

**GRAIN MOULD OF SORGHUM WITH SPECIFIC REFERENCE TO  
GRAIN QUALITY IN SOUTH AFRICA**

**By**

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## PREFACE

This thesis is a compilation of five independent manuscripts. The first chapter is a literature review on sorghum grain mould and its relationship with grain quality. Main topics discussed include the physical and chemical properties of the host, the pathogens involved in the disease complex, weather conditions that favour infection and the role of insects in disease development. The effects of grain mould on grain quality, with specific reference to milling and malting quality as well as the production of mycotoxins, are discussed.

In chapter 2, the incidence of grain mould fungi in sorghum grains from different localities was investigated. The potential of the fungi isolated as grain mould pathogens of sorghum, their role in influencing sorghum grain quality parameters, their effect on sorghum grain processing and the presence of mycotoxins were assessed.

In chapter 3 the influence of weather on the severity of sorghum grain moulds and subsequent grain quality were investigated. The incidence of fungi in grains of sorghum from different flowering dates and locations was evaluated. The study assessed the effect of wetness duration on infection, the effect of fungi on sorghum grain quality with special reference to malting and milling quality, mycotoxin production, seed germination, grain discolouration and kernel mass.

In chapter 4 sorghum grain infection by mould fungi and subsequent grain quality deterioration as affected by insect damage was assessed. Insects were collected from sorghum heads and the frequency of grain mould fungi associated with the insects was determined. The relationship between insect damage and grain mould fungi and the subsequent grain quality was investigated.

Sorghum grain mould fungi are known to affect the malting quality of grains as well as the quality of end products. In chapter 5 the presence of mycotoxins at different malting

stages and the concentrations of mycotoxins in sorghum and other cereal products was examined. The effect of treating sorghum grains with NaOCl concentrations in the malting process with special reference to fungal colonization, germination and root length was evaluated.

The work presented in this thesis will hopefully contribute to a better understanding of grain moulds, the factors that influence the disease and their effect on sorghum grain quality. Due to the fact that each chapter of this thesis represents an independent unit, it has been impossible to avoid some repetition particularly with regard to the introductory remarks and references.

## GENERAL INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth leading cereal, following maize, rice, wheat and barley in the world (FAOSTAT data, 2004) and is the main food source in most underdeveloped countries (Porter *et al.*, 2002). Lower moisture requirements than maize and the ability to tolerate poorly drained soils make sorghum production easier in most regions subject to low rainfall and drought (Maunder, 2002). Sorghum remains one of the more important cereal crops in South Africa, ranked third in terms of total cereal production following maize and wheat during the 2002 cropping season.

Several biotic and abiotic factors affect the production of sorghum. Grain mould is an important disease complex caused by several fungal genera, including *Fusarium thapsinum* Klittich, Leslie, Nelson & Marasas; *F. semitectum* Berk & Rav.; *Curvularia lunata* (Wakk.) Boedijn; *Colletotrichum graminicola* (Ces.) Wilson; *Alternaria alternata* (Fr.:Fr.) Keissl and *Phoma sorghina* (Sac.) Boereman (Singh & Bandyopadhyay, 2000). The disease results in both qualitative and quantitative losses. These include losses in seed mass and percentage germination (McLaren *et al.*, 2002), decreased milling and processing yields, and the quality of sorghum for feed or food (Waniska *et al.*, 2002).

Environmental factors such as weather conditions influence the severity of infection and consequent grain quality. Grain mould is a major problem in areas where flowering and grain maturity coincides with warm weather and high humidity (Rodríguez-Herrera *et al.*, 2000; Singh & Bandyopadhyay, 2000), conditions that are favourable for fungi to infect and sporulate.

Biological factors such as insects play a significant role in infection and may enhance grain mould development either by transporting fungal propagules or by creating a point of entry for the fungus by damaging the grains. Thus, insects can cause an increase in the incidence and severity of grain moulds on sorghum even in more resistant sorghum cultivars

(Marley & Malgwi, 1999). Infection associated with insect damage results in further deterioration of grain quality.

Reductions in grain quality include storage quality, food and feed processing quality as well as market value (Hall *et al.*, 2000). Infection of grains by mould fungi also influences the quality of end products of cereals such as processed food, cereal flour and beverages. Certain grain mould fungi are capable of producing potent mycotoxins that impose health hazards to humans and animals (Bottalico, Logrieco & Visconti, 1989). Mycotoxins produced by sorghum grain mould fungi have received less attention than other cereals, but is nevertheless an important aspect that requires attention.

The above mentioned factors justify the need for the current research and the specific objectives presented in the thesis. Hence fungi associated with sorghum grains were isolated and their pathogenicity was determined. The effect of grain mould fungi on milling and malting quality as well as the production of mycotoxins were assessed. The effects of weather conditions and insects on the development of the disease and the consequences of infection on grain quality were analysed. The study also addressed the presence of mycotoxins at different malting stages and compared the concentrations of mycotoxins in sorghum and other cereal products. Finally the possible reduction of grain mould in steeping during the malt process using NaOCl was investigated.

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# **CHAPTER 1**

## **Literature review**

### **Sorghum grain mould and its relationship with grain quality**

## 1. Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth leading cereal in the world, following maize, rice, wheat and barley (FAOSTAT data, 2004). Despite its importance, sorghum is second only to maize in some parts of South America and is the main food source in most underdeveloped countries (Porter *et al.*, 2002). Sorghum is grown widely in the semi-arid tropics under hot, dry conditions and is a source of food, feed and forage (Reddy, Sharma & Stenhouse, 1995). The crop requires less moisture than maize and is more tolerant of poorly drained soil, making production easier in most agro-ecological regions subject to low rainfall and drought which are unfavourable for most cereals (Maunder, 2002). Sorghum remains an important cereal crop in South Africa. The crop was third in total cereal production following maize and wheat with production at 382,000 tonnes which accounted for 1.37% of the total field crops produced in 2002 (South African Year Book, 2004).

The range of environments in which sorghum is cultivated ensures that biotic and abiotic factors constantly challenge the crop (Frederiksen & Odvody, 2000). Grain mould is one of several destructive diseases and is considered an important disease complex in many parts of the world (Mahaling & Anahosur, 1998). This review emphasizes grain moulds of cereals with specific reference to sorghum and their effect on the grain quality.

Three components of disease development are discussed namely, i) the pathogens involved; ii) the susceptibility of the host, and iii) environmental conditions that favour infection. An outline of the most common symptoms following infection are reviewed, in addition to their effect on grain quality, including germination, grain mass, storage

quality, food and feed processing and quality of sorghum for feed or food. Mycotoxins are a health hazard to both humans and animals and are another important aspect of grain moulds. The most important classes of mycotoxins produced by grain mould fungi, techniques used for their detection and their effect on humans and animals are discussed.

The review focuses on physical characteristics of sorghum grain such as hardness and endosperm texture, colour and biochemical characteristics such as the presence of phenols, anti-fungal proteins and their association with grain mould. Pre- and post-harvest environmental conditions, a component of disease development, that favour grain moulds, are also reviewed.

Grain moulds may be exacerbated by a concurrent attack of panicle feeding insects. The insect-fungus interaction, the role of wounds created by insects as portals of infection and the subsequent deterioration of grain are highlighted. Management strategies and their integration are discussed with special attention devoted to chemical control, breeding for grain mould resistance, post-harvest processing and insect pest control.

## **2. Causal fungi**

Grain mould is a condition of sorghum grain resulting from infection of the developing floret by one or more parasitic fungal species (Esele, 1995). It is one of the most significant biotic constraints to sorghum production and improvement in many parts of the world (Menkir *et al.*, 1996b). Grain mould of sorghum involves a complex of three phenomena which start with field infection of developing grains by parasitic and saprophytic fungi, followed by grain discolouration and weathering that result in loss of grain quality (Murty, 2000).

According to Hall *et al.* (2000) more than 40 fungal genera are associated with mouldy grain. Most are either facultative parasites or saprophytes (Esele, Frederiksen & Miller, 1993). Only a few, including *Fusarium thapsinum* Klittich, Leslie, Nelson & Marasas; *F. semitectum* Berk. & Ravenel; *Curvularia lunata* (Wakk.) Boedijn; *Colletotrichum graminicola* (Ces.) Wilson; *Alternaria alternata* (Fr.:Fr.) Keissl; and *Phoma sorghina* (Sacc.) Boerema, Dorenbosch & Van Kesteren, are regarded as important pathogens (Singh & Bandyopadhyay, 2000). Marley & Ajayi (1999) indicated *Fusarium*, *Curvularia*, *Phoma* and *Cladosporium* spp. as important grain mould fungi of sorghum in West Africa. These grain mould fungi infect sorghum floral tissues during the early stages of grain development (Hall *et al.*, 2000).

Grain mould fungi such as *F. thapsinum* and *C. lunata* may live as saprophytes in field soil or on plant debris. Under favourable environmental conditions these fungi become facultative parasites and attack susceptible cultivars resulting in grain mould (Little, 2000).

### **3. Effect on seed quality**

The most common symptoms of grain mould are pink, orange, gray, white, or black discolourations on the grain surface (Esele *et al.*, 1993). Severe infection produces a thick, dirty, rough black crust on the pericarp that results from mycelial overgrowth (McLaren & Smit, 1996). Symptom development depends on the fungal species involved and the time and severity of infection (Singh & Bandyopadhyay, 2000). Singh & Bandyopadhyay (2000) categorized infected grains into: (i) severely infected grain fully covered with mould, (ii) normal-looking grain with slight discolouration, and (iii) normal

looking grain with no external symptoms where surface sterilization is required to yield grain mould fungi on isolation.

Grain moulds result in both qualitative and quantitative losses. Quantitatively, they result in a decrease in grain yield (Esele *et al.*, 1993), losses in seed mass and percentage germination (McLaren *et al.*, 2002). Grain mould fungi also cause losses in seed viability and subsequent increases in seedling mortality (Williams & McDonald, 1983). According to McLaren & Smit (1996), certain grain mould fungi may cause premature ripening resulting in smaller kernels while other mould fungi such as *F. moniliforme (sensu lato)* may also cause premature sprouting of grains.

Sorghum seeds infected with mould fungi such as *Curvularia lunata*, *Fusarium* spp. and *P. sorghina* are more prone to breakage than healthy seeds. These pathogens also cause a loss of electrolytes from seeds in leachate, with a reduction in seed viability and germination. Seedlings from infected seeds may be less vigorous than those from healthy seeds (Singh & Agarwal, 1989). Prom *et al.* (2003) reported a reduction in seed germination rate and an increased level of grain mould in sorghum cultivars inoculated with two common grain mould fungi, *F. thapsinum* and *C. lunata*. A significant increase in seed dormancy due to infection of grains results in lower seed quality (McLaren *et al.*, 2002). The loss in viability associated with grain mould fungi may cause economic losses to the seed industry. According to Mtisi & McLaren (2002), seed companies and sorghum producers in South Africa lost several million Rand due to poor seed germination associated with grain mould fungi during the 1999/2000 cropping season.

Treating sorghum seed before planting with fungicides such as thiram, or a mixture of thiram and mancozeb, can control mould development on seeds resulting in improved

seed germination and seedling development (Utikar & Shinde, 1989). Lukade (1986) reported a reduction in grain mould incidence in sorghum grains treated with different fungicides at the dough stage as well as increased germination of sorghum seeds subsequent to heads being sprayed with fungicide. *In vitro* evaluation of fungicides such as benomyl and a mixture of thiram + mancozeb or thiram + zineb against major grain mould fungi were found to be effective in inhibiting fungal growth (Mahaling & Anahosur, 1998). Singh & Agarwal (1988) reported a reduction in *F. moniliforme (sensu lato)*, *P. sorghina* and *C. lunta* inoculum with a subsequent increase in seed germination and seedling vigour from seeds treated with carbendazim+thiram. The combination of fungicides provides more effective control of grain mould fungi than the application of a single fungicide (Singh & Agarwal, 1992). Certain plant extracts such as garlic extract have been shown to be effective in reducing grain mould incidence and severity (Navi & Singh, 2004). Control of grain mould fungi with fungicides offers certain levels of protection; however, since it is uneconomical, its use is mostly limited to the protection of breeding material and seed production plots (Marley & Ajayi, 1999).

#### **4. Effect on milling, malting and processing quality**

Aspects of reduced grain quality include storage quality, food and feed processing quality as well as market value (Hall *et al.*, 2000). Fungal growth causes severe discolouration of the grain surface and a break down of grain components. This results in decreased milling and processing yields, and the quality of sorghum for feed or food (Waniska *et al.*, 2002). Grains infected by mould fungi become soft and disintegrate easily; as a result grains deteriorate rapidly during storage (Dogget, 1988). These

deteriorations in grain quality can reduce yield significantly, ranging from 30 to 100% depending on genotype and flowering time (Singh & Bandyopdhyay, 2000).

