Journal of Plant Sciences 3 (1): 52-60, 2008
ISSN 1816-4951
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Head Blight of Wheat in Kenya and Contamination of Grain with Mycotoxin Producing *Fusarium* Species

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Abstract: The study was carried out during the 2006 cropping season in Nakuru district, Kenya. Incidence and severity of head blight were determined and pathogens isolated from diseased wheat heads, wheat and maize kernels. Mycotoxin deoxynivalenol content in grain was determined by direct competitive Enzyme-Linked Immunosorbent Assay (ELISA). Pathogenicity of different *Fusarium* species isolated from wheat was determined by inoculation onto wheat ears in greenhouse. Head blight was highly prevalent (90-100%) and mean incidence and severity ranged from 4 to 9% and 15 to 37%, respectively. *Fusarium* was most prevalent in infected wheat heads while *Epicoccum* was most prevalent in harvested wheat grain. Only *Fusarium* spp. and *Penicillium* spp. contaminated harvested maize grain. The most frequently isolated *Fusarium* species were *F. poae, F. graminearum* and *F. chlamydosporum* in wheat and *F. verticilloides* in maize. Most wheat and maize grain samples were contaminated with mycotoxin (DON), with concentration ranging from 0-1,200 and 0-4,600 μg kg^{-1}, respectively. *Fusarium graminearum* isolates were highly pathogenic, significantly reducing kernel weight. The results suggest that head blight in Nakuru district is due to a complex of *Fusarium* species with *F. graminearum* being the major pathogen. Cross-contamination of wheat and maize is implied, indicating possible contamination of wheat maize products with deoxynivalenol mycotoxin.

Key words: Deoxynivalenol, *Fusarium,* head blight, maize, pathogenicity, wheat

INTRODUCTION

Fusarium head blight (FHB, scab) is a significant disease of small-grain cereals throughout the world where wheat is grown. Up to 17 causal organisms have been associated with the disease (Parry *et al*., 1995). *Fusaria* like *F. avenaceum, F. culmorum, F. graminearum*, *F. poae* (Kriel, 2006) and *Microdochium nivale* are the species most associated with FHB (Simpson *et al*., 2004). These fungi rarely exist in isolation, but occur as a complex with each other and with other *Fusaria* and other fungal genera. Climatic conditions will influence competition among and the predominance of different fungi within this complex (Doohan *et al*., 2003). Similar *Fusaria* species to those causing FHB are thought to cause ear rot of maize. However, three species of *Fusarium* are predominantly responsible for the disease: *F. graminearum*, *F. verticillioides* (moniliforme), *F. subglutinans* (Reid *et al*., 1999; Fandohan, 2005). *Fusarium verticillioides* is likely to be the most common species isolated worldwide from diseased maize (Logrieco *et al*., 2002; Oren *et al*., 2003).

Fusarium head blight may be epidemic over large areas in some seasons but more commonly varies in severity from field to field or between local areas. The prevalence of infection may vary from a trace to virtually 100% of the heads in the field and losses vary correspondingly (Brennan *et al*., 2007). Depending on the species involved *Fusarium* grain infection may result in yield

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reductions (Henriksen and Ellen, 2005). The extent to which yield is affected is influenced by the *Fusarium* species involved. In inoculation trials with *F. graminearum* on Kenyan varieties the yield was reduced by between 23 and 57% and head susceptibility of between 29 and 68% (Muthomi, 2001). *Fusarium* infections can also lead to mycotoxin contamination of the grains thus threatening the health of humans and livestock (Dohlman, 2004; Heier *et al*., 2005). Most frequently found mycotoxins are Deoxynivalenol (DON, also known as vomitoxin) and zearalenone (ZEA) (Llorens *et al*., 2006; Goyarts *et al*., 2007).

Earlier studies in Kenya (Muthomi, 2001; Muthomi *et al*., 2007a; Ndungu, 2006) have found different levels of *Fusarium* and mycotoxin contamination in wheat grains from different parts of the country. There is however, no documentation of the actual spread and the severity of the disease in farmer fields and the DON contamination in grain from different agro-ecological zones. Therefore, this study was carried out to determine the incidence and severity of FHB in farmers' fields in different agro-ecological zones of Nakuru district and to determine the major species involved in infected wheat ears and grains of both wheat and maize.

