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Diversity in Mycotoxins and Fungal Species Infecting Wheat in Nakuru District, Kenya

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Abstract

Mycotoxin contamination of wheat kernels and diversity of fungal pathogens were monitored in a survey of 26 fields in Nakuru district, Kenya, in 2006. Sampling was performed twice; at mid-anthesis in July for leaves, stems and spikelets and at harvest in September for spikelets and kernels. Kernels were analysed for the occurrence of 32 mycotoxins using a high performance liquid chromatography – electrospray tandem mass spectrometry (LC-ESI-MS/MS) method. Fungi were isolated from surface-sterilized tissues and differentiated morphologically to the genus level. *Fusarium* isolates were identified based on the sequence of translation elongation factor 1-alpha gene. Eleven *Fusarium*-related mycotoxins were quantified with deoxynivalenol being detected at highest frequency (69%) and highest concentrations. Occurrence of enniatins in wheat (50%) is reported for the first time in Kenya. Non-*Fusarium* mycotoxins detected included aflatoxin G2, ochratoxin A, alternariol and alternariol monomethyl ether. Prevalence of *Fusarium* species on different wheat parts was 100% at both growth stages. Nineteen *Fusarium* species were identified with seven species – *Fusarium chlamydosporum*, *Fusarium boothi*, *Fusarium poae*, *Fusarium scirpi*, *Fusarium arthrosporioides*, *Fusarium oxysporum* and *Fusarium graminearum* – accounting for 80% of infections. At anthesis, spikelets and leaves were the most and least susceptible tissue, respectively. At harvest, infection of spikelets was 59% higher than that of kernels.

Introduction

Fusarium species are widespread and common pathogens of cereals. They infect wheat causing foot rot, root rot and head blight (Stack 2000; Akinsanmi et al. 2004; Mudge et al. 2006). Wheat can be infected during all growth stages, however, anthesis is the most susceptible developmental stage and rainfall during this stage often results in economically important *Fusarium* head blight (FHB). The disease is often caused by a complex of fungal species with more than 16 *Fusarium* species being able to infect wheat ears (Parry et al. 1994). The number and spectrum of species involved varies with the region and weather conditions during the vegetation period. Diversity of *Fusarium* species may be higher than described earlier as the *Fusarium graminearum* clade has been divided into various species (O'Donnell et al. 2004; Starkey et al. 2007; Qu et al. 2008), which can be differentiated only using nucleic acid-based techniques.

Kenya has a wheat production system where the majority of farmers are small-scale producers (farms <8 hectares), while the rest of production is medium (8–20 hectares) or large-scale (>20 hectares, FAO 2001; Chianu et al. 2008). Most small-scale producers do not use fungicides for fungal disease management, and maize, the country's staple food and known to promote FHB incidence and severity, is grown in the crop rotation or sometimes side-by-side with wheat (FAO 2001). Concerns for *Fusarium* infection of cereals arise from quantitative and qualitative reduction in yield, increased cost of production and mycotoxin contamination, which poses a risk to human and livestock health. Major *Fusarium*-related mycotoxins such as deoxynivalenol, nivalenol (NIV), zearalenone (ZEA), T2-toxin and HT2-toxin have been reported in wheat kernels sampled from fields in different wheat-growing regions of Kenya (Muthomi et al. 2002, 2007a, 2008). It is common practice in the country's production systems to graze or feed livestock on wheat straw after harvesting which acts as a pathway of introduction of mycotoxins to the animal feed chain. Mycotoxin contamination of wheat in Kenya is exacerbated by the tropical climate – high

humidity and temperature – which creates optimal conditions also for the growth of other fungi.

The altitude range of Nakuru district from 1520 to 2500 m above sea level (ASL) is a major factor in variations in temperature and rainfall between different regions (Anonymous 1997). Overall, the district has an annual rainfall of 965 mm (KFSSG 2008). The climatic variations may influence *Fusarium* species diversity as well as FHB and mycotoxin contamination of wheat and other small grain cereals in different localities.

Previous studies have focused on the morphological identification of *Fusarium* species; the quantification of major trichothecenes and ZEA, as well as exploring disease management options is an integrated approach (Muthomi et al. 2002, 2007a, 2008). Despite these efforts, the documentation of mycotoxin contamination of wheat, diversity of associated fungal pathogens and risks associated with *Fusarium* species infection on wheat in Kenya is still at infant stage. Our study provides information on the spectrum of mycotoxins contaminating wheat kernels from Nakuru district, Rift Valley Province, sampled in a monitoring of 26 fields. Additionally, the prevalence and diversity of fungal species colonizing different parts of wheat plants – leaves, stems and ears – were quantified at mid-anthesis and at harvest.