Other detrimental effects on yield and quality caused by grain moulds in sorghum include complete destruction of the grain, reduction in grain size and mass, and mycotoxin contamination (Williams & McDonald, 1983). Infection by grain mould fungi also cause lower test weight, endosperm density and decortication yield, increased amylase, protease and lipase activities as well as a darker colour of milled products (Seetharaman, Waniska & Rooney, 1996). Consumers consider the superficial quality of grain for human consumption as an important factor for acceptance. According to Williams & Rao (1981), discolouration of grains by mould reduces its acceptability and value. Thus, besides the actual weight loss resulting from mould infection, losses in marketable yield may occur due to decreased demand for discoloured grains.

Milling yield of sorghum depends on kernel size and hence larger kernels produce higher yields and flour with high water absorbance (Lee, Pedersen & Shelton, 2002). However, kernel size can be compromised by fungal metabolism within the grain which causes a reduction in dry-matter content and hence kernel density (Cardwell *et al.*, 2000). Successful infection of grains by mould fungi adversely affects grain filling and endosperm texture, hence reducing the milling quality of sorghum (Little & Magill, 2004). Castor & Frederiksen (1980) also observed a reduction in grain moisture content, kernel weight and grain size in *Fusarium* head blight infected sorghum panicles.

Grain mould can also influence the nutrient content of sorghum grains. Singh & Agarwal (1987) reported a reduction in the size of starch granules in sorghum grains infected with *C. lunata*, *Fusarium* spp. and *P. sorghina* when compared with those of

healthy seeds. The amount of soluble carbohydrates and proteins in deteriorated grain is reduced by the fungus which uses it for its own growth and development, and for the synthesis of proteins (Williams & Rao, 1981). Mould fungi that infect field crops or stored grains reduce starch and lipid content, resulting in increased fibre and a reduction in digestible energy, adversely affecting palatability (Blaney & Williams, 1991). Infection of grains by mould fungi also influences the quality of end products of cereals such as beverages. Flannigan *et al.* (1982, cited by Seetharaman, Waniska & Rooney, 1996) reported that moulding of cereals for brewing significantly reduces malt quality.

Avoiding mechanical damage during harvesting and post-harvest processing, which creates an opening and pathway for infection by mould fungi could reduce contamination by grain mould. Schaafsma *et al.* (2001) recommend careful combine adjustment before harvesting and grain cleaning subsequent to harvesting for improved wheat grain and to reduce levels of damage to kernels by *Fusarium* spp. Drying of sorghum grains harvested at physiological maturity or under wet conditions by solarization can reduce grain mould incidence, but the drying process should be economically feasible, particularly for small holding farmers (Bandyopadhyay *et al.*, 2000). According to Bandyopadhyay *et al.* (2000), late infection of sorghum grain by mould fungi often results in infection restricted to the pericarp with little internal colonization of the endosperm, resulting in the superficial mouldy appearance of the grain. Thus, the pericarp of such grains appears clean after mechanical dehulling with reduced mould contamination. According to Hall *et al.* (2000), the major cause of grain mould is rain at harvest and during storage. Improved drying of grains after harvest can thus reduce

mould growth, but its use as a management tool is determined by its economical feasibility.

Grain mould fungi also continue to infect grains in storage especially when environmental conditions favour the growth and multiplication of the fungi. Thus, proper post-harvest management practices, including storing grain at low moisture content will reduce grain mould (Sauer, Storey & Walker, 1984). Navi & Singh (2004) reported a significant reduction in grain mould incidence and severity in pounded grains compared to non-pounded sorghum grains. They also noted a reduction of 60-80% ergosterol content, an indicator of the presence of mould fungi, in pounded grains. Pounding sorghum grains before storage could thus reduce the infection of kernels by mould fungi.

## **5. Mycotoxins**

Moulds account for greater contamination and spoilage of food than any other group of microorganisms. They render contaminated food unpalatable and unsafe for consumption due to the production of mycotoxins (Munimbazi & Bullerman, 1996). Mycotoxins are secondary metabolites of fungi formed by a series of enzyme-catalysed reactions from biochemical intermediates of primary metabolites that exert toxic effects on animals and humans (Bohra & Purohit, 2003). The main sources of mycotoxins in the human and animal food chains are seedborne fungi and contaminated agricultural products such as cereals and oil seeds (Tseng, Tu & Tzean, 1995). Most mycotoxigenic fungi belong to *Fusarium*, *Alternaria*, *Aspergillus* and *Penicillium* spp. Depending on their environmental requirements the mycotoxigenic fungi are categorised into field fungi, which include toxigenic *Fusarium* and *Alternaria* spp., as well as storage fungi that

include *Aspergillus* and *Penicillium* spp. These organisms require high and low moisture contents respectively in the substrate for growth and mycotoxin synthesis (Logrieco *et al.*, 2003). Commonly, substrates are contaminated by more than one mycotoxin (Hussein & Brasel, 2001). This co-occurrence of mycotoxins can result either due to the ability of certain grain mould fungi to produce more than one mycotoxin, or due to the simultaneous contamination of substrates by two or more grain moulds, of the same or different species (FAO, 1994).

### 5.1 Groups of mycotoxins produced by grain mould fungi

Several groups of mycotoxins in agricultural commodities have been reported. Fungi in the grain mould complex are capable of producing mycotoxins, among them *Fusarium* spp. in the Liseola section that produce chemically and biosynthetically diverse mycotoxins (Bandyopadhyay *et al.*, 2000). These cause a range of physiological and pharmacological responses in humans, domesticated animals and plants. Hence, *Fusarium* mycotoxins are of great concern in all cereal-growing areas. The wide range of mycotoxins synthesized by *Fusarium* spp. include trichothecenes, zearalenone and fumonisins (Logrieco *et al.*, 2003).

The trichothecene mycotoxins include more than 45 structurally related groups of fungal metabolites. T-2, deoxynevalenol (vomitoxin, DON), nivalenol, fusarinon-x and their derivatives are most commonly associated with grains moulded by *Fusarium* spp. (Vesonder & Golinski, 1989). The trichothecenes may be divided into type A and type B where T-2 toxins, HT-2 toxins, neosolaniol and diacetoxycirpenol are grouped under type

A, while trichothecenes, including deoxynivalenol, nivalenol and fusarinon-X are grouped into type B (Mello & Macdonald, 1997).

The zearalenone group of toxins is primarily produced by *F. graminearum*. This toxin has been detected in natural sources as well as laboratory substrates, including cereal grains such as maize, rice, sorghum and barley (Shotwell, 1977; Palti, 1978), and in other agricultural commodities such as hay, feed, pig feed and dairy rations associated with illness in farm animals (Shotwell, 1977). Odhav & Naicker (2002) reported the contamination of South African sorghum malt grains by mould fungi where half of the samples tested exhibited positive results for the presence of zearalenone. No grain mould pathogens have been detected in beer, as they are destroyed by cooking during the brewing process. However, Odhav & Naicker (2002) noted the presence of toxins in commercial and home brewed South African beers. This indicates that the toxins are heat-stable and thus, may have an adverse effect on the health of consumers. The presence of zearalenone in mouldy maize, maize porridge, malted sorghum and sorghum beer from Swaziland and Lesotho has also been reported (Sibanada, Marovatsanga & Pestka, 1997).

Several investigators have reported that *F. moniliforme (sensu lato)* is one of mould fungi most commonly isolated from sorghum grain (Onyike & Nelson, 1992; Munimbazi & Bullerman, 1996). The most important mycotoxin produced by *F. moniliforme (sensu lato)* and related species are the fumonisins (Bhat, Shetty & Vasanthi, 2000; Leslie & Marasas, 2002). *F. verticillioides* and *F. proliferatum* are the most prolific fumonisin producers on sorghum (Leslie & Marasas, 2002). Among various fumonisins, fumonisin B<sub>1</sub> is the most important mycotoxin produced by *F. moniliforme* Sheldon (*F.*

*verticillioides* (Sacc.) Nirenburg), *F. proliferatum* (Matsushima) Nirenburg and related species from Liseola group of *Fusarium* spp. (Moss, 2002 b). *F. verticillioides* and *F. nygamai* isolated from sorghum produce high levels of fumonisin which are highly toxicogenic in duckling tests indicating their potential mycotoxicoses (Leslie *et al.*, 2005). This group of fungi (*F. moniliforme sensu lato*) is also associated with other cereal grains. It is one of the most prevalent fungi associated with grain mould of maize and has been reported to cause high rates of human oesophageal cancer in southern Africa (Nelson, Desjardins & Plattner, 1993). Rheeder *et al.* (1992) also reported the association of *F. moniliforme* mycotoxins in mouldy corn from Transkei, South Africa.

Many fusariotoxins are highly stable and persist in food for a long time after all traces of the live *Fusarium* spp. have disappeared (Joffe, 1986). Shephard *et al.* (2002) reported the presence of fumonisins in South African maize meal and porridge prepared from maize meals. Although they observed a reduction in FB<sub>1</sub> levels (up to mean reduction of 23%) in the porridge sampled, the traditional preparation of porridge in South Africa does not include a decontamination procedure. Bhat *et al.* (2000) observed a minimal reduction of fumonisin levels during baking of Indian traditional bread (*roti*) and in porridge prepared with sorghum, suggesting that fumonisins are heat stable mycotoxins. Desjardins *et al.* (2000) also reported the stability of fumonisin mycotoxins. They noted that fumonisin levels showed no change while DON decreased partially during the Nepalese traditional fermentation method for producing maize beer.

Grain mould resistance limits the potential loss in sorghum grain quality by mycotoxin contamination. Preventing the accumulation of toxins produced by *F. moniliforme (sensu lato)* and *F. proliferatum* must be based on controls at the systemic or

*in planta* level. The systemic and endophytic seedborne nature of these fungi prevents their control with fungicides. Breeding for resistance to *Fusarium* spp. in cereals is an option for controlling the pathogen and toxin production in foodstuffs (Bacon & Nelson, 1994). According to Mansuetus, Saadan & Mbwaga (1995) grain mould of sorghum, particularly that caused by *F. moniliforme (sensu lato)* is difficult to control as the strains belong to different mating types and vegetative compatibility groups. Hence they recommend that breeding for resistance should focus on all known strains in order to develop durable resistance against the pathogen.

*Alternaria* spp. are important grain mould fungi that secrete various structural classes of mycotoxins. These are toxic or carcinogenic to animals and cause considerable economic loss to growers and the food processing industry (Zur, Shimoni, Hallerman & Kashi, 2002). A number of mycotoxins produced by *Alternaria* spp. occur naturally in agricultural products including sorghum (Jewers & John, 1990). *A. alternata* may also produce mycotoxins in artificially inoculated cereals such as rice and wheat (Logrieco *et al.*, 1990). The genus *Alternaria* generally produces about 70 secondary metabolites. One of the most important species in this genus, *A. alternata*, produces about seven mycotoxins which are potential food contaminants (Chulze *et al.*, 1995). Secondary metabolite groups of *A. alternata* are classed as the dibenzopyrone derivatives which include alternariol (AOH), alternariol monomethyl ether (AME) and altenuene (ALT), the tetramic acid derivatives that include tenuazonic acid (TA) as well as the perylene derivatives altertoxins I, II and III (Visconti, Logrieco & Bottalico, 1986). According to Seitz *et al.* (1975), there is a definite relationship between location and the production of *Alternaria* mycotoxins. They noted that the levels of AOH and AME in grain moulded

sorghum cultivars were higher at wetter than drier localities. In a survey of toxic *Alternaria* spp. in small grains in the USA, Bruce, Stack & Mislivec (1984) indicated a potential mycotoxin threat in small grains posed by these pathogens. This was deduced from the detection of toxic *Alternaria* species after surface disinfection, indicating penetration, growth and possible toxin production. In addition, at least one of the mycotoxins was found in more than 75% of the isolates examined. Contamination of other crops by *Alternaria* mycotoxins is common. *Alternaria* mycotoxins such as AOH, AME and TA have been reported in olives (Visconti *et al.*, 1986) and in artificially inoculated sunflower seeds (Chulze *et al.*, 1995).

Aflatoxin is a secondary metabolite of moulds that affects a wide range of hosts including sorghum. It is mainly produced by two mould fungi namely *Aspergillus flavus* Link and *A. parasiticus* Speare (Bandyopadhyay *et al.*, 2002). Aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> are the principal compounds associated with plant food products (Eaton & Ramsdell, 1992). *Aspergillus* spp. survive in soil, infect grains in the field and continue to colonize them in storage (Cardwell & Cotty, 2002). High temperatures, irregular rainfall and insect damage are probably amongst the most important factors that allow *A. flavus* and *A. parasiticus* to invade crops in the field (Blaney & Williams, 1991).

Aflatoxin has been linked to health problems in humans and animals and is considered to be the most potent, naturally occurring carcinogen known to man (Windham & Williams, 1998; Hall *et al.*, 2000; Cardwell & Cotty, 2002). Although sorghum can be infected by *Aspergillus* spp., it is not considered a good substrate for the production of aflatoxin compared with other high-risk agricultural commodities such as maize, groundnut (*Arachis hypogaea* L.) and other oil-rich seeds (Bandyopadhyay *et al.*,

2002). The reduced susceptibility of sorghum to infection by *Aspergillus* spp. and aflatoxin contamination is attributed to the physical and biochemical composition of sorghum grains (Ratnavathi & Sashidhar, 2003). Contamination of other cereals, such as wheat, with aflatoxins is not regarded a major problem (Blaney, Moore & Tyler, 1987). However, although the contamination of cereals by *Aspergillus* spp. was low in a study that was conducted in Uganda, 38% of the sorghum samples tested positive for unacceptably high aflatoxin levels ranging from 1 to > 1000µg/kg (Mello & Macdonald, 1997). Thus, the potential of aflatoxins in sorghum grains infected with *Aspergillus* spp. should not be under estimated.

