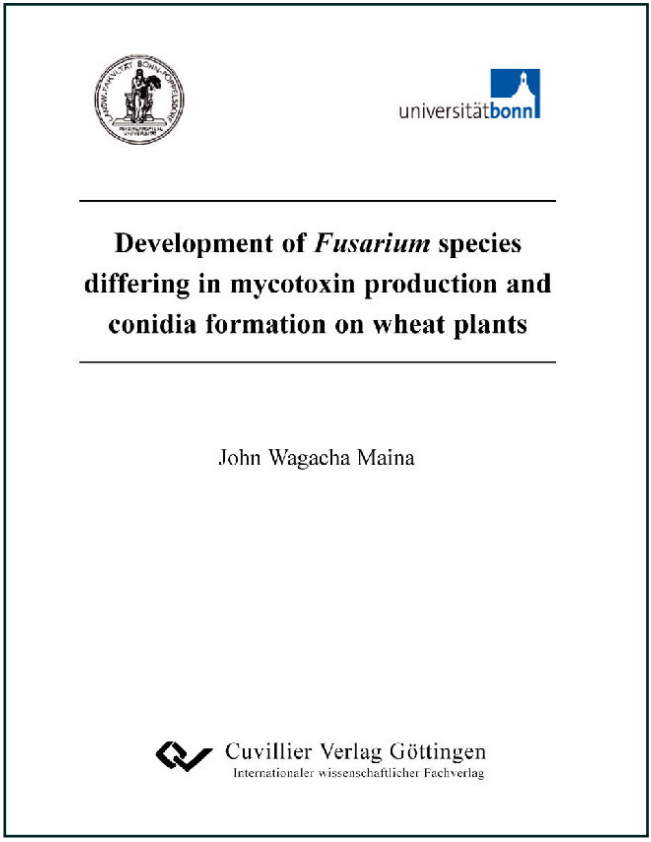

1. Introduction

A number of fungal genera have been described to infect small grain cereals. Such infections on wheat, barley, rye and oats impair quality and quantity causing enormous economic losses to farmers besides the associated immense effects on human and animal health. The most serious consequence of fungal colonization of agricultural products is contamination with mycotoxins, which are produced as secondary metabolites by members of *Fusarium*, *Aspergillus*, *Penicillium*, *Alternaria* and *Claviceps* species (Xu *et al*., 2003). Infection of wheat by *Fusarium* occurs as a complex involving species which differ in mycotoxin production and infection biology. Such complexes result in a broad spectrum of mycotoxins differing in chemistry and toxicology. However, little is known about *Fusarium* infection biology.

Fusarium species have long been recognized as pathogens of many plants. On cereals, they are widespread and common pathogens, causing foot rot, root rot, and head blight (Akinsanmi *et al*., 2004; Ban, 2000; Stack, 2000; Fouly, *et al*., 1996; Parry, 1990). The species have also been reported to infect other aerial plant parts like stems (Mudge *et al*., 2006; Günther and Trail, 2005; Xi and Turkington, 2003) and leaves. Wheat can be infected during all growth stages but the most susceptible and economically important developmental stage is anthesis. Therefore, it is the presence of *Fusarium* spp. on the ears that is most widely studied because of its effects on yield resulting in quantity and quality reduction and by virtue of grain being the consumable part by humans. Infection of the ears occurs as complexes with more than 17 species being implicated in *Fusarium* head blight (Parry *et al*., 1994). Besides their effect on yield reduction, *Fusarium* species produce a wide variety of mycotoxins many of which pose health risks to humans and animals. Although it has been known for many years that other wheat plant parts get infected, there has been little focus on infection of straw material and what possible role that such infections may play in the disease and infection cycles, pathogen spread within and between cropping cycles, and the possible health risk to livestock fed on straw.

