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Occurrence of wheat head blight and *Fusarium* species infecting wheat

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Abstract*:* Survey was carried out during the 2006 cropping season in five agro-ecological zones of Nakuru district, Kenya. Incidence and severity of head blight were determined. Fungi were isolated from diseased wheat spikes, wheat and maize kernels. Pathogenicity of different *Fusarium* species isolated from wheat was determined by inoculation onto wheat spikes in greenhouse. Head blight was highly prevalent (90 –100%) and mean incidence and severity ranged from 4 to 9% and 15 to 37%, respectively. The main fungal genera isolated from wheat spikes and grains were *Fusarium*, *Alternaria* and *Epicoccum*. *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. Main species were *F. poae, F. graminearum* and *F. chlamydosporium* in diseased heads and wheat kernels while *F. verticilloides* was most frequently isolated from maize kernels. *Fusarium graminearum* isolates were highly pathogenic. The results indicated 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 maize with deoxynivalenol mycotoxin.

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

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). *Fusarium spp.*, namely *F. avenaceum, F. culmorum, F. graminearum*, and *F. poae* and *Microdochium nivale* are the most associated species with FHB. (Edwards *et al*, 2001; Kriel, 2006) However the distribution of the different species depends mostly on the climatic conditions (Jenkins *et al*., 1988). *Fusarium graminearum* predominates in the hotter regions of the world while *F. culmorum* and *F. poae* are important in cooler regions (Parry *et al.,* 1995). However, most species produce inocula, grow best, and are most pathogenic to cereal heads at warm temperatures and under humid conditions (Doohan *et al.,* 2003). These fungi rarely exist in isolation, but occur as a complex with each other and with other fungal genera. Climatic conditions will influence competition between, 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, *F. graminearum, F. verticillioides (moniliforme) and F. subglutinans* are predominantly responsible for the disease (Reid *et al* 1999, Fandohan, 2003). *Fusarium verticillioides* is likely to be the most common species isolated worldwide from diseased maize (Munkvold and Desjardins, 1997).

Fusarium head blight may be epidemic over large areas in some seasons but it commonly varies in severity from field to another field or among local areas. The prevalence of infection may vary from a trace to virtually 100% of the heads in the field, and losses vary correspondingly (Schroeder and Christensen 1963). The sporadic nature of infection has been attributed to the environmental requirements of the pathogen. Depending on the species involved, *Fusarium* grain infection may result in yield reduction but the extent of yield reduction is influenced by the *Fusarium* species involved (Henriksen *et al.,* 2005). Mesterhazy (1978) showed that yield reduction was greater after inoculation with *F. culmorum*, the dominant species in Ireland, than after inoculation with other *Fusarium* species. In inoculation trials with *F. graminearum* on Kenyan wheat varieties the yield was reduced in between 23 and 57% and head blight susceptibility in between 29 and 68 % (Muthomi, 2001; Muthomi et al., 2002a). *Fusarium* infections can also lead to mycotoxin contamination of the grains thus threatening the health of humans and livestock (Heier, 2005). Most frequently found mycotoxins are deoxynivalenol (DON, vomitoxin) and zearalenone (ZEA)(Bottalico and Perrone, 2002).

Earlier studies in Kenya (Muthomi, 2001; Muthomi *et al.,* 2002b and 2006; 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 as well as the severity of the disease in farmers' 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). Five agro-ecological zones in which wheat is commonly grown in Nakuru district were

considered: - Upper midland 4 (UM4), Lower highland2 (LH2), Upper highland 2 (UH2), Lower highland 3 (LH3) and Lower highland 4 (LH4). Ten farms were randomly chosen and systematically sampled from each of the five agro-ecological zones. Head blight incidence and severity were determined at soft dough stage (GS85, Zadoks *et al*., 1974). Incidence was determined by marking out a $10m^2$ 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 assessed based on 1-9 scale as proportion of head blighted: -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% (Miedaner, 1996). Diseased heads were collected for isolation of causal agents. At harvest, 1-2kg of the freshly harvested wheat and maize kernels were collected for mycological analysis. The samples were stored at 4^0c until the subsequent studies.