MATERIALS AND METHODS

Determination of Incidence and Severity of Head Blight in Farmers' Fields

The study was conducted during 2006 cropping season (May-December) in Nakuru district of Kenya. Five agro-ecological zones in which wheat is commonly grown in Nakuru district were considered: Upper midland 4 (UM4), Lower highland 2 (LH2), Upper highland 2 (UH2), Lower highland 3 (LH3) and Lower highland 4 (LH4). Ten farmers were systematically sampled from each of the five agro-ecological zones. Both large and small-scale farms were targeted. Head blight incidence and severity were determined at soft dough stage (GS85, Zadoks *et al*., 1974). Incidence was determined by marking out a 10 m^2 quadrants and counting the number of blighted heads in the quadrant out of the total number of heads. Three quadrants were assessed per farm. Disease severity was measured as the average proportion of the heads that were blighted and was determined using the scale by Miedaner *et al*. (1996): -1% = no symptoms, 2 = <5%, 3 = 5-15%, 4 = 16-25%, 5 = 26-45%, 6 = 46-65%, 7 = 66-85%, 8 = 86-95%, 9 = 96-100%. Diseased heads were collected for isolation of causal agents. At harvest, 1-2 kg of the freshly harvested wheat and maize kernels were collected for mycological and mycotoxin analysis. The samples were stored at 4°C until analyzed.

Isolation and Identification of *Fusarium* species

The infected wheat heads were cut into small pieces (0.5 cm long). For the kernels, sub-samples were taken randomly. These were sterilized in 3% sodium hypochlorite for three minutes and rinsed off thrice in sterile distilled water. Plating was on low strength potato dextrose agar amended with mineral salts and antimicrobial agents (Muthomi, 2001): (PDA 17 g, KH_2PO_4 1.0 g, KNO_3 1.0 g, $MgSO_4$ 0.5 g, Agar 10 g). Antibiotics penicillin, tetracycline, streptomycin and pentachloronitrobenzene (PCNB) were added (50 mg) after the media cooled to 45°C. A total of 100 kernels and 50 pieces for the diseased heads were plated per farm. Incubation was at 25°C for 5 to 14 days under 12 h daylight and 12 h darkness cycles.

Fungal colonies were identified based on cultural and morphological characteristics like mycelial colour, pigmentation, spore shape, septation and sporophores. *Fusarium* colonies were sub-cultured onto PDA and Synthetic Nutrient Agar (SNA) (Nirenberg, 1981): (KH_2PO_4 1.0 g, KNO_3 1.0 g, $MgSO_4$ 0.5 g, KCl 0.5 g, Glucose 0.2 g, Agar 20 g) and incubated under-near UV light for seven days to induce sporulation. The cultures were then identified to species level according to Nelson *et al*. (1983) and Seifert (1996).

Determination of Deoxynivalenol Content in Grains

Deoxynivalenol content in the wheat and maize grain was analyzed by direct competitive Enzyme-Linked Immunosorbent Assay (ELISA), (AOAC, 1995; Gathumbi *et al*., 2001). Each sample was homogenized and 100 g ground to fine powder. Five grams of the ground sample was extracted with 25 mL of methanol:water (50:50v/v). The extract was de-fatted with 10 mL hexane and 4 mL of the methanolic layer taken and diluted to 10% using Phosphate Buffer Solution (PBS). Microtitre polystyrene plates were coated with 100 µL of anti-deoxynivalenol antiserum DON143/16 (Usleber *et al*., 1992) in bicarbonate buffer (pH 9.6) per well. Absorbance was determined using spectrophotometer Elisa reader at 450 nm. A calibration curve for the standards for each toxin dilutions were plotted using $\log_{10}$ of standards concentration against the percentage inhibition of the standards.

Pathogenicity of *Fusarium* Species

Isolates of different *Fusarium* species from wheat kernels were inoculated onto spikes of 'Mbuni', a highly susceptible wheat cultivar (Muthomi *et al*., 2002) under greenhouse conditions. Each isolate was cultured separately at 22±5°C on mung bean medium (Bai and Shaner, 1996) for 14 days. Conidia suspension of each isolate was harvested and adjusted to 5×10^5 conidia mL^{-1}. Three drops (0.01%) of Tween 20 was added to ensure uniform conidia dispersion. Wheat ears were inoculated at 50% flowering (GS65, Zadoks *et al*., 1974) by spraying with hand sprayer, exposing all spikelets to the inoculum. Controls were treated similarly with distilled water. After inoculation, the ears were incubated under polythene bags for 48 h to ensure high relative humidity for optimal infection. Each isolate was inoculated separately and replicated four times. Head blight severity was visually assessed using a 1-9 scale (Miedaner *et al*., 1996) as proportion of bleached spikelets after every 5 days on 10 average sized ears per replicate.