Fusarium head blight (FHB) also called wheat scab is a destructive disease of wheat worldwide (Bai and Shaner, 1994) resulting in reduction of grain yield and quality (McMullen *et al*. 1997a). The disease was first described over a century ago and considered a major threat to wheat and barley during the early years of the last century (Dickson and Mains, 1929). In recent years FHB has again increased worldwide (Stack, 2000; Perry *et al*., 1995) and is a potential threat to wheat production wherever the crop is grown (Windels, 2000; McMullen, 1997b). Epidemics in recent years have become more frequent, severe, and widespread in many countries as reported in South Africa (Boshof *et al*., 1999), USA and Canada (Dubin *et al*., 1997) and Japan (Takeda *et al*, 1992). Dubin *et al*. (1997) termed FHB as a re-emerging threat to the world's food supply, with outbreaks in the last decade in Europe, North America and Asia. They

reported losses of more than US$2 billion in wheat production in the last decade in the USA alone. Yield losses ranging from 20 to 100% have been reported in the last decade in Australia (Chakraborty *et al*., 2004; Manning *et al*., 2000).

The International Maize and Wheat Improvement Centre (CIMMYT) has identified FHB as a major factor limiting wheat production in many parts of the world (Dubin *et al*., 1997). During the past decade, several European conferences on *Fusarium* diseases have been dominated by reports on FHB of cereals. It is however the rapid global re-emergence of FHB in the last decade along with contamination of grains with mycotoxins attributable to the disease that have spurred basic research on the fungal causal agents (Goswami and Kistler, 2004). Many of the species responsible for FHB can also infect other plant parts resulting in diseases such as wheat seedling blight and brown foot rot (Parry *et al*., 1995). Mycotoxins produced by the various *Fusarium* species in infected grains are a safety concern in human foods while infected straw could expose livestock to health risks.

Up to 17 *Fusarium* species have been associated with FHB of wheat and other small grain cereals (Akinsanmi *et al*., 2004; Parry *et al.* 1995). The number of species involved has since increased with description of new species as well as molecular separation of species, which were previously classified together, based on morphological characteristics but are genetically different. Although the disease is normally caused by a complex of species, different *Fusarium* species predominate different climatic regions. Worldwide, *F. graminearum* and *F. culmorum* are the predominant species infecting wheat (Parry *et al*., 1995). *F. graminearum* predominates in the warmer, humid areas of the world such as USA (Goswami and Kistler, 2004; Vigier *et al.*, 1997) whilst *F. culmorum* has been shown to be one of the predominant *Fusarium* species in the cooler areas such as north, central and west Europe (Parry *et al*., 1995) and Canada (Demeke *et al*., 2005). Predominance of a species in a region is mostly influenced by climatic conditions especially temperature requirements (Parry *et al.*, 1995). In parts of Northern Europe, for example, *F. culmorum* and *F. avenaceum* are the prevalent causes of FHB (Parry *et al*., 1995; van Eeuwijk *et al*., 1995). It is *F. graminearum*, however, that has caused most of the recent outbreaks of FHB in Southern and Central Europe, the USA and Canada, as well as those in South America, China and Japan (Akinsanmi *et al.*, 2004; Dubin *et al*., 1997; Meidaner *et al*., 1997; Bai and Shaner, 1994). *Fusarium culmorum* has been identified as the predominant species in Western Germany (Muthomi *et al*., 2000) and in the Rhineland region, Germany together with *F. avenaceum* (Lienemann, 2002). Kosiak *et al*. (2003) reported *F. avenaceum*, *F. poae, F. culmorum* and *F. tricinctum* to be the most frequently isolated *Fusarium* species from wheat, barley and oats in Norway. Similarly, *F. avenaceum*, *F. culmorum*, *F. tricinctum* and *F. poae* were the most frequently isolated species from wheat in Finland (Logrieco *et al*., 2002). A

recent study by Clear and Patrick (2006) ranked *F. graminearum, F. culmorum* and *F. avenaceum* as the most dominant FHB causing fusaria in cereals in Canada. A field survey by Muthomi *et al*. (2007a, 2008) in Nyandarua and Nakuru, two major wheat producing districts in Kenya ranked *F. poae*, *F. graminearum* and *F. chlamydosporum* as the most frequently isolated species in Kenyan wheat. Recent surveys in some European countries where *F. culmorum* was predominant some years ago have reported *F. graminearum* to be predominating now (Jennings *et al*., 2004a, b). Waalwijk *et al.* (2003) reported replacement of *F. culmorum* by *F. graminearum* as the predominant ear blight pathogen in the Netherlands. *F. graminearum* has recently also been more common on wheat grain in Germany (Obst and Fuchs, 2000) where high levels of deoxynivalenol have been found (Placinta *et al*., 1999). Shah e*t al*. (2005) found *F. graminearum* to be the dominant species in Italy in a study conducted from 1999 to 2002. This trend could be indicative of a change to warmer weather, a genetic change in the *F. graminearum* population or changes in cropping practices (Bateman, 2005). The trend could also reflect a changing trend of dominance of *Fusarium* species in different parts of the world.