Isolation and identification of *Fusarium* species

The infected wheat heads were cut into 0.5 cm long pieces while sub-samples of the wheat and maize kernels were randomly taken. These were surface sterilized in 3 % sodium hypochlorite 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 17g, KH_2PO_4 1.0g, KNO_3 1.0g, $MgSO_4$ 0.5g, Agar 10g). Antibiotics penicillin, tetracycline, streptomycin and pentachloronitrobenzene (PCNB) were added (50 mg) to the media and cooled to 45^oc. A total of 100 kernels and 50 pieces of the diseased heads were plated per sample. The plates were incubated at 25^0c for 5 to 14 days under 12 hr daylight and 12 hr 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): KH2PO4 1.0g, KNO_3 1.0g, $MgSO_4$ 0.5g, KCL 0.5g, Glucose 0.2g, and Agar 20g (Nirenberg, 1981) 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).

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*., 2002a and 2002b) under greenhouse conditions. Each isolate was cultured separately at 22 ± 5^oC in mung bean medium (Bai and Shaner, 1996) for 14 days. Conidia suspension of each isolate was harvested and adjusted to $5x10^5$ conidia/ml. Three drops (0.01%) of Tween 20 was added to ensure uniform conidia dispersion. Wheat spikes 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 only. After inoculation, the spikes were incubated under polythene bags for 48 hrs to ensure high relative humidity for optimal infection. Each isolate was inoculated separately and replicated four times. Head blight severity was visually assessed on 10 average sized spikes per replicate using a 1 –9 scale (Miedaner *et al*., 1996) as proportion of bleached spikelets after every 5 days.

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 97 % of the farms surveyed and there were significant differences ($P<0.05$) 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 with isolation frequency of 41% while *Epicoccum* was the main contaminant in wheat kernels with isolation frequency of 34%. There were significant differences ($P<0.05$) among the agro-ecological zones in frequency of isolation for the different species. Only *Fusarium* and *Penicillium* species were isolated from the maize kernels. The 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 heads while maize kernels had the least number of species isolated.

Table 1: Prevalence and severity of *Fusarium* head blight on wheat ears in different fields in 5 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 ($P< 0.05$)	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-eco	Wheat heads			Wheat kernels		
zone	*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<0.05)	9.2	10.6	10.6	9.5	11.4	NS

The most frequently isolated *Fusarium* species from wheat heads and kernels, in order of decreasing frequency, were *F. poae*, *F. chlamydosporium*, *F. graminearum*, *F. avenaceum* and *F. equiseti*. However, *F. verticilloides*, *F. graminearum*, *F. solani* and *F. subglutinans* were the most frequently isolated species from maize kernels. *Fusarium graminearum* was found to be a major contaminant in wheat heads, kernels and maize. The different *Fusarium* species significantly differed ($p \leq 0.05$) in severity of head blight induced on inoculated wheat ears (Table 3). However, isolates of *F. graminearum* were the most pathogenic, resulting in the highest disease severity of up to 87% of spikelets bleached.

Table 3: Disease severity over time and kernel weight of wheat ears inoculated with nine isolates of the four most pathogenic *Fusarium* species

Fusarium	Days after inoculation							Kernel
isolate	0	17	22	27	32	37	Mean	weight
F.gram 68.1	0	2.6	12.7	42.5	77.0	85.1	31.4	6.6
F.gram. 60.1	0	2.3	18.5	51.6	76.9	87.5	33.8	8.4
F.gram. 58.4	0	1.0	7.4	30.4	61.5	84.9	26.4	7.7
F.aven. 69.7	0	4.9	9.8	19.6	37.0	41.0	16.0	11.1
F.aven. 45.4	0	.3	1.0	5.0	21.4	43.8	10.2	10.0
F.vert. 69.7	0.2	0.2	1.3	5.0	18.3	32.9	8.3	14.1

F.gram = *Fusarium graminearum*; F.aven = *F. avenaceum*; F.vert. = *F. verticilloides*;
F.poae = *F. poae*