## 5.2 Detection and assay of mycotoxins

Fungi occur extensively in nature and this may lead to the presence of mycotoxins in food and feed. Preventing the formation of toxins in the field and during storage can control mycotoxin production (Edwards, Callaghan & Dobson, 2002); however, technology does not yet exist for the complete prevention or removal of mycotoxins from food and feeds (Chu, 1992). Measures for the control of mycotoxins rely on thorough monitoring programs that are sensitive, specific and simple for mycotoxin detection in various ecological systems (Sydenham *et al.*, 1996).

A number of methods have been developed for the determination of mycotoxins in mould contaminated grains, grain-based animal feeds, and grain or liquid based media. The techniques that are commonly used include enzyme-linked immunosorbent assay (ELISA), high performance liquid chromatography (HPLC) and thin layer chromatography (TLC) (van der Gaag *et al.*, 2003). The effectiveness of the techniques

differs according to the mycotoxin group targeted. For example, the HPLC technique employs pre-column derivatization with ophthaldialdehyde, reverse-phase separation and fluorescence detection, which have been reported as accurate in providing reproducible data on the levels of FB<sub>1</sub> and FB<sub>2</sub> in cereals and in feed samples (Thiel *et al.*, 1991). However, new immunochemical analytical techniques that are more effective have been developed. The Polyclonal antibody (PAb)-based competitive direct enzyme-linked immunosorbent assay (CD-ELISA) method has been reported to be more effective than HPLC in screening of fumonisins from maize. Sixteen of 18 samples showed between 85% and 100% higher concentrations than the corresponding HPLC results (Sydenham *et al.*, 1996). Despite the sensitivity of HPLC, it is the most costly and time consuming of the methods used, as a result of the required extraction and clean up methods (van der Gaag *et al.*, 2003).

Schnerr, Vogel & Niessen (2002) reported the use of a surface plasmon resonace based indirect inhibitive immunoassay for the rapid quantification of deoxynivalenol (DON) concentrations in wheat. The analysis is completed within 15 minutes including both sample preparation and mycotoxin quantification. Pettersson & Aberg (2003) investigated the use of near infrared spectroscopy for detection of mycotoxins in cereals and speculated on its possible use to develop a calibration model to screen DON in wheat. Plattner & Maragos (2003) developed a liquid chromatography/mass spectrometry method to detect DON and nivalenol from ground wheat and maize grains that require no sample cleanup and can detect the toxins at 0.05 µg/g. Blaney *et al.* (1987) implemented two techniques to assay DON namely HPLC and gas chromatograph-mass spectrometry (GC-MS). Wilson & Abramson (1992) also

reported GC-MS as the best methods for trichothecene analysis. Extreme selectivity and sensitivity of GC-MS for the determination of derivatives of trichothecene is obtained when used in the selected ion-monitoring mode, eliminating problems associated with interfering substances (Wilson & Abramson, 1992). Hart & Schabenberger (1998) recommended the use of ELISA, which is rapid and simple to determine levels as well as the presence or absence of vomitoxin.

Several aflatoxin determination methods have been developed. These include a wide variety of analytical methods such as TLC, HPLC, GC-MS and capillary electrophoresis (CE) as well as immunochemical methods such as ELISA (Maragos & Greer, 1997). According to Wilson & Abramson (1992), conventional methods of mycotoxin analysis such as TLC or HPLC require preliminary purification steps as losses occur before the determination step. Immunochemically based methods are less sensitive to impurities in the extract as they selectively bind the targeted compound before the removal of interfering substances. Maragos & Greer (1997) developed capillary electrophoresis with laser-induced fluorescence detection (CE-LIF) for the analysis of AFB<sub>1</sub> in contaminated maize that had been isolated using a silica gel column technique and an immunoaffinity column technique. The CE-LIF method is effective at detecting AFB<sub>1</sub> at levels as low as 0.5 or 1 ppb. It is more advantageous than HPLC as less organic solvent is used for the determination steps (Maragos & Greer, 1997).

According to Schade & King (1984), the most commonly used method for the identification and quantitative analysis of major and minor *Alternaria* toxins has been TLC. Gas-liquid chromatography (GLC) and HPLC are generally used for the major toxins of *Alternaria* spp. such as AME, AOH, ALT and TA. Visconti *et al.* (1986)

implemented TLC for the analysis of *Alternaria* spp. mycotoxins and HPLC for mycotoxin quantification and for the confirmation of TLC findings. Lau *et al.* (2003) reported the detection of AOH and AME from several juices by liquid chromatography-mass spectrometry and liquid chromatography-tandem mass chromatography with higher sensitivity and specificity.

In comparison to other agricultural food products, little attention has been given to the possible effects of toxins associated with sorghum grain mould. The different methods mentioned in this review should be readily adaptable for the extraction of mycotoxins from sorghum grains and processed sorghum products to provide an accurate assessment of the hazard posed by grain mould metabolites in the food and feed supply. The investigation of natural contamination of sorghum grains by mould and the analysis of fungal mycotoxins should thus be encouraged.

### *5.3 Effect of the mycotoxins on humans and animals*

In addition to being pathogenic, certain grain mould fungi are capable of producing potent mycotoxins that impose health hazards to humans as well as animals and thus are of concern in cereal growing areas all over the world (Bottalico, Logrieco & Visconti, 1989; Hall *et al.*, 2000). The threat of mycotoxins becomes more important with increased population pressure and limited food supplies that could lead to more mycotoxin-contaminated foodstuffs being consumed (Nelson *et al.*, 1993).

Many strains within *Fusarium* section *Liseola* are capable of producing significant levels of fumonisin B<sub>1</sub>. Their presence in symptomless, healthy grain suggests a potential for widespread contamination of human food and animal feeds

(Leslie *et al.*, 1992). The most widely reported animal disease syndromes associated with the ingestion of *F. moniliforme (sensu lato)*-contaminated feed is equine leukoencephalomalacia (LEM) which is a neurotoxic disease of Equidae. It causes apathy, nervous disorders, paralysis of the lower lip and mobility problems (Thiel *et al.*, 1991; Sydenham *et al.*, 1992). Disease syndromes associated with the ingestion of *F. moniliforme*-contaminated feeds are not restricted to equines only. Sydenham *et al.* (1992) reported acute toxicity of *F. moniliforme* isolates obtained from contaminated corn-fed to ducklings. Disease symptoms of poultry fed a diet contaminated with fumonisin include black sticky diarrhoea, reduced weight gain, drastic drop in egg production, lameness and mortality (Bhat *et al.*, 2000). Piramanayagam & George (2002) also reported a reduction in feed conversion rate, live weight reduction, poor performance, diarrhoea and dullness in fumonisin-fed chickens. Fumonisin B<sub>1</sub> can cause the death of pigs by chronic accumulation of fluid in the lungs (porcine pulmonary oedema) and of rats by necrosis of the liver. Fumonisin B<sub>1</sub> is also thought to be the aetiological agent of oesophageal carcinoma in humans (Desjardins *et al.*, 2000; Moss, 2002 b). In Transkei, South Africa, human oesophageal cancer has been attributed to mouldy maize ears being used for brewing of beer. Contaminated maize is also used for feed during poor crop years (Marasas *et al.*, 1981).

The contamination of grains by more than one mycotoxin can affect toxicity as some naturally occurring combinations of mould fungi, such as *Fusarium* spp. produce toxins that are synergistic in laboratory animals (FAO, 1994). Swamy *et al.* (2002) reported reduced growth, altered brain neurochemistry, and increased serum immunoglobulin concentration in pigs fed with *Fusarium* contaminated swine diets.

They also noted a synergistic toxicity of *Fusarium* mycotoxins such as deoxynivalenol, acetyldeoxynivalenol, fusaric acid and zearalenol.

Zearalenone (ZEA), a mycotoxin commonly produced by *F. graminearum*, causes estrogenic responses and a wide variety of reproductive problems in livestock when animals consume zearalenone-contaminated feed or grain (Bandyopadhyay *et al.*, 2000). ZEA is also produced by *F. culmorum*, *F. equiseti* and *F. semitectum* and is among the most widely distributed mycotoxins. ZEA is found at high concentrations in agricultural commodities particularly in maize (Logreico *et al.*, 2003). Pigs are more sensitive than other species to zearalenone, a pseudo-oestrogen, which can thus have significant impact on the pig industry (Blaney *et al.*, 1987). In swine, it causes vulvovaginitis, low birth weights, fetal reabsorption, aborted pregnancies, reduced litter size, abnormal estrus and feminization of males (Shotwell, 1977). This toxin also has an estrogenic effect on poultry. The most common symptoms in poultry are, swelling of the vent, cystic development of the genital tracts, eversion of cloaca and enlargement of the oviduct (Palti, 1978).

Trichothecenes such as nivalenol and DON are known protein synthesis inhibitors and consumption of grain contaminated with these mycotoxins can cause anemia and immunosuppression, haemorrhage, diarrhoea and emesis (Desjardins *et al.*, 2000). The effect of deoxynivalenol as immunosuppressant in animals is significant even at low levels of the mycotoxin (Sibanda *et al.*, 1997). Human and animal exposure as low as 10  $\mu\text{mol/L}$ , as well as greater or equal to 100  $\mu\text{mol/L}$  DON can cause aqueous and inflammatory diarrhoea, respectively through the inhibition of the intestinal D-glucose/D-galactose sodium-dependent transporter (Marc *et al.*, 2002). Vomitoxin produced

commonly by *F. graminearum* causes outbreaks of acute gastrointestinal illness in humans. Vomiting, feed refusal, immune suppression, diarrhoea and weight loss occur in animals that consume vomitoxin-contaminated feed with consequent economic losses due to low feed intake (Bandyopadhyay *et al.*, 2000).

T-2 toxin, produced by various *Fusarium* spp., has been reported to be the cause of alimentary toxic aleuka in humans and its immunosuppressive activity has adverse effects on many animals (FAO, 1994). T-2 intoxication of poultry leads to reduced weight gain, beak lesions, poor feathering, increased susceptibility to infection, a drastic and sudden drop in egg production, eggs with thin shells and mortality (Jacobsen *et al.*, 1993). Damage caused by T-2 is irreversible and its intoxication is congruent with widespread tissue and organ damage including haematological, hepatic, renal, pancreatic, muscular and cardiac effects (Bandyopadhyay *et al.*, 2000). The liver is a major site for trichothecene toxicity and the common biological mode of action of trichothecene toxins results from inhibition of protein synthesis and tissue necrosis (Smith, 1992).

Ingestion of mycotoxins produced by *Alternaria* spp. results in a wide spectrum of toxicological effects that include foetotoxicity, teratogenicity and possible carcinogenicity in humans and animals (Jewers & John, 1990). Watson (1984) suggested that the concurrent presence of different *Alternaria* mycotoxins in food samples leads to synergistic toxicity and reported that the mutagenicity of *Alternaria* compounds is greater than the sum of the mutagenicity of the individual compounds. It has been reported that the administration of alternariol and alternariol-monomethyl-ether caused foetotoxin as well as teratogenic and visceral necrosis in mice respectively but were more effective when administered in a 1:1 ratio (Jewers & John, 1990). Tenuazonic acid has been

shown to be toxic to poultry. The LD<sub>50</sub> for day old chicks is 37.5 mg/kg of body weight and will result in the death of the animal within three to four days as a result of hemorrhagic lesions in the musculature of the thighs, breast, heart and subcutaneous tissue (Jewers & John, 1990). Another common grain mould fungus, *Phoma sorghina*, has been implicated in the production of tenuazonic acid which causes a haematological disorder in humans, onyalai, in Africa with distinctive hemorrhagic bullae in the mouth (Steyn & Rabie, 1976).

Mould contaminated and damaged grains are frequently channelled into animal feeds or may be consumed by people particularly in areas that experience food shortages. In South Africa, about 200 people suffer from an unnamed hemorrhagic disease with additional teratogenic (neurotoxic) effects, and associated liver and kidney symptoms each year. The disease complex is suspected to result from drinking home-brewed native sorghum beer, contaminated with tenuazonic acid produced by *A. alternata* and *P. sorghina*, and cytochalasin produced by *A. flavus* (Kendrick, 1992).

Aflatoxins have a wide range of substrates with acute and chronic toxic effects that make them significant in human and animal health. In laboratory rats, aflatoxin B<sub>1</sub> has been identified as one of the most carcinogenic metabolites. Sensitivity to aflatoxin varies among animal species as well as between sexes of the same species, the male generally being more susceptible than the female (Moss, 2002a). According to McDonald *et al.* (1996), young animals are more susceptible than adults of the same species. The animals are classed into three categories based on their response to aflatoxin namely highly susceptible such as turkey and poults; susceptible such as calves and pigs and resistant such as mice and sheep. Aflatoxins have been reported to be hepatotoxic

and bind DNA, disrupting genetic coding resulting in tumorigenicity (Sibanda *et al.*, 1997). The principal target organ of AFB<sub>1</sub> is the liver and it has been suggested that the mycotoxin's interference with ATP production may play a role in its hepatotoxicity (Eaton & Ramsdell, 1992).