Data Analysis

All data were subjected to analysis of variance (ANOVA) using the PROC ANOVA procedure of Genstat (Lawes Agricultural Trust Rothamsted Experimental station 2006, version 9) and differences among the treatments means were compared using the Fisher's protected LSD test at 5% probability level.

RESULTS

Fusarium head blight was found in most farms (97%) but there were significant differences ($p\leq0.05$) among the agro-ecological zones in both head blight incidence and severity (Table 1). *Fusarium*, *Alternaria* and *Epicoccum* were isolated from both diseased heads and wheat kernels (Table 2). However, *Fusarium* was the major contaminant in the heads (41%) while *Epicoccum* was the main contaminant in wheat kernels (34%). There were significant differences ($p\leq0.05$) among the agro-ecological zones. Only *Fusarium* and *Penicillium* were isolated from the maize kernels. The

Table 1: Prevalence and severity of Fusarium head blight on wheat ears in farmer's fields in different agro-ecological zones of Nakuru district

Agro-ecological zone	Incidence	Severity
UH2	9.0	37.4
LH4	9.3	24.5
UM4	6.5	23.1
LH3	5.8	20.2
LH2	4.4	15.0
Mean	7.0	24.0
LSD ($p\leq0.05$)	3.8	13.3

Table 2: Percentage isolation frequency of major fungi contaminating wheat ear and kernels in different agro-ecological zones of Nakuru district

Agro-ecological zone	Wheat heads			Wheat kernels		
	Fusarium	*Alternaria*	*Epicoccum*	*Fusarium*	*Alternaria*	*Epicoccum*
UH2	42.2	37.2	20.5	21.2	12.8	32.4
LH3	50.9	18.9	22.0	25.1	42.0	33.0
LH4	38.3	38.4	21.2	42.7	20.8	32.8
LH2	46.0	19.9	25.2	11.8	50.1	32.4
UM4	30.3	30.2	34.9	24.4	15.9	42.8
Mean	41.5	30.0	24.8	25.0	28.3	34.7
LSD ($p \leq 0.05$)	9.2	10.6	10.6	9.5	11.4	NS

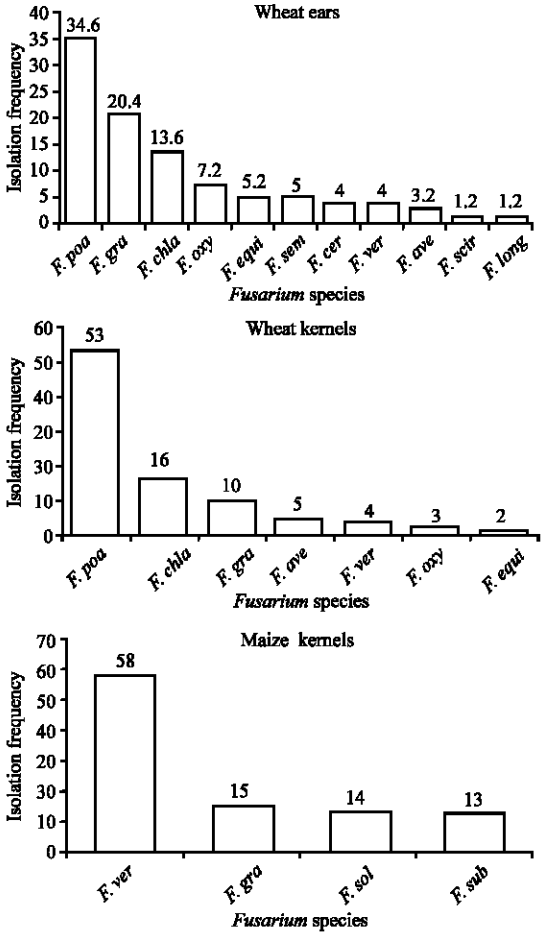

Fig. 1: *Fusarium* species isolated from head blight infected wheat ears, harvested wheat kernels and harvested maize kernels from Nakuru district (*F. gra* = *F. graminearum*; *F. poa* = *F. poae*; *F. chla* = *F. chlamydosporum*; *F. oxy* = *F. oxysporum*; *F. equi* = *F. equiseti*; *F. sem* = *F. semitectum*; *F. cer* = *F. cerealis*; *F. ver* = *F. verticillioides*; *F. ave* = *F. avenaceum*; *F. scir* = *F. scirpi*; *F. long* = *F. longipes*; *F. sub. F. subglutinans*; *F. sol* = *F. solani*)

infected wheat heads, wheat and maize kernels differed in the spectrum of *Fusarium* species isolated (Fig. 1). However, the highest number of *Fusarium* species was isolated from the diseased wheat