Worldwide, *F. graminearum* (teleomorph *Gibberella zeae*), *F. culmorum*, *F. poae*, *F. avenaceum* (teleomorph *G. avenaceae*) and *Microdochium* species (teleomorph *Monographella nivalis*) are the most common pathogens of wheat (Kriel 2006; Simpson *et al*., 2004; Tekauz *et al*., 2000; Miedaner *et al*., 1997; Parry *et al*., 1995; Schaffnit, 1912). *F. tricinctum* (teleomorph *G. tricincta*) is also a species with a worldwide distribution even though more common in temperate parts of the world (Leslie and Summerell, 2006). Occurrence of *Fusarium* species rarely occurs in isolation but rather as a complex with other fusaria and fungal genera. The existence of high variability in fungal community at ecological niches indicates inter-species interactions for nutrients and space. Weather conditions in combination with host susceptibility influence the growth, survival, dissemination and hence the incidence of *Fusarium* species and their virulence as well as interaction among different fungi within this complex thereby determining the predominant species (Doohan *et al*., 2003). The influence of climatic factors on FHB is complicated by the fact that *Fusarium* species can cause disease individually or in complex infections (Doohan *et al*. 1998), and there are numerous reports on how species differentially respond to different environmental variations, particularly temperature and relative humidity. It has been suggested that spore germination of different fungi is triggered by internal and external factors such as inherent characteristics of a fungal species (Glenn 2006; Harris 2005), presence of water (Hamer *et al*. 1998) as well as presence of nutrients such as carbohydrates (Beyer *et al*. 2004; Osherov and May 2000).

Predominance of a *Fusarium* species is as a result of complex interaction of pathogen, host and environmental factors. Understanding the interactions of different *Fusarium* species

during germination, invasion and early colonization of the host is critical because these early events are important in understanding aggressiveness and hence predominance of particular species in the FHB complex on wheat and other small grain cereals. The *Fusarium* species in such a complex have been shown to possess different characteristics such as competitive ability, aggressiveness and the secondary metabolites that they produce. Therefore, while studying the infection process of *Fusarium* species, it is paramount to include different isolates or species to understand the implications of their diversity on the process. More importantly, such inclusions could be necessary in providing data that may explain the predominance of low aggressive *Fusarium* species such as *F. poae* in the presence of highly aggressive species like *F. culmorum* and *F. graminearum* under field conditions. Such studies should also consider that interactions can occur during the early stages (spore germination and penetration) or later (tissue colonization) using various mechanisms to occupy the ecological niches. Conditions favourable for *in vitro* growth of *Fusarium* species are also generally the most favourable on cereal grains. Understanding the behaviour of individual *Fusarium* species is an important preliminary step in getting insight into the complex interactions among *Fusarium* species.

All *Fusarium* species are capable of surviving saprophytically on crop residues (Parry *et al*., 1994), which is considered the main source of inoculum for infection of wheat ears (Sutton, 1982). *Fusarium* species overwinter on infected cereal crop debris on which they produce ascospores or conidia (or both), which are then dispersed by wind or splashing to new infection courts providing secondary inoculum. Even if no FHB was present, the crop residue may be colonized after harvest providing carryover inoculum (Wang and Miller, 1988). Despite understanding the role played by crop debris in disease cycle, few studies have investigated infection of straw during the growth period of wheat and whether such infections contribute secondary inoculum for infection of ears and pose a health risk of mycotoxin contamination to animals. Such knowledge would be important in understanding the role of straw infection in disease cycle; pathogen survival and spread; and the associated risks to animal health.