Materials and Methods

Field sampling

A field monitoring was conducted between June and September 2006 in Nakuru district in mid-western Kenya, one of the country's leading wheat-growing regions. A total of 25 fields were sampled out of which 15, 2 and 8 fields were small (<8 ha), medium (8–20 ha) and large (>20 ha) scale, respectively. Random sampling was carried out at mid-anthesis (growth stage, GS 65–69) and harvest (GS 92) in three agro-ecologically diverse divisions – Rongai (1850 m ASL, seven farms), Njoro (2200 m ASL, seven farms), and Mau Narok (2500 m ASL, 11 farms). At mid-anthesis, leaves, stems and spikelets were sampled, while at harvest, spikelets and kernels were sampled. At harvest, kernels from an additional field in Rongai division were sampled resulting in a total of 26 kernel samples. At least 30 plants or 0.5 kg of kernels were sampled per field from which fungal isolations were carried out from leaves, stems, kernels and spikelets. From large fields, a maximum of double the amount sampled from small-scale fields was taken. A semi-structured questionnaire was used to generate information on production data. Half a kilo kernel sample was taken from each field at harvest for mycotoxin analysis.

Microbiological assays

Samples of leaves (1 cm^2) and stems (1 cm long) as well as kernels and spikelets were surface sterilized with 1.3% sodium hypochlorite for 2 min and subsequently rinsed three times for 2 min in sterile distilled water. The plant sections were then plated on half strength potato dextrose agar (PDA) modified with salts and antibiotics (Muthomi et al. 2002). A total of 150 plant parts were plated from each field (five plant sections per petri-dish replicated 30 times). After 5–7 days, colonies growing from the plant sections were marked and sub-cultured on PDA and synthetic nutrient agar (SNA, Nirenberg 1981) with two replications. Cultures on SNA were incubated under near UV-light to facilitate *Fusarium* sporulation while those on PDA were incubated at room temperature (23 ± 2°C) for 14–21 days. Manuals from Nelson et al. (1983) and Leslie and Summerell (2006) were used for the identification of *Fusarium* species based on morphological and cultural characteristics. From all typical cultures identified morphologically ($n = 8365$) at least one representative isolate was selected for confirmation through molecular identification. Other fungal pathogens infecting wheat parts were identified to genus level and their isolation frequency was established.

DNA extraction for sequencing

Single-spore *Fusarium* isolates grown on PDA for 5–7 days were lyophilized in 2 ml Eppendorf tubes for 12 h and stored at room temperature. Freeze-dried mycelium (18–22 mg) was transferred into a sterile 2 ml Eppendorf tube. After adding a sterile steel bead, a mechanical shaker was used to crash the mycelium into fine powder. The 'Wizard magnetic DNA purification system for food' (Promega, Mannheim, Germany) was used for DNA extraction according to the manufacturer's instructions, and the DNA was stored at −20°C.

PCR for *tef* gene sequencing

Polymerase chain reaction (PCR) was carried out for partial sequencing of translation elongation factor 1-alpha (*tef*) gene of *Fusarium* isolates. Sequences of *tef* were obtained by performing PCRs using primers EF1T (ATGGGTAAGGAGGACAAGAC) and EF2T (GGAAGTACCAGTGATCATGTT, O'Donnell et al. 1998). The reactions were performed in a Biometra® T Gradient PCR system (Göttingen, Germany). Amplification reactions were carried out in 50 μl containing 20 ng of template DNA in 2, 5 μl PCR buffer, 4 μl dNTPs (600 μM), 1.5 μl of each primer (6 μM) and 0.3 μl Taq DNA Polymerase (Roche Diagnostics, Mannheim, Germany). The amplification protocol was one cycle of 2 min at 95°C, 35 cycles of 30 s at 95°C (denaturation), 30 s at 54°C (annealing), 1 min at 72°C (extension) and one cycle of 10 min at 72°C. Amplification and concentration of DNA was checked by gel electrophoresis, and where necessary, DNA in the sample was diluted to a final concentration of 10 ng/μl.

Sequencing reactions were performed in volumes of 10 μl including 4 μl of amplified DNA, 2 μl buffer, 2 μl of DYEnamic ET terminator Cycle sequencing kit (GE Healthcare, Freiburg, Germany) and 0.7 μl of one primer (EF1T or EF2T). Thermocycler program for sequencing was 24 cycles of 20 s at 94°C, 15 s at 50°C and 1 min at 60°C.

Sequencing and blasting of *tef* gene

Automated sequencing was run on ABI 3700 (Applied Biosystems, Foster City, CA, USA) equipment of the Greenomics® facility at Plant Research International, Wageningen (The Netherlands). Sequences of *tef* gene were utilized to search for matches of the isolates using available information in two gene banks – FUSARIUM–ID version 1.0 database (http://fusarium.cbio.psu.edu/) and the National Centre for Biotechnology Information database (http://www.ncbi.nlm.nih.gov/BLAST/). Sequenced isolates were deposited at the Institute of Crop Science and Resource Conservation (INRES) culture collection, University of Bonn, Germany and at Biodiversity (Mycology and Botany) Center of the Agriculture and Agri-Food, Canada.