#### 5.4 Plant pathogenesis

Certain mycotoxins have been implicated in the pathogenicity of *Fusarium* spp. In addition to being toxic to animals, some mycotoxins such as T-2 toxin have multiple effects and may cause phytotoxic and antimicrobial syndromes (Chelkowski, 1989). Harris *et al.* (1999) reported the production of various mycotoxins other than trichothecenes, including zearalenone and fusarins by *F. graminearum* and noted their involvement in plant pathogenesis. Mańka *et al.* (1985) also reported that the most virulent *Fusarium* spp. on cereal hosts were those species that produced high levels of trichothecene and zearalenol with a possible synergistic effect in the disease process. After inoculation with a non-trichothecene producing mutant and trichothecene-producing *Giberella zae* in studies of wheat ear infection, Nicholson *et al.* (1997) found that the amount of mutant isolated was generally less than the wild type in the inoculated plants. This indicates that colonization of grain is reduced when trichothecene production is inhibited.

### 6. Grain characteristics and grain mould severity

Morphological and physical characteristics of the seed, glume and/or panicle that block the introduction of fungal conidia into the infection site are the primary sources of

resistance to grain mould fungi (Little, 2000). Sorghum varieties are generally classified as hard or soft, based on the relative proportion of the outer, hard, translucent endosperm to the inner, soft and opaque endosperm (Mazhar & Chandrashekar, 1993). These sorghum varieties differ in the proportion of the relative areas of corneous and floury endosperm that influences grain hardness (Waniska *et al.*, 2001). Hence, varieties with hard sorghum grains are more resistant to fungal attack than varieties with soft grains (Kumari, Chandrashekar & Shetty, 1992). Jambunathan, Kherdekar & Bandyopadhyay (1992) reported that grains of resistant groups take longer to grind than mould susceptible grains. Sorghums with low and medium endosperm hardness are most vulnerable to deterioration while harder grains exhibit lower mould ratings even when watered with sprinklers or inoculated with mould fungi (Waniska *et al.*, 2001). In maize, cultivars resistant to mould fungi possess thicker pericarps than susceptible maize crops (Hoenisch & Davis, 1994).

Sorghum grains exhibited a range of colours as a result of several interacting factors such as pericarp thickness, presence or absence of a testa as well as endosperm texture and colour. The pigmentations of pericarp and testa are caused by phenolic compounds (Hahn & Rooney, 1985). Sorghum with a red pericarp contains phenolics that prevent fungal growth on the grain surface. White grains with a corneous, hard endosperm resist fungal colonization internally, but are unable to suppress late infection and sporulation by grain mould fungi on the pericarp (Bandyopadhyay *et al.*, 2002). According to Waniska *et al.* (2001), sorghum grain with a red pericarp and/or tannins do not need to have a hard endosperm to resist fungal invasion in comparison to grain having a white pericarp. Sharma *et al.* (2000) reported a pigmented testa as an important

trait in resistance to grain moulds and observed that hybrid sorghums with a pigmented grain colour were more resistant to head bugs and grain mould infection. Audilakshmi *et al.* (1999) reported that a harder grain, darker glumes and higher phenol levels play an important role in resistance to grain mould. Menkir *et al.* (1996a) associated darker coloured sorghum kernels with increased resistance against grain mould infection. According to Pedersen & Toy (2001), sorghum cultivars with a red pericarp are more resistant to mould than non-pigmented seeds, which enables them to easily germinate and consequently their seedlings emerge without being affected by mould or soil-borne fungi.

Endosperm protein level is associated with grain hardness. Reddy *et al.* (2000) reported the presence of higher levels of protein and prolamine in the endosperm of hard grains, which are mould resistant compared with soft grains which are susceptible to grain mould. According to Kumari *et al.* (1992), levels of these compounds are higher in hard varieties than in soft varieties during all stages of development. Deposition of prolamine in hard grains reduces intercellular spaces for the growth of fungal hyphae particularly in the peripheral cells of the endosperm (Kumari *et al.*, 1992).

All sorghums contain phenols and flavanoids, but not all sorghums contain tannins. Sorghum thus can be classified based on tannin occurrence. They are classified as type I, no tannins; type II, tannins in pigmented testa; or type III, tannins in pigmented testa and pericarp (Waniska *et al.*, 2002). Unlike other cereals, the structure of the sorghum head exposes the grain to insect and mould damage. Sorghums with phenols, especially tannins which are able to inhibit fungal enzyme activity, may confer a degree of resistance against invasion by mould fungi (Hahn, Faubion & Rooney, 1983). Doherty

*et al.* (1987) also reported that grain mould and insect resistant caryopses contain higher free phenolic compounds and tannins than susceptible cultivars (Doherty *et al.*, 1987).

The concentration of flavan-4-ols is higher in mature grains of mould-resistant sorghum cultivars than mould-susceptible cultivars when grown under controlled environments (Jambunathan *et al.*, 1986) and under field conditions (Jambunathan, Kherderkar & Bandyopadhyay, 1990). However, some genotypes with high levels of flavan-4-ols become susceptible to grain mould while some genotypes with low flavan-4-ols show relative resistance. This could be due to other internal factors such as the effectiveness of flavan-4-ols at certain stages of kernel development or external environmental factors (Melake-Berhan *et al.*, 1996). In most plants, antibiotic phenols are formed in response of pathogen attack, which is considered an active defence mechanism. They may also occur constitutively and thus function as preformed pathogen inhibitors (Nicholson & Hammersmidt, 1992). Rodriguez-Ballasteros *et al.* (2002) reported that phenolic compounds, which are free or bound to the glumes, play a crucial role in resistance to pathogens. They indicated that an increase in the concentration of phenolic compounds in artificially inoculated plants was induced by the presence of mould fungi and made an important contribution to grain mould resistance.

The physical characteristics and fat content of grains plays a role in the accumulation of aflatoxin. Ratnavathi & Sashidhar (2003) reported that certain white sorghum genotypes low in fat, with average starch and high protein content showed maximum aflatoxin resistance. This could be attributed to the corneous nature of the endosperm in combination with the low fat content of the genotypes. They have also noted that grains with high polyphenol levels are more resistant than those with a floury

endosperm and low levels of polyphenols. Stack & Pedersen (2003) demonstrated that sorghum hybrids with a tannin-content testa layer had the lowest incidence and severity of grain mould. According to Menkir *et al.* (1996a), the concentrations of apigeninidin and luteolinidin, which inhibit fungal growth, are higher in red sorghums while white sorghums, which are devoid of a testa, have the lowest concentrations. Hahn *et al.* (1983) also reported the relative resistance of sorghums with high tannin-content (pigmented) testa to grain mould fungi than non-pigmented sorghum. However, they also noted that tannins produce an unpleasant flavour and reduce the grain's quality.

Preformed or induced secondary metabolites, hydrolytic enzymes, and antifungal proteins (AFP) that act individually or in combination are important components of the defence mechanisms of plants against pathogens (Little, 2000). Several studies have been conducted to determine the availability and activity of antifungal proteins *in vitro*. Seetharaman *et al.* (1997) reported inhibition of spore germination and hyphal rupture of *F. moniliforme* and *C. lunata in vitro*, and with certain speculation *in vivo*, by AFP in sorghum seed. AFP's include sormatin, which increases the permeability of fungal cell membranes, hydrolases such as chitinase, which degrade structural polysaccharides of the fungal cell wall, and ribosomal-inhibiting protein (RIP), which modify and inactivate foreign ribosomes. Rodríguez-Herrera, Waniska and Rooney (1999) also reported higher levels of sormatine,  $\beta$ -1,3-glucanase and RIP in grain mould resistant sorghum lines suggesting that AFP's may contribute to grain mould resistance.

In field experiments, Bueso *et al.* (2000) associated grain mould resistance with the amount of AFP synthesis induced by fungal inoculation in the field, and observed less fungal infection when AFP levels increased. Pathogenesis-related proteins such as

chitinase and  $\beta$ -1,3-glucanases can be induced in sorghum plants exposed to various stresses such as fungal infection, insect infestation and mechanical wounding. The increase in the level of stress induced proteins in sorghum plants is thought to limit the spread of pathogens or other opportunistic microorganisms (Krishnaveni *et al.*, 1999). Antifungal proteins could be more effective when they act synergistically. Guo *et al.* (1997) found almost equal concentrations of RIP in both resistant and susceptible maize kernels and noted that other proteins may act synergistically with RIP to confer resistance to *A. flavus*. Although these proteins play an important role in grain mould resistance, antifungal proteins on their own only confer partial resistance (Rooney *et al.*, 2002).

The use of grain mould resistant cultivars is the preferred and most feasible method of controlling and minimizing damage to grains by grain mould fungi (Menkir *et al.*, 1996a) as no extra effort would be required to control the disease (Marley & Ajayi, 1999). Although conventional breeding methods have been partially successful in producing sorghum genotypes with tolerance to grain mould, high levels of resistance have not been incorporated into white-grain genotypes with high yield potential (Bandyopadhyay *et al.*, 2002). Biotechnology could contribute to grain mould resistance, but because of its resource intensive nature, may not provide new cultivars in the short term (Reddy *et al.*, 2000). Hence, Reddy *et al.* (2000) highlight the need for complementary approaches at the field and pre-consumption grain processing levels, with genetic enhancement for holistic grain mould management.

The identification and screening of cultivars for mould resistance forms the basis in breeding for resistance. Various screening techniques have been developed for this purpose. Grain mould resistant sorghums can be identified in the field by using sprinkler

irrigation to wet panicles during the grain filling period, artificially inoculating the heads to ensure pathogen presence and bagging to maintain high humidity in the panicles (Bandyopadhyay & Mughogho, 1988). Sorghum heads are then assayed for mould presence. Where field-screening is not reliable, such as white-grained photoperiod sensitive material, a laboratory-based screening method has been developed (Reddy *et al.*, 2000). The degree of mould development on different sorghum cultivars is assayed *in vitro* by incubating harvested grains on moist blotting paper at 25°C in an alternating 12 h light and 12 h dark regime (Williams & Rao, 1981).

## **7. Influence of environmental factors on grain mould**

Most sorghum is grown in the semi-arid tropical regions under rain-fed conditions. These conditions favour the development of grain moulds (Jambunathan *et al.*, 1992). Grain mould is a major problem in areas where flowering and grain maturity coincide with warm weather and high humidity (Rodriguez-Herrera *et al.*, 1999; Singh & Bandyopadhyay, 2000; Rooney *et al.*, 2002). Grain mould development on sorghum has two critical stages namely, spore germination, and mycelial growth (Hahn *et al.*, 1983). High relative humidity during the critical periods (early plant growth as well as between the end of flowering and harvesting periods) favours grain mould fungi by providing suitable conditions for infection or by enhancing sporulation (Ratnadass *et al.*, 2003a). Bhat *et al.* (2000) reported that sorghum harvested during unseasonal rain and left in piles in the field became visibly mouldy and contaminated with fumonisin B<sub>1</sub> due to the high moisture. They also noted that the incidence of fumonisin mycotoxins in sorghum samples was higher in rain-affected grains compared to normal samples. Rodriguez-

Herrera *et al.* (1999) reported severe grain mould as a result of unusually heavy rainfall shortly after physiological maturity in sorghum lines known to be resistant.

High humidity during anthesis in cereals such as wheat has also been reported to be conducive to the development of mould fungi and consequently to significant increases in mycotoxin contamination of grain (Turner, Jennings & Nicholson, 1999). Rapid development of *A. alternata* and subsequent mycotoxin contamination of sorghum may be attributed to excessive rainfall accompanied by high temperatures late in the growing season (Ansari & Shrivastava, 1990).

Certain moulds are favoured by dry weather conditions. In addition to insect damage to the ears and low levels of host resistance, the greatest potential for disease development in maize kernels caused by *A. flavus* is under dry conditions. This results in reduced grain quality and potential aflatoxin contamination of the grain (Hamblin & White, 2000).

The structure of sorghum heads influences the microclimate and plays an important role in infection by grain mould fungi. Sorghum cultivars with compact heads are more prone to infection by grain mould fungi, as their closely arranged panicles retain moisture that creates a suitable environment for the growth of airborne moulds and those from other sources. Less fungal contamination occurs in sorghum cultivars with open heads as little moisture is retained due to air movement through the head (Reddy & Nusrath, 1985). This may be especially important in areas where sorghum matures during periods of high rainfall and relative humidity.

Cultural practices such as time of harvesting in relation to weather conditions may also influence the incidence and severity of sorghum grain mould. Bandyopadhyay *et al.*

(2002) reported that grain mould incidence and severity increases after physiological maturity when it coincides with delayed harvesting and/or when wet conditions persist. Manjusha *et al.* (2003) also noted an increased threshed grain mould score and high grain mould incidence accompanied by reduced seed germination in sorghum grains subjected to delayed harvesting.

