoxin accumulation. *Phytopathology, 89*, 1028–1037.

Reid, L., Zhu, X., & Ma, B. (2001). Crop rotation and nitrogen effects on maize susceptibility to gibberella (*Fusarium graminearum*) ear rot. *Plant and Soil, 237*, 1–14.

Rheeder, J., Sydenham, E., Marasas, W., Thiel, P., Shepard, G., Schlechter, S., Stockenstrom, S., Cronje, D., & Viljoen, J. (1995). Fungal infestation and mycotoxin contamination of South Africa commercial maize harvested in 1989 & 1990. *South African Journal of Science, 91*, 127–131.

Sumner, D., Doupnik, B., & Boosalis, M. (1981). Effects of reduced tillage and multiple cropping on plant diseases. *Annual Review of Phytopathology, 19*, 167–187.

Waalwijk, C., de Vries, P., Köhl, J., Xu, X., van der Lee, T., & Kema, G. (2008). Development of quantitative detection methods for *Fusarium* in cereals and their application. In J. Leslie, R. Bandyopadhyay, & A. Visconti (Eds.), *Mycotoxins: detection methods, management, public health and agricultural trade* (pp. 195–205). Wallingford: CAB International.

Ward, J., & Nowell, D. (1998). Integrated management practices for the control of maize grey leaf spot. *Integrated Pest Management Reviews, 3*, 177–188.

Watkins, J., & Boosalis, M. (1994). Plant disease incidence as influenced by conservation tillage systems. Managing Agricultural Residues pp. 261–283.

Winnepenninckx, B., Backeljau, T., & van der Peer, Y. (1993). Extraction of high molecular weight DNA from molluscs. *Trends in Genetics, 9*, 407.

Youssef, M. (2009). Natural occurrence of mycotoxins and mycotoxigenic fungi on Libyan corn with special reference to mycotoxin control. *Research Journal of Toxins, 1*, 8–22.

Zinedine, A., Soriano, J., Molto, J., & Manes, J. (2007). Review on the Toxicity, Occurrence, Metabolism, Detoxification, Regulations and Intake of Zearalenone: An Oestrogenic Mycotoxin. *Food and Chemical Toxicology, 45*, 1–18.