Mycotoxin analysis

Kernels sampled at harvest (GS 92) were ground and 250 mg of the flour weighed in 1.5-ml vials in two replications. All sample replicates were analysed for mycotoxins based on liquid chromatography – electrospray tandem mass spectrometry (LC-MS/MS) technique. The technique has been optimized for the detection of 32 toxins in a single run (Herebian et al. 2009).

Production data and data analysis

A semi-structured questionnaire was used to generate information on production systems such as farm sizes, source of seeds, rotation programs, straw disposal and disease management practices. For the isolation data, infection frequency from each plate was pooled and mean frequency determined. Analysis of variance was based on PROC ANOVA procedure of Genstat Discovery 2 statistical software (Lawes Agricultural Trust, Rothamsted Experimental Station 2006, version 9) and mean differences compared using the Fisher's protected least significant difference test at 5% significance level.

Results

Diversity and frequency of fungal species isolated from wheat at different growth stages

Above average rainfall was recorded during the cropping season 2006 in the district. A broad diversity of fungal pathogens was isolated at varying frequencies from different wheat parts either at anthesis or at harvest (Table 1). Five fungal genera – *Fusarium*, *Alternaria*, *Penicillium*, *Aspergillus* and *Epicoccum* – were common on all plant parts. *Alternaria* species were more common on leaves, while *Fusarium* species were isolated at a higher frequency from stems and spikelets at both growth stages. Colonization by *Fusarium* species was highest for spikelets and lowest for leaves. Among wheat fields, *Fusarium* infection of kernels varied from 6.7 to 100% (mean 42.2%). *Alternaria* species were isolated at high frequency from all plant parts at both growth stages. *Penicillium* and *Aspergillus* species, common storage fungi were identified on all plant parts in the field and showed opposite distribution; where one was frequent, the other was low. Frequency of *Epicoccum* spp. decreased from anthesis to harvest by a factor of 2.7. Other fungal species isolated included *Trichoderma* sp. and *Pythium* sp. Although molecular techniques were not used routinely, sequencing of the *tef* gene proved handy in distinguishing genetically divergent *Fusarium* species which could not be distinguished based on morphological characteristics. Infection frequency by *Fusarium* species was significantly lower ($P \leq 0.05$) in large-scale fields where fungicides were used for FHB management. However, this was not the case in small-scale fields where fungicides were normally applied at rates below the recommended rates.

Fusarium species identified at anthesis

At anthesis, 16 *Fusarium* species were identified from wheat leaves, stems and spikelets (Fig. 1a). The prevalence of *Fusarium* infection on the field basis was 100%. Frequency of *Fusarium*-infected leaves ranged from 3 to 67% (mean 29%), the range for stems was 7–73% (mean 35%) and that for spikelets was 7–83% (mean 39%). The most frequent species on spikelets (2–8%) were *Fusarium chlamydosporum*, *Fusarium poae*, *Fusarium boothi*, *Fusarium arthrosporioides*, *Fusarium scirpi*, *Fusarium oxysporum* and *F. graminearum*. The most frequent species on stems and leaves were *F. chlamydosporum*, *F. boothi*, *F. scirpi*, *F. oxysporum*, *F. poae*, *Fusarium avenaceum* and *F. graminearum*. Overall, the six most frequent species on the three plant parts – *F. chlamydosporum*, *F. boothi*, *F. poae*, *F. scirpi*, *F. oxysporum* and *F. arthrosporioides* – accounted for more than three quarters of all *Fusarium* species identified at anthesis. Other common *Fusarium* species included *Fusarium equiseti* and *incarnatum incarnatum*. Species identified at low frequencies

Table 1
Frequency of fungal genera isolated from different parts of wheat plants at anthesis (GS 65) and at harvest (GS 92), respectively

Growth stage	Plant part	Frequency (%) of fungal pathogens					
		Fusarium spp.	*Alternaria* spp.	*Penicillium* spp.	*Aspergillus* spp.	*Epicoccum* spp.	Other fungi
GS 65	Leaves	29.1 ± 5.5[a]	43.2 ± 7.7	24.3 ± 6.5	16.7 ± 5.3	21.7 ± 4.3	8.7 ± 2.8
	Stems	39.3 ± 7.0	24.7 ± 5.4	21.7 ± 5.2	6.9 ± 1.9	12.0 ± 2.3	10.9 ± 3.1
	Spikelets	43.5 ± 7.8	36.8 ± 6.9	29.9 ± 7.1	10.5 ± 3.8	16.7 ± 3.9	6.5 ± 2.3
GS 92	Spikelets	67.1 ± 8.0	21.3 ± 2.9	7.4 ± 3.2	13.3 ± 4.2	8.6 ± 2.4	6.8 ± 1.4
	Kernels	42.2 ± 8.1	25.0 ± 5.2	21.2 ± 4.2	9.9 ± 4.0	10.1 ± 2.2	2.7 ± 1.5

[a]Mean ± SD (n = 30 plates with five plant parts each).