Table 3: Deoxynivalenol contamination of wheat and maize grain from different agro-ecological zones of Nakuru district

	Wheat			Maize		
		DON			DON	
Agro-ecological zone	No. of farms	Content (μg kg^{-1}) range	Mean	No. of farms	Content (μg kg^{-1}) range	Mean
UH2	5	200-1,000	320	8	50-4,600	634
LH2	5	50-1,200	340	6	50-200	33
LH3	4	0-200	150	6	50-300	162
LH4	6	100-265	99	6	50-400	125
UM4	4	265-400	369	4	0-20	14

Table 4: Average disease severity over time on wheat ears inoculated with *Fusarium* species isolated from wheat kernels

Fusarium species	Days after inoculation						
Fusarium isolate	0	17	22	27	32	37	Mean
F. graminearum	0	2.0	12.9	41.5	71.8	85.8	35.7
F. avenaceum	0	2.6	5.40	12.6	29.2	42.4	15.4
F. verticillioides	0	0.2	1.30	5.0	18.3	32.9	11.5
F. poae	0	1.5	2.40	5.6	8.1	18.8	6.1
F. sporotrichioides	0	0.3	1.10	2.3	9.6	17.7	5.2
F. solani	0	0.1	0.50	0.8	4.9	16.9	3.9
F. oxysporum	0	0.2	0.30	2.3	6.8	10.5	3.4
F. equiseti	0	0.2	0.60	0.8	3.4	10.2	2.5
F. sambucinum	0	0.1	0.40	1.2	2.7	9.0	2.2
F. chlamydosporum	0	0.0	0.00	0.9	4.1	7.7	2.1
F. semitectum	0	0.0	0.00	0.0	0.3	11.3	1.9
F. scirpi21.3	0	0.0	0.20	0.5	1.7	4.3	1.1
F. lateratium	0	0.0	0.00	0.0	0.4	2.8	0.5
F. cerealis	0	0.0	0.00	0.0	0.0	1.8	0.3
Control	0	0.0	0.00	0.0	0.0	0.0	0.0

heads while maize kernels had the least number of species isolated. The most frequently isolated *Fusarium* species from wheat heads and kernels were *F. poae*, *F. chlamydosporum*, *F. graminearum*, *F. avenaceum* and *F. equiseti*. *Fusarium verticilloides*, *F. graminearum*, *F. solani* and *F. subglutinans* were most isolated from maize kernels. *Fusarium graminearum* was found to be a major contaminant in wheat heads, kernels and maize.

Most of the wheat and maize samples were contaminated with mycotoxin deoxynivalenol (DON) but the different agro-ecological zones differed the amount of the toxin (Table 3). Wheat samples had higher mean levels of DON (249 μg kg^{-1}) compared to maize (mean 221 μg kg^{-1}). However, the highest DON content was detected in maize samples with up to 4,600 μg kg^{-1} compared to 1,200 μg kg^{-1} in wheat. The different *Fusarium* species significantly differed ($p \leq 0.05$) in severity of head blight induced on inoculated wheat ears (Table 4). However, isolates of *Fusarium graminearum* were the most pathogenic, resulting in the highest disease severity of up to 87% of spikelets bleached.

DISCUSSION

Fusarium head blight was found in all the agro ecological zones surveyed (AEZS) with 100% prevalence. The disease severity was highly positively co-related to incidence ($r = 0.647$, $p \leq 0.001$). The varying severity and incidence over the different AEZS could be due to the different environmental conditions that impact on numerous aspects of FHB epidemiology (Doohan *et al*., 2003; Brennan *et al*., 2005; Chen *et al*., 2006; Klahr *et al*., 2007a). The major wheat varieties grown in Kenya have been found to be susceptible to FHB (Muthomi *et al*., 2002, 2007; Ndung'u, 2006) Currently, no source of complete resistance is known but varieties with partial resistance have been reported (Wisniewska and Kowalczk, 2005; Browne, 2007; Semagn *et al*., 2007).