The moisture content of grain during or after harvesting affects grain mould infection and consequent mycotoxin contamination. Higher frequencies of grain mould fungi occur during storage in grains with a higher moisture content (Sauer *et al.*, 1984). Grains stored under poor conditions are susceptible to grain mould fungi and fungal activity can cause losses in nutritional value, produce unpleasant odours, losses in germination, deterioration in baking and milling quality and contamination with mycotoxins (Magan *et al.*, 2003). Jurjevic *et al.* (2002) reported an increase in grain mould fungi from pearl millet with an increase in storage humidity, temperature and grain moisture content. Consequently they recorded increased aflatoxin contamination of up to 800 ng/g. Waniska *et al.* (2002) also reported a relationship between high moisture levels in sorghum during storage and in other cereals, with high levels of mycotoxin production. Aflatoxin levels can greatly increase in stored, wet sorghum grains, even in grains with low frequencies of *Aspergillus* species (Blaney & Williams, 1991). High aflatoxin production occurs as a result of inadequate post-harvest storage when high moisture content and warm temperatures enhance rapid growth of mould fungi (Moss, 2002a). Dry matter loss as a result of respiration by mould fungi in stored grain is influenced by moisture, temperature, degree and type of kernel damage as well as the amount and type of mould fungal inoculum (Seitze *et al.*, 1982).

Various cultural practices to avoid disease, such as adjusting planting time can be implemented to minimize infection by grain mould fungi. According to Bandyopadhyay (2000), avoidance of grain mould can be achieved by delaying sowing dates or by growing medium to late maturing cultivars. In this case grain filling and maturity occur after the end of the rains. Marley & Ajayi (1999) also reported the influence of adjusting planting time on grain mould incidence and severity. They noted that traditionally photosensitive cultivars mature under conditions of low relative humidity and hence escape infection by grain mould fungi. However, this cultural practice requires irrigation because delayed flowering may lead to yield losses because of severe insect attacks by pests such as head-bugs (*Eurystylus* spp.) and midge (*Contarinia sorghicola* Coquillett) (Bandyopadhyay *et al.*, 2000). Although it may not always be practical due to the need to maximize yield by sowing early and the unpredictable nature of rainfall, Bandyopadhyay & Mughogho (1988) recommended the planting of sorghum so that it matures during dry weather after the end of the rainy season or humid period.

Other cultural practices such as timely harvest should also be considered in the management of grain mould fungi. Grain mould is exacerbated by the exposure of the mature crop to high humidity and rainfall and it would thus be advisable for harvesting to be conducted at physiological maturity (Garud *et al.*, 1998), or as soon as possible thereafter (Williams & Rao, 1981). This can be achieved by synchronizing planting dates such that anthesis and grain filling occur during the dry season (Duncan & de Milliano, 1995). In addition to reduced grain mould pressure, harvesting at physiological maturity will also result in producing high market value grains (Palakshappa & Chetana, 2001).

## **8. Influence of insects on sorghum grain mould**

An insect-fungus interaction occurs when either or both populations exert a definite influence on the other. The classification of these interactions is based on the type of insect involved, i.e. whether the insect transports the fungal pathogen to the host, or damages the host by feeding and thus provides an entrance for the fungus, or it participates in symbiotic relations (Mills, 1983).

Insects puncture grains in order to obtain food or to lay eggs. In most cases the direct damage caused by insects is increased by fungi that invade injured seeds and cause infection (Agrios, 1980). Grain moulds are, therefore worsened by concurrent attacks of panicle feeding insects (Murty, 2000). Among insects that feed on sorghum grains, head-bugs are most commonly associated with grain mould fungi. Both feeding and oviposition punctures by head bugs in maturing sorghum caryopses result in severe quantitative and qualitative losses, and favour secondary infection by grain mould fungi (Ratnadass *et al.*, 1995; Marley & Ajayi, 1999). Marley & Malgwi (1999) observed an increase in the incidence and severity of grain mould on sorghum grains that had previously shown a very low incidence and noticed that grain with insect punctures had a higher incidence of mould than the same grain without punctures. In addition to providing a site through which mould fungi can enter and colonize the grain, sap exuded from head-bug injuries provides a substrate for fungal growth on the grain surface (Bandyopadhyay *et al.*, 2002). Minor head-bug injury renders most mould-resistant genotypes susceptible to infection, significantly increases the number of grain mould fungal colonies and alters their relative frequency (Bandyopadhyay *et al.*, 2002). Bandyopadhyay & Mughogho (1988) reported that insect excreta also serve as a medium

for mould growth on infested sorghum heads. Although penetration by grain mould fungi may occur under favourable weather conditions, infection can be aggravated by other biological factors such as infestation by head-bugs (Ratnadass *et al.*, 2003b). Showemimo (2003) reported the head bug *Eurystylus oldi* Poppius as the most devastating insect pest in the sub-Saharan region.

Sorghum head structure influences the degree of infestation by head bugs. According to Teetes & Pendleton (2004), open panicles are less affected by head bugs than compact panicles. Head-bug-aided infection of sorghum panicles is associated with the growth stage of sorghum grains. Infection mostly occurs during the early milk stage of grains, when they emerge from the glumes and become accessible to insects (Ratnadass *et al.*, 2003a). According to Ajayi *et al.* (2001), maximum head bug infestation is mostly observed during the dough stage. Soft dough presents a growth stage where severe grain mould infection occurs (Tarekegn, McLaren & Swart, 2004).

Insects that feed on kernels break the testa of grains, the natural barrier to fungal growth (Sétamou *et al.*, 1998). Dowd *et al.* (1999) reported that rupturing of the kernel pericarp either by insects or other means breaks down kernel defences against mould fungi. They noted a significant correlation between *Fusarium* mould of maize at harvest with insect incidence. Insects that feed by rasping and sucking, such as thrips on maize kernels, provide an ideal point of entry for fungi through the wound (Farrar & Davis, 1991; Hoenisch & Davis, 1994). Under normal circumstances, infection of maize ears by *A. flavus* occurs through the silk; however, biological factors such as insect damage provide portals for infection at damaged sites and facilitate the proliferation of fungi (Beti, Philips & Smalley, 1995). Besides directly damaging maize kernels, insects like

*Sitophilus zeamais* Motschulsky increase *A. flavus* infection by transporting spores (McMillan *et al.*, 1980). Mechanical damage to grain other than by insects is also conducive to the entry of grain moulds and subsequent contamination particularly in insufficiently dried grains (Magan *et al.*, 2003).

The relationship between insect damage to grain and grain mould has been extensively studied in maize. Maize kernel feeding by the European corn borer (*Ostrinia nubilalis* Hübner) leads to infection by grain mould fungi, including the mycotoxin producers, *F. verticillioides* and *F. proliferatum* resulting in increased Fusarium ear rot severity and fumonisin concentrations in kernels (Munkvold, Hellmich & Rice, 1999). Feeding by the larvae of this insect causes severe physical damage resulting in yield loss and delivery of fungal inoculum from the plant surface into the plant (Magg *et al.*, 2002). Sétamou *et al.* (1998) reported that insects enhance infection of maize by *A. flavus* before harvest in Benin and suggested that the levels of aflatoxin in preharvest maize is determined by the extent of damage caused by insects. Windham, Williams & Davis (1999) also reported higher aflatoxin levels and kernel infection in maize hybrids inoculated with southwestern corn borer combined with *A. flavus* than in hybrids inoculated with *A. flavus* alone, regardless of whether the hybrids were resistant or susceptible to the pathogen. Insect-fungi associations have also been reported in other crops such as soybeans. Shortt *et al.* (1982) reported the role of bean leaf beetles in the transmission and inoculation of *Alternaria* spp. to favourable sites such as wounds created by the insect. As a result the fungi infect the seeds easily, resulting in significant seed germination losses.

Insects are a major cause of damage both in the field and during storage where they predispose grain to fungal infection and subsequently to mycotoxin production (Mello & Macdonald, 1997). Maize weevils provide ideal infection sites for *A. flavus* in the postharvest maize crop by mechanically damaging the kernels, increasing moisture content and creating an increased surface area for the growth of the fungus (Beti *et al.*, 1995). Sauer *et al.* (1984) reported an increasing frequency of grain mould fungi in stored cereal samples with increasing live insect infestation. Sun, Zhang & Li (2003) reported the involvement of acaroid mites in carrying and transmitting grain mould fungi during storage. The association of grain-infesting insects with grain mould fungi also varies depending where metamorphosis occurs. For example, larvae and pupae of some insects, such as weevils, develop within the infested kernels, carrying spores of mould fungi with them. The infestation of grains by these insects creates a favourable microclimate for the rapid growth of grain mould fungi in stored grains (Sauer, Meronuck & Christensen, 1992). Storage insects can grow and multiply in drier conditions than fungi. The insects' metabolic heat generates water via condensation on surfaces of grains creating a favourable environment for fungal spoilage (Magan *et al.*, 2003).

Insect involvement is not only limited to kernel wounding and/or fungus transmission, as certain fungi can also promote insect growth. Cardwell *et al.* (2000) observed an increase in the number of coleopteran pests in *F. verticillioides* inoculated compared with uninoculated maize plants including those resistant to the beetles. In addition insect feeding exposes maize grain to fungal invasion by microorganisms such as *A. flavus*, resulting in higher potential aflatoxin levels of grain in both the field and during storage. Thus, the presence of *F. verticillioides* in a plant promotes infestation by

insects which in turn render the grain susceptible to *A. flavus* and subsequently to potential increases in aflatoxin and fumonisin levels (Schulthess, Cardwell & Gounou, 2001).

Although most mould pathogens can penetrate plant tissues directly, mechanical as well as insect damage provides additional entry sites, facilitating infection and the development of grain mould. To avoid this, proper harvest and post harvest grain handling and effective insect management strategies during the growing season as well as during post-harvest shipping and storage should be implemented to minimize mould and mycotoxin contamination (Stack, 2000). Dowd *et al.* (1999) found that appropriate insecticides applied at the correct rate and time could reduce mould incidence directly by controlling insects. Dowd *et al.* (2000) reported a reduction in insect incidence and visible moulding caused by *Fusarium* spp. in maize plants treated with insecticides (chlorpyrifos and malathion) with a concomitant reduction in fumonisin concentration.

Control of insects in stored grains, and a subsequent reduction in the risk of moulds occurring, requires an integrated pest management approach (Magan *et al.*, 2003) that includes sanitation, control of grain moisture and temperature, frequent monitoring and chemical treatment (Munkvold, 2003).

Weed control, particularly weeds that harbour sorghum insect pests can contribute to reduced grain damage. Castor bean (*Ricinus communis* L.) acts as a source of sorghum panicle infestation by *E. oldi*, and proper management of this alternate host within the vicinity of sorghum plants reduces both infestation and damage to sorghum (Ratnadass *et al.*, 2001).

Where fungal invasion is primarily dependent on insect damage, development of insect resistant crops could minimize grain mould infection and reduce associated mycotoxin problems (Dowd, 2000). Since head bug damage to sorghum grains increases the severity of grain mould, planting of cultivars with resistance to both would reduce the severity of grain moulds (Sharma *et al.*, 2000). Bandyopadhyay *et al.* (2000) reported the relationship between grain hardness and resistance to grain mould and head bugs. Loose panicle types, allow for good aeration, low humidity within the head, and easy access to natural enemies of head bugs and as a result harbour fewer head bugs (Leuschner, 1995). Hence it is crucial to consider the nature of the sorghum head during selection in such a way as to minimize head bug damage to the panicles, which would ease the entry of mould pathogens.

## **9. Conclusions**

Grain mould of sorghum is a serious disease caused by a complex of fungi. It affects sorghum quality and yield severely. The involvement of several fungal genera makes it a unique disease where many fungal species play relative roles in the etiology of the disease.

The most frequently encountered genera are *Fusarium*, *Curvularia*, *Alternaria* and *Phoma*. Symptoms of grain mould that develop as a result of infection by mould fungi in the field depend upon the fungal genera involved, the time and severity of infection. Grains severely infected are completely covered with pink and /or black mould growth and disintegrate easily during threshing and processing. Deteriorated grain is

lighter in weight, discoloured and darkened which renders the grain unacceptable to most consumers.

Infection of grain decreases feed value through the removal of starch and the hydrolysis of protein. If appropriate cultural practices are not implemented, mould invasion continues during storage. This results in an increase in the relative amount of fibre in the grain in proportion to a decline in starch and protein content. Damaged grain that has undergone fungal growth during storage may also exhibit unpleasant odours and flavours rendering it unpalatable. In general, changes in palatability and nutrient content of grain infected with mould, pre-and post-harvest, results in deterioration of grain quality. Reduced seed germination, loss of seed viability and subsequent seedling mortality could also be associated with infection by grain mould fungi.

Invasion of cereal grains and their products by grain mould fungi results in the production of mycotoxins. *Fusarium* spp. from the section *Liseola*, are most prolific mycotoxin producers. Other mould fungi such as *Alternaria* and *Phoma* spp. have also been implicated in the production of metabolites toxic to both humans and animals. Mycotoxins can lead to decreased productivity and in severe cases death to animals and humans. Sensitive, specific and simple effective procedures for mycotoxin detection should be exploited to identify the toxins and minimize the devastating losses that can follow contamination.