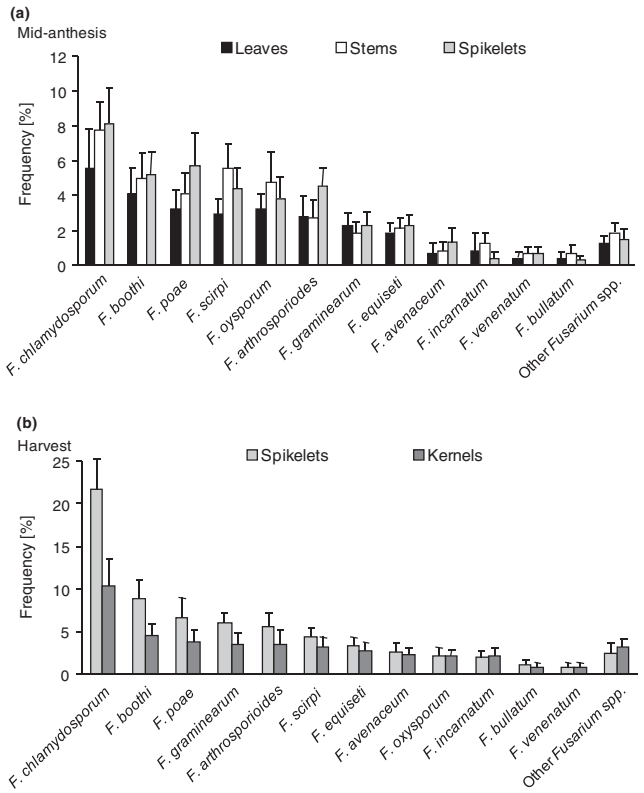

Fig. 1 Frequency of *Fusarium* species on different parts of wheat plants at two growth stages. (a) GS 65, (b) GS 92; bars represent standard deviation. Summary of data from 25 (GS 65) and 26 (GS 92) fields sampled in Nakuru district, Kenya

(<1%) were *Fusarium venenatum*, *Fusarium bullatum*, *Fusarium longipes*, *Fusarium solani*, *Fusarium sporotrichioides* and *Fusarium verticillioides*. Infection of leaves, stems and spikelets by *F. boothi* was higher than by *F. graminearum* by a factor of 1.4. Tissue susceptibility to *Fusarium* infection decreased in the order: spikelets, stems and leaves, respectively. However, there was variation depending on individual *Fusarium* species. Distinction between species within *F. graminearum* clade, *F. avenaceum* and *F. arthrosporioides* as well as recently described *Fusarium* species such as *F. venenatum*, *F. bullatum* and *F. incarnatum*, was only possible through sequencing of the *tef* gene.

Fungal species isolated from ears at harvest

The frequency of the genera *Alternaria*, *Penicillium* and *Epicoccum* infecting wheat spikelets at harvest was considerably lower than at anthesis; in contrast, the frequency of the genus *Fusarium* increased by an average of 24% (Table 1).

A total of 18 *Fusarium* species were isolated from kernels at frequencies ranging from <1 to 10.4%. *Alternaria* spp., *Penicillium* spp., *Aspergillus* spp. and *Epicoccum* spp. were also common (Table 2). Nineteen *Fusarium* species were identified from the spikelets and kernels of wheat ears (Fig. 1b). The frequency of *Fusarium*-infected kernels ranged from 7 to 100% (mean 42%) while that of spikelets ranged from 10 to 100% (mean 67%). The most frequent species on spikelets and kernels were *F. chlamydosporum* (average 16%), *F. boothi* (7%), *F. poae* (5%), *F. graminearum* (5%), *F. arthrosporioides* (5%), *F. scirpi* (4%) and *F. avenaceum* (3%). *Fusarium* species described only recently, such as *F. venenatum*, *F. bullatum* and *F. incarnatum* were also detected commonly. *Fusarium longipes*, *F. verticillioides*, *F. solani*, *F. cerealis*, *F. pallidoroseum*, *F. sporotrichioides* and *F. chlamydosporum* var. *fuscum* were identified at low frequencies. *Fusarium* infections of spikelets increased from anthesis to harvest by 72%.

The frequency of plant tissue infected by more than one *Fusarium* species ranged from 1.6% (leaves) to 3.6% (spikelets) at GS 65. For spikelets, this frequency increased to 9.1 ± 2.0% at harvest. Incidence of multiple infections was lowest in leaf tissues at anthesis (mean 1.6%) and highest in spikelets at harvest (mean 9.1%).