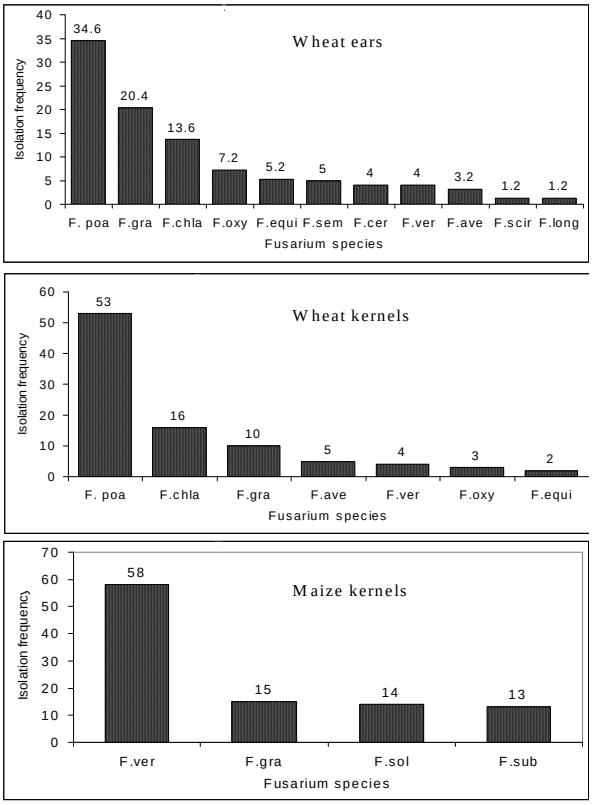

Fig. 1. Fusarium species isolated from head blight infected wheat ears, harvested wheat kernels and harvested maize kernels from Nakuru district

Discussion

Fusarium head blight (FHB) was found in all the agro ecological zones surveyed with 100% prevalence. The disease severity was highly positively co-related to incidence ($r = 0.647$, $P < 0.001$). 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 (Hershman *et al*., 1999; Gilbert, 2002; Chen *et al.*, 2006). The major wheat varieties grown in Kenya have been found to be susceptible to FHB (Muthomi *et al*., 2002 and 2007; Ndung'u 2006). Currently, no source of complete resistance is known but varieties with partial resistance have been reported (Musket *et al.*, 2003).

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). Therefore, the co –occurrence of several fungi could have a synergistic effect on disease severity and higher yield reductions in wheat. In addition, some species of *Alternaria* are known to produce mycotoxins altanariol

and altenuene (Moss, 1996). It was noted that the isolation frequency of *Alternaria* or *Epicoccum* was high, the level of *Fusarium* was low. These findings are in agreement with those of Gonzalez *et al*. (1999), Muthomi *et al*. (2006) and Ndung'u (2006).

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 as the main causal agents of head blight of wheat in Kenya. *Fusarium graminearum* is known to cause severe blighting of wheat heads resulting in visually damaged kernels (Parry *et al*., 1995). 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 *et al*. (1991) *F. 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, 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 with different crops, usually maize, sorghum or barley and therefore, cross-contamination of the crops with *Fusarium* spp. is possible (Vincent *et al.,* 2006). *Fusarium graminearum* the predominant causal agent of head blight (Kriel, 2006) but it is also a major pathogen in ear rot of maize (Parry *et al*., 1995; Sutton 1982). *Fusarium verticilloides* is also known to infect both maize and wheat (Dill-Macky, 2000). The co –occurrence of *Fusarium* species in maize and wheat kernel suggests co-occurrence of mycotoxins. *Fusarium.graminearum* and *F. poae* produce type B trichothecenes such as nivalenol, deoxynivalenol, zearalenone and fusarenon-x (Jennings *et al.,* 2000; Magan and Olsen, 2004) whereas *F. verticilloides* produces fumonisins and moniliformin (Moss,1996). Ndung'u (2006) reported co-occurrence of mycotoxins deoxynivalenol, zearalenone and aflatoxin B1 in harvested wheat grain 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 (Edwards *et al*., 2001). Contamination of maize with fumonisins has been reported in Kenya (Kedera, 1999).

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

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