Diseased wheat heads and kernels were contaminated with high levels of *Alternaria* and *Epicoccum*. *Epicoccum* and *Alternaria* species are mainly saprophytes or weak pathogens that grow on senescencing plant tissues (Vincent *et al*., 2006). However, *Alternaria alternata* is one of the most common wheat pathogens, causing huge reductions in yield worldwide (Williamson, 1997). The co-occurrence of several fungi could have synergistic effect on disease severity and higher reductions of wheat yield. In addition, some species of *Alternaria* are known to produce mycotoxins altanariol, altenuene, tenuazonic acid and altertoxin I-III (Weidenbörner, 2001; Sab *et al*., 2007). It was noted where the isolation frequency of *Alternaria* or *Epicoccum* was high that of *Fusarium* was low similar to results by Kosiak *et al*. (2004), Ndung'u (2006) and Sab *et al*. (2007).

Over 10 different *Fusarium* species were isolated from infected wheat ears and harvested kernels, indicating that head blight is due to a complex of *Fusarium* species. Species isolated at high frequencies were *F. poae*, *F. oxysporum*, *F. graminearum* and *F. chlamydosporum*. However, results from inoculation studies showed that only *F. graminearum* was highly pathogenic, suggesting that this species could be implicated in the possible head blight of wheat in Kenya. *Fusarium graminearum* is known to cause severe blighting of wheat heads resulting in visually damaged kernels (Brennan *et al*., 2005). Other species including *Fusarium poae*, *F. avenaceum*, *F. equiseti* and *F. sporotrichioides* do not blight the heads but sometimes cause damage to the spikelets, resulting in low disease severity and latent seed infection. According to Marasas (1991) *Fusarium equiseti*, *F. graminearum*, *F. poae*, *F. verticillioides* and *F. sporotrichioides* are considered the most toxic *Fusarium* species.

Although wheat and maize differed in the spectrum of *Fusarium* species isolated, both were contaminated with *F. graminearum* and *F. verticilloides*. Maize is the staple grain in Kenya and it is grown alongside wheat by most farmers. Wheat fields are surrounded by fields used for different crops, usually maize, sorghum or barley wheat are therefore potentially exposed to cross-contamination by other plants (Vincent *et al*., 2006). *Fusarium graminearum* the predominant causal agent of head blight (Kriel, 2006) is also a major pathogen in ear rot of maize (Parry *et al*., 1995; Silva *et al*., 2007). The co-occurrence of *Fusarium* species in maize and wheat kernels suggests co-occurrence of mycotoxins, *F. graminearum* and *F. poae* produce type B trichothecenes such as nivalenol, deoxynivalenol, zearalenone and Fusarenon-x (Geraldo *et al*., 2006; Kriel, 2006) whereas *F. verticilloides* produces fumonisins and moniliformin (Marasas, 2001; Ramos *et al*., 2006). Ndung'u (2006) reported co-occurrence of mycotoxins deoxynivalenol, zearalenone and aflatoxin B1 in harvested wheat in Kenya. Deoxynivalenol was the major mycotoxin while the rest were at low levels. These mycotoxins cause a wide range of acute and chronic effects in humans and animals (Tiemann and Daenicke, 2007). Sab *et al*. (2007) suggested a possible role played by zealarenone in competitive interactions between *F. graminearum* and *A. alternata*. Contamination of maize with fumonisins has been reported in Kenya (Kedera, 1999). Consumption of fumonisin has been associated with elevated human esophageal cancer incidence in various parts of Africa, Central America and Asia (Marasas *et al*., 2004). Some correlation studies have suggested a link between the consumption of maize with high incidence of *F. verticillioides* and fumonisins and the high incidence of human oesophageal carcinoma in certain parts of S. Africa (Yoshizawa *et al*., 1994; Anonymous, 2000). *Fusarium verticilloides* is also known to infect both maize and wheat (Dill-Macky and Jones, 2000; Ramos *et al*., 2006).

The study indicated that FHB is present in significant levels in Kenya and that it is due to a complex of different *Fusarium* species. However, *F. graminearum* and *F. avenaceum* could be main pathogenic species involved in pathogenicity. The presence of *F. graminearum* in both wheat and maize indicated that maize is a reservoir for the head blight pathogens. This could be due to the growing of wheat and maize in the same or neighbouring fields and lack of proper rotation programmes. Due to the health risks posed by mycotoxin contamination, there is need for continuous surveillance for the major *Fusarium* mycotoxins, especially deoxynivalenol to prevent carry over to food and animal feeds.

Further characterization of *F. graminearum* isolates from maize and wheat would be necessary to determine whether similar population infect both crops. In addition, the resulting yield losses and possible management strategies for FHB need to be determined.

ACKNOWLEDGMENT

This study was funded by the International Foundation for Science (IFS) grant No.E/3654-1.

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