Spectrum and concentration of mycotoxins

Fifteen mycotoxins were detected in the samples of wheat kernels (Table 3). Fields varied in the spectrum and concentration of mycotoxins. Samples from three fields had the broadest spectrum (eight mycotoxins above level of quantification). In kernels from field 6, although infected by several pathogens, no mycotoxin could be detected. The majority of samples (14 out of 26) were contaminated with two or three toxins. Eleven compounds detected in the samples are known to be produced by *Fusarium* species. The presence of alternariol (AOH) and its monomethyl ether (AME) in seven samples (27%) was related to the occurrence of *Alternaria* species while ochratoxin A (OTA), also detected in seven samples is known to be produced by *Aspergillus* and *Penicillium* species. Aflatoxin G2 produced by *Aspergillus* species was detected in three wheat samples.

The most frequent *Fusarium*-associated mycotoxins and their incidence were DON (69%), enniatin B (50%), 3-acetyl-deoxynivalenol (3-AcDON) (35%), ZEA (27%) and fusarenone X (15%). By far, the highest concentrations were detected for DON and 3-acetyl DON (1310 and 1703 ng/g, respectively). In fields, where both DON and 3-AcDON were detected, 3-AcDON was in higher concentration than DON except in two fields. However, DON was detected in 42% of the fields where its acetylated derivative 3-AcDON was not detected. Moniliformin (MON) and NIV were detected only in two fields each.

Spatial distribution of *Fusarium* species in Nakuru

Incidence of *Fusarium* species varied significantly ($P \leq 0.05$) among the three sampling areas. Rongai division had the highest diversity of *Fusarium* species while Njoro had the lowest (Table 4). With an overall low incidence of *Fusarium* species, *F. scirpi* was missing only in this division. There were no significant differences ($P \geq 0.05$) in *Fusarium* species diversity among farms of different sizes. The number of fungal species colonizing kernels was not correlated to the number of mycotoxins measured in the samples ($R^2 = 0.056$, $n = 26$).

Table 2
Number of fungal isolates identified from 150 wheat kernels per field sampled from 26 fields in Nakuru district at harvest, by species

Division	Altitude[a] (m ASL)	Rainfall[b] (mm/a)	Field	FCHL	FBOO	FPOA	FGRA	FART	FSCI	FEQU	FAVE	FOXY	FINC	FBUL	FVEN	FSPP	ALSP	PESP	ASSP	EPSP	OSPP
Rongai	1520–1890	< 760	1	19	0	5	0	6	11	10	4	6	4	0	0	9	84	49	34	17	9
			2	38	14	0	0	0	6	7	4	0	10	0	0	6	38	39	0	29	22
			3	36	16	10	0	40	4	14	10	10	0	0	0	7	42	31	6	29	0
			4	18	11	0	0	0	0	6	0	6	0	0	0	4	53	48	0	0	0
			5	21	6	6	0	6	4	0	0	5	0	0	0	6	58	47	41	17	0
			6	21	14	0	10	11	25	20	12	9	6	6	0	5	35	64	0	26	0
			7	9	6	9	11	5	7	7	0	5	0	0	0	0	53	31	68	6	0
			8	15	14	7	24	17	5	5	7	7	0	0	0	5	13	24	49	39	0
Njoro	1800–2400	760–1270	9	15	0	4	0	0	0	10	0	3	0	0	0	4	59	48	34	6	4
			10	0	0	12	0	9	0	0	6	6	11	0	0	0	58	61	30	11	0
			11	0	5	0	6	0	0	0	5	0	0	0	0	0	29	34	24	0	32
			12	9	0	0	5	0	0	0	0	0	5	0	0	5	93	48	23	7	0
			13	0	6	4	0	10	0	5	6	0	6	4	0	0	28	26	0	34	0
			14	0	11	5	4	6	0	0	5	0	0	0	0	0	17	48	36	9	7
			15	0	0	0	0	0	0	6	0	5	5	0	0	0	68	53	0	12	0
Mau Narok	> 2400	1270	16	16	0	6	5	0	0	0	0	0	8	0	9	6	53	21	25	9	0
			17	15	6	0	6	5	12	4	4	5	0	0	4	14	40	56	0	4	7
			18	68	11	29	6	0	9	6	0	7	7	0	0	11	21	11	0	9	0
			19	0	5	0	0	0	0	0	6	0	4	0	0	0	44	35	0	27	0
			20	6	9	6	5	6	0	0	0	0	0	0	6	4	0	7	0	11	0
			21	9	0	9	0	0	0	0	0	5	4	0	0	14	4	6	0	14	5
			22	19	24	0	14	7	15	5	7	0	12	5	10	4	0	14	0	17	0
			23	21	6	0	16	12	11	7	6	0	0	0	0	5	0	0	0	28	6
			24	19	4	11	6	0	12	6	4	6	0	8	0	9	11	0	0	7	19
			25	24	5	0	5	0	0	0	5	6	4	7	0	5	25	14	0	16	0
			26	11	5	19	14	5	7	0	6	0	0	0	0	0	29	0	10	18	0