Various morphological and physical characteristics of the glume and/or panicle reduce infection. Sorghums that are more resistant to grain moulds contain higher concentrations of antifungal proteins and phenolic compounds than susceptible cultivars.

Sorghum is grown in a wide range of climatic regions from tropical, sub-tropical to temperate regions. High humidity during anthesis and unseasonal rains during sorghum harvesting may create a conducive environment for the development of grain moulds. Most grain mould pathogens colonize grains in the field, however, favourable temperature, relative humidity and grain moisture may also allow the moulds to grow post-harvest during transporting, storage and processing.

Many biotic or abiotic factors may lead to stress, which in turn may predispose sorghum grains to mould pathogens. Physical surface damage to grains, either as a result of mechanical injury or insect damage allows fungal penetration into the grain and consequent mycelial growth and mycotoxin production. Head bugs are recognised as one of the most widespread insect pests of sorghum and have been implicated in the severity of grain mould infection. Insects, particularly head bugs attack sorghum kernels at the dough stage. This is the stage at which grain mould fungi infect the plant, with resultant implications for grain quality and yield.

Grain mould is a growing constraint in sustainable production of sorghum in most parts of the world. Studies have shown that yield losses as high as 100% occur as a result of grain mould infection which in turn impacts negatively on the economy. In Africa and Asia crop losses due to grain moulds is estimated to be valued at ± \$129 million (deVries & Toenniessen, 2001). There is therefore, a need to develop strategies for the management of grain mould.

The complexity of sorghum grain mould and the numerous causal fungi make it difficult to manage using a single method of control. Bandyopadhyay *et al.* (2002) noted that most research on the control of sorghum grain mould has focussed on a single

method of control at inopportune times. They recommended the integration of various control strategies that also consider the socio-economic, marketing, organizational as well as institutional framework under which the farmers operate. It would thus be worthwhile to integrate all the possible control measures in to a holistic approach to manage grain mould and retain the quality of sorghum grain. Hence the long-term aim to limit the deterioration of sorghum by grain mould fungi could be realised, to enable its use in food products.

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## **CHAPTER 2**

### **Grain mould fungi and their effect on sorghum grain quality**

## Abstract

The major grain mould fungi of sorghum in South Africa were determined by assaying five sorghum cultivars from three localities in 2002 and eight localities in 2003. The predominant fungal isolates from all the cultivars and localities were *Alternaria*, *Curvularia*, *Fusarium* and *Phoma* spp. in order of frequency isolated. The relative frequency of grain mould fungal isolates differed with locality. Grain mould fungi were least frequently encountered at Bethlehem followed by Heilbron. The incidence of grain mould fungi correlated negatively with milling quality and certain malting properties of sorghum grain cultivars were affected. The effect of fungal isolates on various grain quality parameters was examined by inoculating sorghum panicles under glasshouse conditions. All inoculated fungi reduced grain germination in a range of 5.0-50.1 % with *Curvularia lunata* showing the highest reduction (50.1%) followed by a mixed population of fungi (30.2%). *Fusarium proliferatum*, *A. alternata*, *F. graminearum*, *P. sorghina* and a fungal mixture significantly ( $P \leq 0.05$ ) reduced 1000 kernel mass by 31.5, 26.2, 21.3, 10.6 and 7.4% respectively. *Fusarium thapsinum* and *C. lunata* yielded a 3.8 and 0.5% reduction in 1000 kernel mass respectively. *Fusarium thapsinum*, *F. proliferatum* and the mixed fungal population followed by *P. sorghina*, *C. lunata* and *A. alternata* resulted in higher levels of grain discolouration in glasshouse studies. Mycotoxin production on grains inoculated with different grain mould fungi was significant. These results indicated that various fungi are involved in the grain mould disease complex and that both qualitative and quantitative losses of sorghum may result.

## Introduction

Grain mould of sorghum (*Sorghum bicolor* (L.) Moench) is a complex disease caused by several fungi from various genera, including *Fusarium*, *Alternaria*, *Phoma* and *Curvularia* (Singh & Bandyopadhyay, 2000). Grain mould disease symptoms are often manifested on sorghum kernels in the field, the most common of which are pink, grey, white, or black discolouration of the grain surface (Esele *et al.*, 1993). The disease is one of the leading constraints for the production of optimum quality sorghum grain worldwide (Little & Magill, 2003). Damage caused by grain mould on yield and quality of sorghum grain includes reduction in grain size, kernel mass, nutritional quality and complete destruction of the grain itself (Williams & McDonald, 1983). These decreases in grain quality render the grain unacceptable by consumers and thus, result in a reduction in market value. Mould fungi also cause a reduction in endosperm density and decortication yield resulting in lower milling quality of sorghum grains. The influence of mould fungi in the reduction of nutritional quality could be either due to the size reduction of starch granules as a result of infection by mould fungi (Singh & Agrawal, 1987) or the reduction of soluble carbohydrates and proteins which are utilized by the mould fungi as an energy source and for the synthesis of fungal proteins, respectively (Williams & Rao, 1981). Infection of sorghum grains by mould fungi leads to contamination in storage and also influences the quality of end products of cereals such as beverages by deteriorating malting quality (Seetharaman, Waniska & Rooney, 1996).

In addition to the association of grain mould with grain quality aspects, grain mould fungi can also affect the germination and viability of sorghum seeds. The loss of seed germination is directly proportional to an increased level of grain mould severity (Navi, Bandyopadhyay & Hall, 2002). The loss of seed viability results from infection of

grain by mould fungi, which subsequently leads to seedling mortality, the main cause of a several million rands loss to seed companies and sorghum farmers in South Africa (Mtisi & McLaren, 2002).

In addition to being plant pathogens, certain grain mould fungi are capable of producing mycotoxins which account for partial reduction in grain quality (Harris *et al.*, 1999). Various sorghum grain mould pathogens among them *Fusarium* spp. (Leslie *et al.*, 1992), *Alternaria* spp. (Jewers & John, 1990) and *Phoma* spp. (Steyn & Rabie, 1976) are reported to be implicated.

The objectives of the present study were: (i) To determine the incidence of grain mould fungi in sorghum grains harvested from experimental plots at different localities, (ii) to assess the potential of fungi isolated as grain mould pathogens of sorghum, and (iii) to assess the role of these mould fungi in influencing sorghum grain quality parameters such as discolouration, grain weight and mycotoxins.

## **Materials and methods**

### ***Fungal isolation and colony counts***

Sorghum grain from five cultivars at three (Table 1) and eight localities (Table 2) during 2002 and 2003 respectively from the National Cultivar Trials was provided by the ARC-Grain Crops Institute. Randomly selected 100-kernel samples were surface sterilized in a 1% NaOCl solution for 3 min, rinsed three times in sterile water and plated onto Petri dishes (10 grains per plate) containing 0.3 ml streptomycin sulphate per liter of a 1% malt extract agar (MEA) medium (Biolab<sup>®</sup>). A selective medium for the isolation of *Fusarium* spp. (Van Wyk *et al.*, 1986) was also used. Plates were incubated at 25°C with fluorescent light for 12 h day<sup>-1</sup> for 7 days. Fungi growing from kernels on MEA were

identified to genus level and fungal colonies were counted. Fungi growing on the selective medium were transferred onto carnation leaf agar (CLA) for single spore production and subsequent identification.

### ***The effect of grain mould fungi on malting and milling qualities of sorghum***

The effect of grain mould fungi on malting and milling qualities of sorghum was determined using sorghum grains from five cultivars harvested from seven locations in 2003.

### ***Germinative vigour (GV) and germinative energy (GE)***

Germination of grain samples was evaluated in triplicate according to the technique described by Dewar, Taylor & Joustra (1995). One hundred sorghum kernels were evenly spread on moistened filter paper in a Petri dish. Petri dishes were placed in a germination cabinet for 72 h (Specht Scientific SG-330), with a temperature set at 25°C and a relative humidity of 95%. The number of germinated kernels was counted after 24, 48 and 72 h of germination. After 48 h, GV% was assessed and GE% after 72 h. After 72 h of germination, both germinated and chatted kernels were counted. The latter are kernels that had started to germinate, but with only the tip of the root visible through the pericarp of the grain.

### ***Water absorption and malting loss***

Malting of the samples was done according to the technique described by Dewar *et al.* (1995). The technique was modified by washing samples before steeping in a solution of 25 ml Biocide IO<sub>4</sub> (IO Dophor based reagent) (SABS 1081) (Lever Industrial) in 16 l of

water and after steeping in a solution of 0.16% NaOCl m/v (bleach) solution. Samples (50 g) of sorghum were weighed in nylon bags, then washed in the Biocide IO<sub>4</sub> solution, by means of a 5 min scrubbing - 10 min soaking process, followed by rinsing in sterile water three times. After rinsing, samples were spinned for 3 min in a Miele WZ 259 Spinner. Steeping of the samples was done in a Labotec Model 101 Steeper at 25°C for 24 h. The steeping cycle of 24 h consisted of 3 h intervals of soaking and a 1 h period during which water flowed from the steeper, to allow aeration of the grain. The samples were washed but not scrubbed in the bleach solution after steeping, followed by soaking, rinsing and spinning. Water absorption (WA) was determined after the steeping period, by weighing the grain following washing and was calculated as follows:

$$\text{WA (\%)} = \frac{\text{soaked mass of grain (g)} - \text{original mass (g)}}{\text{original mass (g)}} \times 100$$

Germination was done in a Specht Scientific SG-330 germination cabinet for 5 days, during which the samples were soaked in water (10 min) and spinned (3 min) twice daily. The germinated grain was dried in a Memmert UL 80 drying oven at 50°C for 24 h. Samples were weighed after the drying process and the malting losses (ML) were determined as follows:

$$\text{ML (\%)} = \frac{\text{original mass of grain (g)} - \text{dried mass (g)}}{\text{original mass (g)}} \times 100$$

After malting, the samples were milled in a Janke and Kunkel mill to determine the diastatic power.

*Diastatic power (DP)*

The moisture content of malt was determined in a Memmert UL 80 drying oven set at 105°C for 3 h according to the method described by Dewar *et al.* (1995). The DP assay was performed on the samples according to the micro-method described by Gomez *et al.* (1997) with peptone as extractant. A malt sample of 0.5 g was mixed with a peptone extract, by adding 10 ml of 2% peptone (bacteriological, Biolab®) solution and heating it for 150 min at 30°C, whilst covering the solution with Parafilm® and shaking it every 20 min. The samples were centrifuged at 3000 rpm for 2 min in a Heraeus Christ Labouge centrifuge. Supernatant (0.2 ml) from each sample was added to a buffered starch solution at 30 seconds intervals in a water-bath at 30°C and after 30 min the reaction in the water-bath was stopped by the addition of 4 ml 0.5 N sodium hydroxide (0.5 N ampule, Merck reagent no. AC001252.500). The starch solution was prepared by dissolving 20 g starch (guaranteed reagent, Merck) in water over heat and adding 20 ml buffer before filling the solution up to the mark of a 1000 ml volumetric flask. The solution was maintained at 30°C and the starch buffer was prepared by diluting 68 g sodium acetate (guaranteed reagent, Merck) and 1 N acetic acid (guaranteed reagent, Merck) to 1 l with distilled water. In the case of the blank, sodium hydroxide was added to the buffered starch solution before the addition of the malt extract. One blank was prepared for each sample. A volume of 4 ml 0.05 N alkaline ferricyanide (guaranteed reagent, Merck) was pipetted into an Erlenmeyer flask and 2 ml of the digest starch solution was added. The flasks were kept in a bath of boiling water for 20 min. After cooling the solution, 10 ml 1 N acetic acid salt solution, prepared from glacial acetic acid (guaranteed reagent, Merck), and 0.4 ml potassium iodide (guaranteed reagent, Merck) were added before it was titrated against 0.05 N sodium thiosulphate (0.1 N ampule, Merck). The potassium iodide indicator was prepared by the addition of two drops of

concentrated sodium hydroxide solution to 50 g of potassium iodide dissolved in 60 ml distilled water and diluting the solution to 100 ml. The DP was determined as follows:

$$DP = \frac{TB-TA}{100 - M} \times \frac{VE \times VD \times 2000 \times f}{W \times AE \times AD}$$

Where TA = titre of thiosulphate used for the samples;

TB = titre of thiosulphate used for the blank;

AD = aliquot of digest for sugar determination, i.e. 2.0;

AE = aliquot of extract for sugar determination, i.e. 0.5;

f = normality of thiosulphate, i.e. 0.05;

MC = % moisture content of malt;

VD = volume of digest for sugar determination, i.e. 20.5;

VE = volume of digest for sugar determination, i.e. 0.5, and

W = weight of malt extract

(Gomez *et al.*, 1997).

The DP was corrected for the moisture content of the malt and the titration volume of the blank. The DP of the sample was measured in sorghum diastatic power units (SDU)/g malt.