Many studies have predominantly focused on mycotoxin production on cereal grain foodstuffs and detrimental effects on human health. There needs to be greater focus too on straw since straw and chaff are used as feedstuff and bedding material and therefore, might contribute to mycotoxin exposure of farm animals. This might constitute a risk to livestock health and draw attention especially to organic farming, which has gained significance in recent production and consumption trends. Infection of straw material may play two crucial roles – a) in the pathogen/disease cycle and b) exposure of animals to health risks if infection results in mycotoxin production. The ability of *Fusarium* species to survive saprophytically on crop debris implies that the pathogen is able to survive in the plant debris between cropping seasons and may

also provide secondary inoculum within a cropping season where vegetative plant parts get infected. It has been reported that mycotoxins are virulence factors and are produced on stems during host colonization (Mudge *et al*., 2006). Such reports raise the need to investigate the level of mycotoxin production in straw material vis-à-vis the kernels to understand the level of exposure of mycotoxins to animals and humans, which feed on straw and kernels, respectively. It was therefore, necessary to study mycotoxin production and quantify fungal biomass in leaf, stem and kernel matrices of wheat in order to document whether differences exists in their colonization and mycotoxin contamination. Such knowledge would be important in expounding the role of straw infection in exposure of animals to mycotoxins and exploring necessary interventions in the *Fusarium* management strategies.

Mycotoxins are toxic secondary metabolites produced by fungi and contaminate various agricultural commodities either before harvest or under post-harvest conditions (FAO, 1991). Among the mycotoxigenic fungal pathogens infecting wheat are *Aspergillus*, *Penicillium*, *Alternaria* and *Fusarium* species. Out of the 300-400 mycotoxins known, the most important are aflatoxins, ochratoxins, trichothecenes (e.g. deoxynivalenol, nivalenol, T-2 toxin and T-2 like toxins), zearalenones, fumonisins and moniliformin (Desjardins, 2006; Xu, 2003). Recently, a number of studies in temperate and tropical environments have reported high prevalence of enniatins (Kulik *et al*., 2007; Jestoi *et al*., 2004; Logrieco *et al*., 2002). *Fusarium* species produce different types and amounts of mycotoxins. Trichothecenes, zearalenone, fumonisin, moniliformin, enniatins and fusarin C are the most important mycotoxins produced by *Fusarium* species. The major mycotoxins produced by *Fusarium* species on wheat and other small grain cereals are shown in table 1.

A decade ago, the Food and Agricultural Organization of the United Nations (FAO) estimated that up to 25% of the world's food crops are significantly contaminated with mycotoxins (WHO, 1999). Accumulation of these mycotoxins in wheat grain increases the importance of *Fusarium* species to wheat production (Charmley *et al*., 1994). Food and feed safety are by far the greatest concerns as scabby wheat grain is often contaminated with trichothecenes and oestrogenic mycotoxins. When contaminated grain is consumed, the mycotoxins produced by the fungus have been shown to cause feed refusal and reproductive disorders in cattle, swine, and poultry at levels as low as 1 - 3 ppm (De Wolf, 2003; O'Donnell *et al*., 2000 and Dubin *et al*., 1997). The European Union (EU), which has the most stringent standards on mycotoxins, limits deoxynivalenol and zearalenone to 1250 and 100 ppb, respectively, in, unprocessed wheat for human consumption. On the other hand, the EU only has guidance values for the mycotoxins in feedstuffs, which range from 900 to 8000 ppb and 100 to 2000 ppb for DON and ZEA, respectively for different animals (EC, 2006). The Federal

Department of Agriculture, USA has recommended that deoxynivalenol levels should not exceed 1 ppm in processed human food (De Wolf, 2003). In view of the standards available, it appears that some remarkable progress has been made regarding human food but a lot needs to be done to set mycotoxin standards in feedstuffs. Whereas high mycotoxin exposure in tropical developing countries may be attributed to inadequate storage and processing facilities coupled with conducive weather conditions for fungal proliferation, the major problem in developed countries arises from feeding of animals with mouldy feeds.