FCHL, *Fusarium chlamydosporum*; FBOO, *Fusarium boothi*; FPOA, *Fusarium poae*; FGRA, *Fusarium graminearum*; FART, *Fusarium arthrosporioides*; FSCI, *Fusarium scirpi*; FEQU, *Fusarium equiseti*; FAVE, *Fusarium avenaceum*; FOXY, *Fusarium oxysporum*; FINC, *Fusarium incarnatum*; FBUL, *Fusarium bullatum*; FVEN, *Fusarium venenatum*; FSPP, other *Fusarium* species; ALSP, *Alternaria* spp.; PESP, *Penicillium* spp.; ASSP, *Aspergillus* spp., EPSP, *Eppicoccum* spp., OSPP, other fungal species.
[a]Source – Anonymous (1997).
[b]Source – Kenya Meteorological Department.

Table 3
Diversity of mycotoxin contamination of wheat kernels from 26 fields in Nakuru district, Kenya, at harvest; concentration (ng/g) of 15 mycotoxins detected in ground kernels

Division	Field	Deoxynivalenol 20[c]	3-AcDON[a] 5[c]	Nivalenol 20[c]	Fusarenon X 3[c]	HT2-toxin 3[c]	15-AcO-[b] scirpenol 10[c]	Neosolaniol 5[c]	Zearalenone 5[c]	Monoliformin 2[c]	Enniatin B 2[c]	Beauvericin 2[c]	Aflatoxin G2 2[c]	Alternariol 4[c]	Alternariol Omethyl 2[c]	Ochratoxin A 1[c]
Rongai	1	1310	1445	–[d]	164	161	42	28	10	–	–	–	96	–	–	–
	2	45	285	–	–	–	–	–	–	–	5	–	–	–	5	–
	3	1116	1703	–	294	124	–	20	7	–	–	–	76	–	–	–
	4	243	–	–	14	–	–	–	–	–	74	–	–	37	52	88
	5	40	154	–	–	–	–	–	–	–	2	–	–	–	–	–
	6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	7	220	–	–	–	–	–	–	–	–	113	–	–	–	–	–
	8	1159	661	60	–	239	107	51	–	–	–	–	103	–	–	8
Njoro	9	–	–	–	–	–	–	–	10	–	–	–	–	–	–	–
	10	–	–	–	–	–	–	–	16	–	29	–	–	–	9	–
	11	328	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	12	677	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	13	40	–	–	–	–	–	–	–	–	47	–	–	–	–	–
	14	–	–	–	–	–	–	–	9	17	256	–	–	–	–	–
	15	–	–	–	–	–	–	–	–	–	3	–	–	–	5	–
Mau Narok	16	37	80	–	–	–	–	–	–	5	171	–	–	–	–	–
	17	628	–	–	–	–	–	–	–	–	–	–	–	–	–	18
	18	33	–	–	–	–	–	–	–	–	–	–	–	–	3	–
	19	146	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	20	–	234	–	–	–	–	–	–	–	–	–	–	–	13	28
	21	–	120	25	–	–	–	–	55	–	51	13	–	71	174	255
	22	52	–	–	–	–	–	–	–	–	59	15	–	–	–	–
	23	–	–	–	–	–	–	–	10	–	151	–	–	–	–	–
	24	25	–	–	–	–	–	–	–	–	8	–	–	–	–	–
	25	73	–	–	19	–	–	–	–	–	–	–	–	–	–	15
	26	188	125	–	–	–	–	–	–	–	–	–	–	–	–	13

Values with shaded background are above recommended limits in cereals (EC 2006; total of DON and 3-Ac-DON).
[a]3-AcDON, 3-acetyl-deoxynivalenol.
[b]15-AcO-, 15-monoacetoxy-.
[c]LoQ, level of quantification.
[d]Below LoQ.

Table 4
Diversity of fungi infecting wheat kernels and frequency of kernels infected by *Fusarium* species and other fungi in Nakuru district, Kenya, by division and by farm size

	Species richness (mean ± SEM)		Frequency of infected kernels (%)	
	Fusarium spp.	Other genera	*Fusarium* spp.	Other genera
Division				
Rongai (8[1])	8.3 ± 1.3 a[2]	3.8 ± 0.3 ab	59.1 ± 9.1 a	91.8 ± 7.3 a
Njoro (7)	4.6 ± 0.7 b	4.0 ± 0.3 a	19.8 ± 2.9 b	90.0 ± 7.1 a
Mau Narok (11)	7.6 ± 1.7 a	3.0 ± 0.2 b	48.2 ± 7.9 a	37.7 ± 6.9 b
Farm size[3]				
Small (15)	6.4 ± 1.6 A[2]	3.7 ± 0.2 A	40.8 ± 7.1 A	79.9 ± 7.8 A
Medium (3)	6.3 ± 2.4 A	3.7 ± 0.7 A	42.2 ± 25.5 A	84.9 ± 8.7 A
Large (8)	8.1 ± 0.9 A	3.0 ± 0.4 A	47.5 ± 7.4 A	34.6 ± 9.7 B

[1]Number of fields investigated.
[2]Means followed by the same letters within columns not significantly different ($P \leq 0.05$).
[3]Small, < 8 hectares; medium, 8–20 hectares; large, > 20 hectares.