### ***Pathogenicity tests***

NK 283, the most common commercial hybrid sorghum in South Africa, and PAN 8706W, a white, tan cultivar were used for pathogenicity tests in the glasshouse. Plastic pots (5 l) were filled with steam-sterilized soil and four seeds were planted per pot which were thinned to two plants per pot after seedlings emerged. Commencing two weeks after emergence, plants were fertilized biweekly with limestone ammonium nitrate (LAN 28%) at a rate of 1g/kg soil. Fungal species that were previously isolated from sorghum kernels, namely *A. alternata*, *C. lunata*, *P. sorghina*, *F. thapsinum*, *F.*

*graminearum* and *F. proliferatum*, were used to inoculate panicles at soft dough stage. An additional treatment consisted of a mixture of the fungi (MMF) listed above. Eight panicles were inoculated with each treatment.

All isolates were grown on MEA at 25°C and spore suspensions were prepared from 10-day-old cultures. Cultures were flooded with sterile distilled water and lightly agitated using a sterile glass rod. Spore suspensions of each isolate were decanted through sterilized cheesecloth to remove mycelial fragments. Conidial suspensions were adjusted to 10<sup>5</sup> conidia/ml using a haemocytometer. The spore suspensions were sprayed onto panicles using a hand-operated sprayer. Heads were inoculated at the soft dough stage of grain development, which is the most susceptible stage of grain development (Tarekegn, McLaren & Swart, 2004). Each inoculated panicle was covered with a plastic bag for five days to create a humid environment conducive to infection. Control plants were sprayed with sterile water and enclosed in plastic bags for the same period. Plants were kept in the glasshouse until maturity, harvested and hand threshed.

To fulfill Koch's postulates, re-isolations of test fungi were made from kernels that had been inoculated and sprayed with water (control). All grain samples were surface sterilized before plating onto growth media as described above. One hundred kernels per replicate (two heads per replicate) were surface sterilized and plated onto 1% MEA amended with 0.3 ml streptomycin sulphate per liter of medium. Kernels from panicles inoculated with *Fusarium* spp. were plated onto *Fusarium* selective medium (Van Wyk *et al.*, 1986). Seven to 10 days after incubation, the frequency of the respective pathogens was recorded.

### ***1000 kernel mass***

The effect of infection on the grain density was determined by weighing 1000 kernels from each treatment on an electronic sensitive balance (Scout II) Ohatsu® Corporation, Pine Brook, NJ.).

### ***Germination percentage (%GER)***

One hundred kernels per replicate obtained from pathogenicity tests were placed on moistened sterile filter paper (Copeland & McDonald, 2001) in glass Petri dishes (90 mm) (10 grains per plate) and incubated at room temperature in the dark. Grains were regularly monitored and recorded as germinated when roots and shoots emerged from the grains.

### ***Threshed grain mould score***

A sample of 30 g per replicate of threshed grain from glasshouse inoculated plants was spread in a 90-mm-diameter Petri dish and scored visually for mould severity on the grain surface following the methods of Audilakshim *et al.* (1999). Grain mould severity was assessed using a stereo microscope (x30 magnification) as a visual estimate of the percentage of grain surface, moulded or discoloured. Samples were scored using a 1-5 scale, where 1 = no mould; 2 = slight superficial mould growth up to 10% of the grain surface covered by mould; 3 = moderate mould growth and 11-25% of the grain surface moulded; 4 = considerable mould growth with 26-50% of the grain surface moulded and 5 = extensive mould growth with more than 50% of the grain surface covered by mould (Audilakshmi *et al.*, 1999).

## ***Mycotoxin analyses***

### *Zearalenone test*

Sorghum grain samples from glasshouse inoculated plants were blended in a laboratory blender so that at least 75% of the ground material would pass through a 20 mesh sieve. A ground sample (5 g) was added to 25 ml of 70% methanol/water and agitated vigorously for 3 min. The extract was filtered by pouring 5 ml through a Whatman #1 filter and the filtrate was collected for analysis. The sample was analyzed for zearalenone presence and quantified using a Veratox® zearalenone quantitative test kit (Neogen® Corporation, 620 Lasher Place, Lansing, MI 48912, USA).

### *Fumonisin test*

Sorghum grain samples were ground in a laboratory blender so that at least 75% of the material would pass through a 20 mesh sieve. A ground sample (25 g) was blended with 125 ml of 70% methanol/water solution for 2 min in a high speed blender. The extract was filtered through a Whatman #1 filter and the filtrate was diluted by adding 100 µl of extract into a pre-filled sample dilution bottle from the test kit. The sample was analyzed for fumonisin presence and quantified using a Veratox® fumonisin quantitative test kit (Neogen® Corporation, 620 Lasher Place, Lansing, MI 48912, USA).

### *Deoxynivalenol (DON)*

A 10 g ground grain sample was blended with 50 ml of distilled water for 3 min in a high speed blender. The extract was filtered through a Whatman #1 filter and 5 ml of the

filtrate was collected. This was analyzed for DON using a Veratox® DON quantitative test kit (Neogen® Corporation, 620 Lesher Place, Lansing, MI 48912, USA).

### ***Statistical analysis***

Data from each experiment were subjected to an analysis of variance (ANOVA), and Tukey-Kramer multiple comparison test was used to separate treatment means. All data analyses were performed using the statistical program NCSS 2000 (BMDP Statistical Software Inc., Los Angeles, CA). Correlation between data sets was performed using the statistical package Minitab (Minitab Inc. 1998).

## **Results**

### ***Fungal isolation and colony counts***

Grain mould fungal species isolated from kernels of sorghum cultivars from different localities are listed in Tables 2.1 and 2.2. The predominant fungal species isolated from all cultivars and localities during both seasons were *Alternaria*, *Epicoccum*, *Curvularia*, *Fusarium* and *Phoma* spp. *A. alternata* was the most frequently isolated fungus from all the regions from where the sorghum cultivars were collected. The frequency of grain mould fungi isolated differed between localities. In 2003, the highest fungal frequency was recorded in Potchefstroom, while grain mould fungi were infrequently encountered at Bethlehem with the lowest number of grain mould fungi isolated, followed by Heilbron. The frequency of fungal species from the same cultivar also varied between locations. For example, a higher frequency of *A. alternata* was recorded from grains obtained from Potchefstroom while grains from Cedara had a higher frequency of *C. lunata*.

### ***The effect of grain mould fungi on malting and milling qualities of sorghum***

Milling and malting quality parameters of sorghum cultivars from different locations are presented in Table 2.3. Grain mould fungi were significantly ( $P \leq 0.05$ ) negatively correlated with certain milling and malting properties (Table 2.4). Diastatic power and abrasive hardness were the most negatively affected grain quality parameters. A strong negative correlation with malt loss and abrasive hardness was evident for *A. alternata*. A significant ( $P \leq 0.05$ ) negative correlation with abrasive hardness was also recorded for *P. sorghina*. Increased water uptake (%) was correlated positively with the presence of grain mould fungi while the lowest malting loss was recorded in cultivars from areas with higher mould fungal frequencies.

### ***Pathogenicity tests***

Inoculation of sorghum panicles with fungal pathogens, either singly or in combination, resulted in grain mould in both the cultivars used (Table 2.5). Lesions caused by *F. thapsinum* and *F. proliferatum* had tan to greyish brown discolourations while brown to dark grayish discolourations developed in sorghum heads inoculated with *A. alternata*, *C. lunata* and *P. sorghina*. No grain mould symptoms were observed on control plants although some extraneous saprophytes such as *Penicillium* and *Alternaria* were occasionally isolated from plated kernels. No mould fungi other than the inoculated genera were observed on plated grains.

### ***1000 kernel mass***

Glasshouse inoculation of sorghum grains with grain moulds reduced 1000 kernel mass significantly ( $P \leq 0.05$ ) (Table 2.6). In NK 283, *F. proliferatum*, *A. alternata*, *F.*

*graminearum*, *P. sorghina* and the grain mould mixture reduced 1000 kernel mass by 31.47, 26.24, 21.26, 10.58 and 7.44% respectively. *F. thapsinum* and *C. lunata* had the least effect on 1000 kernel mass with 3.82 and 0.48% reduction respectively. In PAN 8706W, *F. proliferatum*, *P. sorghina* and *A. alternata* reduced grain mass by 36.95, 21.63 and 17.28% respectively, while *C. lunata* and *F. thapsinum* showed very little decrease.

### ***Germination percentage***

Germination of sorghum grain from panicles that were inoculated with grain mould fungi in the glasshouse varied significantly ( $P \leq 0.05$ ) (Table 2.6). All inoculated mould fungi reduced grain germination. In cultivar NK 283, inoculated fungi reduced germination by between 4-48 % with *C. lunata* having the greatest effect (48.03%) followed by MMF (27.82%). In the cultivar PAN 8706W, inoculation of panicles with mould fungi reduced germination by 5-50 %. *C. lunata* had the greatest effect on germination (50.14%) followed by MMF fungi (30.19%). Grain germination was negatively correlated with threshed grain mould score (TGMS) (Table 2.7).

### ***Threshed grain mould score***

Significant ( $P \leq 0.05$ ) differences were recorded in visual grain damage using TGMS caused by grain mould fungi (Table 2.6). *F. thapsinum* and the MMF culture, followed by *P. sorghina*, *C. lunata* and *A. alternata* resulted in the highest level of grain discolouration in glasshouse inoculated grains of NK 283. In PAN 8706W, *F. proliferatum* (TGMS=4), followed by the MMF, *C. lunata* and *F. thapsinum* (both scored 3.75) had the highest TGMS.

### ***Mycotoxin Analyses***

Equal concentrations of deoxynivalenol (DON) were detected in NK 283 from *F. graminearum* and MMF inoculated panicles with concentrations of 0.27, and 0.25 ppm, respectively (Table 2.8). *F. thapsinum* inoculated grains produced the highest (0.55) concentration of fumonisin while *F. proliferatum* inoculated grains yielded 0.17 ppm. Zearalenone was produced by both *F. graminearum* and MMF inoculated sorghum grains of NK 283 and PAN 8706W cultivars.

### **Discussion**

Plating and incubating whole sorghum grains on blotting paper or selective and non-selective media can provide an indication of frequencies of grain mould infection (Bandyopadhyay *et al.*, 2000). In the present study, the frequency of grain mould fungi isolated from different localities and cultivars varied significantly. For example, *A. alternata* was highest in Potchefstroom while the highest frequency of *C. lunata* was observed in grains grown at Cedara. This variation in fungal frequency could be due to the effect of different environmental conditions and varying selection pressure on fungal species.

According to Hall *et al.* (2000), *Fusarium* spp., *C. lunata* and *P. sorghina* are considered the most important grain mould pathogens. However, the frequency of these mould fungi from mature sorghum grains plated onto non-selective growth media is relatively low in comparison to many other mould fungi (Hall *et al.*, 2000). In the present study, the relative frequency of *C. lunata* was lower than that for *A. alternata* but, the negative effect on grain germination was more detrimental when compared to all the other grain mould fungi.

Damage related to grain quality includes storage quality, food and feed processing quality as well as market value (Hall *et al.*, 2000). According to Waniska *et al.* (2002), fungal contamination results in decreased milling and processing yields as well as the quality of sorghum for feed or food. According to Dogget (1988), grains infected by mould fungi become soft and easily disintegrate. In the present study, mould fungi caused a disintegration of sorghum kernels. This was indicated by a negative correlation between the frequency of mould fungi isolated and the abrasive hardness and dehulling indexes. This deterioration in quality and disintegration of sorghum grains is not only limited to milling quality of the grains, but also the malting quality.

Diastatic power (DP) indicates the combined activity of alpha- and beta- amylase present in sorghum malt, which results in the generation of fermentable extract, an important indicator of the final malt quality of sorghum (EtokAkpan, 2004). DP is expressed as sorghum diastatic units (SDU) per gram malt and a value of 28 SDU/g malt is regarded as an acceptable value in the beer-brewing industry (Taylor, 1998). In the present study, DP values from the same sorghum cultivar grown at a different location differed significantly in accordance with the variation in fungal frequency from the same cultivar. The cultivar PAN 8446 in particular displayed different DP values at different localities. The SDU of the cultivar was higher in Bethlehem compared to Potchefstroom and Cedara, both locations associated with higher fungal populations in this cultivar. This tendency was recorded with all cultivars and locations. Abiodun (2002) reported low diastatic activity in sorghum cultivars which were mould infected and weathered. The carbon dioxide released during respiration of the grain mould fungi may have resulted in the inhibition of alpha amylase formation, hence the low DP recorded. Aeration during steeping process is required as adequate oxygen is necessary for the

formation of alpha-amylase where excessive carbon dioxide inhibits its formation (Dewar, Taylor & Berjak, 1997).

Other malting properties of sorghum that were examined and correlated with mould fungi in this study were malting loss and water uptake. In most cases, grain mould fungi correlated negatively with malt loss and positively with water uptake. Cultivars from locations with higher frequencies of mould, such as Potchefstroom and Cedara, showed the least malting loss while locations with lower fungal populations, such as Heilbron, had higher malting losses. Conversely, increased water uptake was recorded in cultivars from locations with higher mould fungal populations while cultivars from locations with lower mould fungal incidence had lower water uptake. Similar phenomena were reported by Abiodun (2002) where cultivars characterized by grain mould infection had the lowest malting loss.