Table 1: Mycotoxigenic *Fusarium* species and the mycotoxins they produce

Fusarium sp.	Tricho-thecenes	Zearale-none	Monili-formin	Enni-atins	Fumo-nisins	Equi-setin	Fusa-rins	Chlamy-dosporol	Beau-verin
F. avenaceum	○		●	●	○	○	●	●	●
F. culmorum	●	●					●	●	
F. graminearum	●	●			○		●	●	
F. poae	●		◐	●	○		●		●
F. tricinctum	○						●	●	●
F. chlamydosporum			●	●				●	
F. equiseti	●	●	●			●			●
F. semitectum	●	●	●			●			
F. oxysporum	●	○	●	●	●				●
F. solani	●	○		○					
F. sporotrichioides	●		●	●	○		●		●
F. venenatum	●						●		●
F. subglutinans	○		●	●	◐		●		
F. proliferatum	○		●	●	●				●
F. cerealis	●	●		○			●		○

● Production confirmed ○ No production described ◐ Production by some isolates

Adapted from Desjardins, 2006

Of all the *Fusarium* mycotoxins discovered to date, trichothecenes have been the toxins most strongly associated with chronic and fatal toxicoses of humans and animals (Desjardins, 2006). Chemically, trichothecenes are a large group of sesquiterpene epoxides with potent activity against ribosomal protein synthesis (Tag *et al*., 2001). They are characterized by the presence (type A trichothecenes) or absence (type B trichothecenes) of a keto group at the C-8 position. The trichothecenes, including deoxynivalenol, acetyldeoxynivalenol, nivalenol, and fusarenone X, are common fungal contaminants of cereals (Magan and Olsen, 2004; Jennings *et*

al., 2000) and occur naturally worldwide on cereals (Dalcero *et al*., 1997; Müller *et al.*, 1997; Park *et al*., 1996; Ryu *et al*., 1996; Kim *et al*., 1993; Fujisawa *et al*., 1992; Abbas *et al*., 1988). According to trichothecene production, trichothecene-producing *Fusarium* strains have been divided into two chemotypes: the nivalenol chemotype, which includes isolates producing nivalenol and fusarenone X, and the deoxynivalenol chemotype, which includes isolates producing deoxynivalenol and acetyldeoxynivalenol (Bakan *et al*., 2001; Sydenham *et al*., 1991; Ichinoe *et al*., 1983). However, the culture conditions, matrix and environmental conditions may influence the type and amount of toxin produced by a *Fusarium* isolate (Llorens *et al.,* 2006; O´Neill *et al*., 1993).

Zearalenone is a macrocyclic secondary metabolite with anabolic and uterotropic characteristics, which leads to hyperestrogenism and infertility in swine, poultry and cattle. Zearalenone is the primary toxin causing infertility, abortion or other breeding problems, especially in swine (Diekman and Green, 1992; Flannigan, 1991). This group of mycotoxins differ in the presence and reduction state of hydroxyl groups and in their acetylation. Zearalenone is the major homologue produced by *Fusarium* species, but other metabolites, such as α-zearalenol, β- zearalenol, and 4-acetylzearalenone, can also occur at low levels in naturally contaminated grain or under optimal culture conditions in the laboratory (Desjardins, 2006).

Moniliformin is a small ionic molecule that occurs naturally as a sodium or potassium salt whose production is widespread but not universal. This toxin has been associated with high rate of Keshan heart disease especially in China (Yu *et al.,* 1995).

Enniatins are nonribosomal, cyclic depsipeptides with general antibiotic and phytotoxic activities (Desjardins, 2006). Enniatins have been associated with growth inhibition of wheat seedlings (Burmeister and Plattner, 1987; tomato wilting (Stoessl, 1981); and dry rot of potato tuber tissues (Hermann *et al*, 1996).

Fusarium mycotoxins have been detected in kernel and stem tissues of wheat. Mycotoxins such as DON have been reported to play a role in *Fusarium* pathogenesis (Hestbjerg *et al*., 2002; Mesterhazy, 2002). Although the life cycle of *Fusarium* species is well understood (Parry *et al*., 1994; Sutton, 1982), little is known about their infection process and colonization of wheat tissues, especially the vegetative parts. On wheat ears, the fungus is thought to infect flowers by infecting the anthers first and then using these, and their pollen, as a food base and energy source to invade ovaries and developing kernels (Cook and Veseth, 1991). Studies on the infection process of wheat ears by *F. culmorum* and *F. avenaceum* indicated that the species did not penetrate wheat spikes directly but developed a dense mycelium on the inner surfaces of the spikes before invasion of lemma, glume, palea and ovary by penetration pegs (Kang *et al*., 2005; Kang and Buchenauer, 2002). Kang and Buchenauer (2002) also reported inter- and intracellular