Data on the production systems provided information on their possible contribution to build-up of *Fusarium* inoculum in the region. The majority (46%) of farmers sourced their seeds from neighbours while 29 and 25% relied on certified and own seeds, respectively. Ninety per cent of farmers prepared their fields mechanically compared to 10% who did it manually. More than 50% used crop rotation as the preferred method for fungal disease management compared to 36 and 9% depending on fungicides or fallow rotation, respectively. However, 32% of the farmers included maize in the crop rotation, although at different crop cycle intervals ranging from 0 to 3 years. The most popular method for straw disposal was plough-in practiced by 39% of farmers. Thirty-one per cent and 15% of them fed the straw to livestock and burnt it, respectively, while another 15% used other methods of disposal.

Discussion

Field monitoring in Nakuru demonstrated that FHB on wheat was the result of a complex of at least 19 *Fusarium* species, the widest *Fusarium* species diversity documented on wheat in Kenya from a single study. *Fusarium chlamydosporum*, *F. boothi*, *F. poae*, *F. sciripi*, *F. arthrosporioides*, *F. oxysporum* and *F. graminearum* accounted for 80% of all infections on leaves, stems, spikelets and kernels. Even in a situation of a *Fusarium* complex, there is dominance of a few species which was in agreement with Akinsanmi et al. (2004). Previous studies which relied on morphological characteristics described *F. graminearum* as the dominant species. *Fusarium boothi* (O'Donnell et al. 2004) was found to be the dominant species within the *F. graminearum* clade in Nakuru district in this study. Additionally, *F. arthrosporioides* – a newly described species previously classified as *F. avenaceum* (Yli-Mattila et al. 2002) – was found to be more prevalent than *F. avenaceum*. The dominance of *F. boothi* and *F. arthrosporioides* compared to *F. graminearum* and *F. avenaceum*, respectively, provided new status on *Fusarium* species on wheat in Nakuru. A number of newly described *Fusarium* species such as *F. bullatum* and *F. venenatum* (Geiser et al. 2004; O'Donnell et al. 2004) were also identified in the study although at a low frequency. This is the first report of these species in Kenya and underlines the importance of molecular techniques in the identification of *Fusarium* species with morphological and cultural similarities but which are genetically divergent. The study therefore highlights the limitations of reliance on morphological characteristics in the establishment of *Fusarium* species diversity. The wide *Fusarium* species diversity has implications both on disease management strategies as well as health risks to humans and livestock.

Although previous studies in the country reported a similar ranking of *Fusarium* species, there were variations in diversity and frequency of isolation. Ranking of *Fusarium* species in Nakuru district through a field monitoring study during the same year (2006) was in decreasing order: *F. poae*, *F. graminearum* and *F. chlamydosporum*, respectively (Muthomi et al. 2008). Another field monitoring in the same district ranked *F. poae*, *F. oxysporum*, *F. graminearum*, *F. chlamydosporum* and *F. verticillioides* as the major FHB causes species in wheat, while *F. poae*, *F. chlamydosporum* and *F. oxysporum* were the most prevalent in all the agro-ecological zones in Nakuru and Nyandarua districts (Muthomi et al. 2007a). In a field monitoring on wheat and maize by Muthomi et al. (2007b) in Nakuru district, prevalence of FHB was 90–100%, which concurs with the current findings. They identified 14 *Fusarium* species with *F. poae*, *F. graminearum*, *F. chlamydosporum* and *F. oxysporum* being the most frequent in wheat and *F. verticillioides* in maize, with *F. graminearum* and *F. verticillioides* infecting both crops. Co-occurrence of *Fusarium* species in wheat and maize kernels implies co-occurrence of mycotoxins. The authors reported that most wheat and maize grain samples were contaminated with DON with concentrations of up to 1200 and 4600 μg/kg, respectively. The current study and that by Muthomi et al. (2007b) elucidated potential spread of *Fusarium* inoculum from maize to wheat (and vice versa), which are regular rotation crops or grown alongside each other in most Kenya small-scale production systems. Isolation of *F. verticillioides* from wheat – described as a pathogen of maize by Leslie and Summerell (2006) – even though at low frequencies highlights the role of maize in FHB

of wheat. The differences in ranking of *Fusarium* species could be attributed to reliance on morphological identification by previous studies in contrast to the current study, which employed both morphological and molecular tools. Additionally, differences in sampling times, seasons and agro-ecological zones could have a bearing on species diversity and prevalence.

Similar to the species diversity, this report documents the widest spectrum of mycotoxins in Kenyan wheat from a single monitoring study. *Fusarium* species identified in this study are well-known mycotoxin producers (Desjardins 2006; Leslie and Summerell 2006). *Fusarium boothi*, *F. graminearum*, *F. poae* and *F. equiseti* produce type B trichothecenes such as NIV, DON and its acetylated derivatives as well as ZEA and fusarenone while *F. avenaceum*, *F. chlamydosporum*, *F. equiseti* and *F. oxysporum* are known to produce MON (Desjardins 2006). However, there is little information on mycotoxins produced by newly described species such as *F. bullatum*, *F. arthrosporioides*, *F. venenatum*, *F. incarnatum* and *F. pallidoroseum*, which were identified in this study. Such species may have a similar or different spectrum of mycotoxins to the species with which they are morphologically similar.

The detection of several mycotoxins in wheat samples from Nakuru district is in agreement with reports on cereals in Kenya with a 35% co-occurrence of DON, ZEA and T2-toxin (Muthomi et al. 2007a). Other studies reported a narrower spectrum of mycotoxins in Kenya wheat (Muthomi et al. 2002, 2008). This is the first report of wheat contamination with enniatins in Kenya. Enniatins are produced by a wide range of *Fusarium* species and on a variety of host crops (Logrieco et al. 2002; Thrane et al. 2004). The *Fusarium* species reported to produce enniatins and identified in this study include *F. chlamydosporum*, *F. poae*, *F. arthrosporioides*, *F. avenaceum*, *F. oxysporum*, *F. scirpi*, *F. proliferatum*, *F. sporotrichioides* and *F. verticillioides* (Herrmann et al. 1996; Nicholson et al. 2004; Desjardins 2006). Although little is known about the occurrence of enniatins, a recent study by Dornetshuber et al. (2007) reported that short-term exposure to very low concentrations of the toxin might have tumour-promoting functions based on growth stimulation. Elevated enniatin concentrations exerted profound cytostatic and cytotoxic activities especially against human cancer cells, suggesting a potential quality of enniatins as an anticancer drug (Dornetshuber et al. 2007; Lee et al. 2008).

Although the prevalence of *Fusarium* species was 100%, no mycotoxins were detected in the kernel sample from one field. This could be attributed to superficial infection of spikelets, mycotoxin levels below the toxin-specific limits of detection, or production of other *Fusarium* mycotoxins besides the 32 whose detection had been optimized using LC/MS/MS system, or a combination of these factors. Mycotoxins known to be produced by other fungal species such as AOH and AME, aflatoxin G2 (AFG2) and OTA aggravate the risk of mycotoxin exposure to humans and animals. OTA is produced by fungi of the genera *Aspergillus* and *Penicillium* (Benford et al. 2001; Weidenbörner 2001). Although OTA contamination increases during storage, it was clear in this study that the primary contamination occurs under field conditions. This is the first report of OTA contamination of wheat in Kenya. In all samples where they were detected, AFG2 and OTA were above the recommended limits of 4 and 5 ppb, respectively in cereals according to the European Union standards (EC 2006), implying that there is a danger of high exposure of the two mycotoxins to consumers.

Lower frequency of *Fusarium*-infected kernels in Njoro division was associated with a lower mycotoxin contamination of wheat kernels although the colonization of kernels by other fungal species in this mid-altitude area was similar to that in Rongai the division at the lowest altitude. Low fungal colonization of wheat kernels from Mau Narok the highest altitude division is likely to result from lower temperature as well as from fungicide use on large-scale farms dominating in this division. Variation in moisture index among the three study regions during the period of study (Mau Narok = <10; Njoro = 10–30; Rongai = 30–42) as well as differences in precipitation (Mau Narok = 1270 mm; Njoro = 760–1270 mm; Rongai = 760 m) could have influenced *Fusarium* infection levels and mycotoxin contamination of kernels (Kenya Meteorological Department, 2006). In Mau Narok, a region where most of the fields are large-scale, fungicide application for FHB management was widely practiced. Common FHB control fungicides included tebuconazole (Folicur®; Bayer Crop Science, Research Triangle Park, NC, USA) and propiconazoles (Tilt®; Syngenta Crop Prop Protection, Greensboro, NC, USA and Bumper®; Mana Crop Prop Protection, Raleigh, NC, USA). However, in some of the small-scale fields, application rates were up to 26% below the recommended rates thereby compromising their efficacy levels.

OTA, however, was detected in five out of 11 samples. Similar to the low correlation between the *Fusarium* mycotoxins and the frequency of *Fusarium* species, OTA levels were hardly correlated to the frequency of *Aspergillus* and *Penicillium* species.

The study emphasizes the need to include other mycotoxigenic fungal pathogens into strategies for the management of *Fusarium* species causing FHB, at least under tropical and subtropical conditions. Continuous monitoring of *Fusarium* species and mycotoxin contamination is necessary to understand their dynamics and long-term trends with a view to establish effective management strategies.

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