Kernel size, with its cluster of variables including kernel mass, volume, area and length can be used in determining the end-use quality characteristics of cereals (Nielsen, 2003). In the present study grain mould inoculated panicles yielded grains with reduced kernel mass. Singh & Agarwal (1989) observed a reduction in 100-kernel mass of sorghum grains infected with grain mould fungi. They also noted that such grains were more prone to breakage than uninfected, healthy grains. Milling yield of sorghum depends on kernel size and larger kernels produce high yields, flour with high water absorbance, brighter colour and larger particle size (Lee, Pedersen & Shelton, 2002). Kernel size can be reduced in grains infected with grain mould fungi as fungal metabolism decreases dry-matter content which results in a loss of kernel density (Cardwell *et al.*, 2000). The effect of fungi on grain metabolism will also reduce the nutrient value of the crop. Castor & Frederiksen (1980) also reported a reduction in grain

moisture content, kernel weight and size of grains from panicles infected with *Fusarium* head blight compared to healthy panicles.

Healthy seed is essential for healthy, vigorous seedlings that result in robust tillering, booting, inflorescence emergence and eventual heading (Little, 2000). In the present study, seed germination percentage decreased drastically in grains inoculated with grain mould fungi. Prom *et al.* (2003) similarly reported that seed viability and germination decreases with an increase in infection.

Grain mould of sorghum is caused by a range of fungal species belonging to various genera thus rendering it a complex disease (Williams & Rao, 1981; Bandyopadhyay *et al.*, 2000). In the present study, MMF treatment of panicles in both cultivars reduced germination more than individual fungi, with the exception of kernels inoculated with *C. lunata*. This could be due to the synergistic effect of the mould fungi resulting in a greater reduction in germination. Prom *et al.* (2003) reported a reduction of seed germination and increased level of grain mould severity in sorghum cultivars inoculated with two common grain mould fungi, *F. thapsinum* and *C. lunata*. In the present study, *C. lunata* was the greatest germination inhibitor with a 50% reduction in germination. McLaren *et al.* (2002) also reported a reduction in seed germination of up to 60% in glasshouse grown sorghum seeds artificially inoculated with grain mould fungi.

In a natural environment, grain mould fungi such as *F. thapsinum* and *Curvularia* spp. may live as saprophytes in the soil or plant debris resulting in reduced seed germination and subsequent lowering of seedling vigour (Little, 2000). These fungi could become opportunistic pathogens in an appropriate environment such as the sorghum floret and infecting sorghum heads. Sometimes seed from infected grains may

germinate, but produce blighted seedlings or exhibit poor seedling vigour (Singh & Agarwal, 1989).

The variable TGMS is a measure of grain mould response and a visual assessment of the extent of kernel discolouration (Audilakshmi *et al.*, 1999). Discolouration of sorghum grain may lead to the blackening of milled products, thus lowering sorghum grain quality (Seetharaman *et al.*, 1996). The fungi used in the present study produced different degrees of grain discolouration. Prom *et al.* (2003) reported an increase in TGMS in sorghum kernels inoculated with grain mould fungi and that cultivars responded differently. In the present study, fungal recovery was positively related to TGMS, suggesting that a greater superficial infection leads to poorer appearance. According to Williams & Rao (1981), sorghum grain quality is considered very important for human consumption. However, grain discolouration resulting from infection reduces its acceptability and value.

In the present study, grains from kernels inoculated with *F. proliferatum* and *F. thapsinum* produced 0.17 and 0.55 ppm fumonisin respectively. Rheeder, Marasas, & Vismer (2002) reported the production of fumonisin by these fungi. According to Leslie & Marasas (2002), *F. proliferatum* is one of the most prolific fumonisin producers from sorghum. The acceptance level of mycotoxins for human consumption and animal feed varies from country to country. In the USA, the tolerance level for fumonisin is 5 ppm in horse feed and 10 ppm for swine feed, while the level of acceptance in South Africa for human consumption is 0.3 ppm (Buitendag, 2002). The concentrations of fumonisin found in the present study are higher than the tolerance level for South Africa, particularly for human consumption. *Fusarium* section *Liseola* are capable of producing significant levels of fumonisin B<sub>1</sub> and their ability to be internally seedborne in

symptomless healthy grain suggests a pronounced potential for widespread contamination of human food and animal feeds (Leslie *et al.*, 1992). Grain in the current study was assayed after harvest suggesting that fumonisins can survive post-harvest and that the amount will increase if favourable conditions are created in storage. According to Bacon & Nelson (1994), fumonisins are produced in the field due to the infection of plants and the concentration of the mycotoxin may increase due to improper storage conditions. The natural occurrence of fumonisins in maize associated with human esophageal cancer in South Africa is 0 - 0.0105 mg/g for maize rated as “good” and 0.0006 - 0.0632 mg/g for maize rated as “mouldy” (Rheeder *et al.*, 1992). The fumonisin level that was found in the present study (0.0005 mg/g) confirms its potential for causing esophageal cancer if consumed by humans.

The acceptance level of deoxynivalenol (DON) is less than 2 ppm for animal feed and 1 ppm for commodities used for human consumption (Buitendag, 2002). Although the DON produced in the present study was relatively insignificant, the potential of higher levels of DON being produced if grain is stored improperly does exist. Furthermore, according to Sibanda, Marovatsanga & Pestka (1997) the effect of DON as an immunosuppressant in animals is significant and chronic even at low levels.

Sorghum grains inoculated with *F. graminearum* in the present study produced concentrations of zearalenone which could trigger mycotoxicoses in small farm animals. Although abortion in pigs could result from zearalenone in feed at a concentration of 50 ppm, levels greater than 10 ppm may reduce litter size and weight of piglets (Anonymous, 2004). Zearalenone contamination has been reported in South African sorghum malt grains, home and commercially brewed beer (Odhav & Naicker, 2002). Sibanda *et al.* (1997) also reported the presence of zearalenone in malted sorghum and

sorghum beer from Swaziland and Lesotho. This indicates the potential of the fungi to contaminate the grains and the stability of zearalenone even in processed sorghum products.

The results of the current study indicated the involvement of various fungi in the grain mould complex. Association of these fungi with losses in grain quality was evident, indicated by losses in kernel mass, grain germination and discolouration. In previous studies more emphasis was given to the importance of sorghum grain mould fungi in agriculture. In addition to its agricultural importance, the present study has highlighted the importance of grain mould fungi in the food industry as indicated by their effect on milling and malting qualities and the production of mycotoxins.

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Table 2.1 Relative frequency (%) of major mould fungi associated with sorghum grains (2002)

Location	Cultivar	Grain mould fungus					
		<i>A. alternata</i>	<i>C. lunata</i>	<i>P. sorghina</i>	<i>Fusarium</i> spp.	<i>Epicoccum</i> spp.	<i>Penicillium</i> spp.
Bethlehem	NK 283	29.5	5.0	3.0	3.0	5.5	5.5
Bethlehem	Buster	20.5	0.0	1.0	5.5	2.0	9.5
Bethlehem	PAN 8564	43	1.0	1.0	3.0	6.5	2.0
Bethlehem	SNK 3939	43.5	0.0	1.5	3.5	9.0	4.0
Bethlehem	PAN 8446	37.5	2.5	0.0	2.5	5.5	2.5
Cedara	NK 283	25.0	15.0	7.5	16.5	18.0	6.0
Cedara	Buster	43.0	9.0	10.0	4.0	19.5	2.5
Cedara	PAN 8564	38.5	20.0	5.0	7.5	12.0	3.5
Cedara	SNK 3939	48.0	16.0	4.5	6.5	13.5	5.5
Cedara	PAN 8446	36.0	7.8	5.0	9.0	24.5	5.0
Potchefstroom	NK 283	25.5	2.5	1.5	1.5	3.0	6.5
Potchefstroom	PAN 8564	50.0	5.0	3.0	0.0	3.0	4.5
Potchefstroom	SNK 3939	39.0	6.5	1.5	4.0	0.0	4.0
Potchefstroom	PAN 8446	44.0	0.0	1.0	4.5	0.0	6.5

Table 2.2 Relative frequency (%) of major mould fungi associated with sorghum grains (2003)

Fungus and locality	Cultivar					Mean
	NK 283	Buster	PAN 8564	PAN 8446	PAN 8706W	
<i>A. alternata</i>						
Bethlehem		13.0		3.0 e	9.0 e	8.3
Cedara	35.5 a	28.5	38.5 c	30.0 d	41.5 c	34.8
Delmas	60.5 b		53.5 b	46.5 c	52.5 b	42.6
Gladdedrif	32.5 c		28.5 d	30.0 d	29.0 d	53.3
Heilbron	16.5 d		10.5 e	7.5 e	11.5 e	11.5
Platrand	62.0 b		56.5 b	57.0 b	53.0 b	57.1
Potchefstroom	74.5 a		66.5 a	67.5 a	77.0 a	71.3
Potch. (Irr.)	61 b		72.5 a	73.5 a	67.0 a	68.5
<b>Mean</b>	48.9	20.75	46.25	39.38	42.56	
<i>C. lunata</i>						
Bethlehem		4.0		0.0 b	1.0 cd	1.6
Cedara	19.0 a	16.0	18.5 a	13.0 a	15.5 a	16.4
Delmas	0.0 b		4.0 c	3.5 b	3.5 bc	2.75
Gladdedrif	3.5 b		0.0 d	0.0 b	0.0 d	0.8
Heilbron	1.5 b		1.5 cd	0.0 b	0.5 cd	0.8
Platrand	3.5 b		3.0 c	3.5 b	4.5 b	3.6
Potchefstroom	2.0 b		7.0 b	0.0 b	2.0 bcd	2.7
Potch. (Irr.)	1.5 b		0.0 d	0.0 b	0.0 d	0.4
<b>Mean</b>	4.43	10	4.86	2.5	3.38	
<i>Fusarium spp.</i>						
Bethlehem		1.0		0.3 a	0.0 c	0.4
Cedara	5.3	3.3	6.0 a	2.0 ab	11.0 a	5.5
Delmas	4.0		3.7 ab	5.0 ab	4.7 b	4.3
Gladdedrif	3.7		3.7 ab	3.7 bc	5.3 b	4.0
Heilbron	5.3		1.7 b	2.3 bc	1.0 c	2.5
Platrand	5.3		5.0 a	4.7 abc	6.3 b	5.3
Potchefstroom	4.3		5.7 a	4.0 abc	4.3 b	4.5
Potch. (Irr.)	6.7		6.6 a	8.3 z	9.0 a	7.6
<b>Mean</b>	4.95	0.5	4.62	3.79	4.67	
<i>P. sorghina</i>						
Bethlehem		0.0		0.0 c	0.0 b	0.0
Cedara	1.5	1.0	2.5	1.5 c	2.0 b	1.7
Delmas	4.0		6.0	8.5 a	3.0 b	5.4
Gladdedrif	1.5		1.5	3.0 bc	3.0 b	2.2
Heilbron	0.5		0.0	0.0 c	0.5 b	0.1
Platrand	4.0		4.5	8.5 a	6.5 a	5.8
Potchefstroom	2.0		0.5	0.0 c	1.5 b	1.0
Potch. (Irr.)	4.5		3.5	7.0 ab	8.5 a	5.8
<b>Mean</b>	2.57	0.5	2.31	3.56	3.06	

Means of cultivars in a column followed by the same letter do not differ significantly ( $P \leq 0.05$ ) according to Tukey-Kramer multiple comparison test

Table 2.3 Milling and malting quality of sorghum grains (2003)

Location	Cultivar	Milling quality		Malting quality				
		Abrasive hardness index	Dehulling index	Germination vigour (%)	Germination energy (%)	Diastatic power (SDU/g malt)	Water uptake (%)	Malt loss (%)
Bethlehem	PAN 8446	6.10	0.90	70.30	92.7	43.2	40.2	12.6
Cedara	NK 283	6.06	1.60	73.23	79	26.53	43.87	13.67
Cedara	BUSTER	7.25	1.12	75.85	85.05	25.13	40.67	13.97
Cedara	PAN 8564	8.16	1.14	70.92	78.88	20.8	37.56	13.5
Cedara	SNK 3939	7.44	1.20	73.52	83.46	22.54	39.6	12.75
Cedara	PAN 8446	6.38	1.80	77.98	90.35	33.1	40.18	13
Delmas	NK 283	5.29	2.70	-	-	29.82	-	-
Delmas	PAN 8564	4.92	9.08	-	-	33.85	-	-
Delmas	PAN 8706W	5.59	0.07	-	-	-	-	-
Delmas	PAN 8446	4.99	14.33	-	-	29.34	-	-
Heilbron	NK 283	-*	-	43.67	68	26.34	36.73	17.27
Heilbron	PAN 8564	-	-	63.00	79.67	39.98	35.25	16.35
Heilbron	PAN 8706W	-	-	39.33	68.33	31.29	37.52	17.09
Heilbron	PAN 8446	-	-	57.33	75.67	38.57	37.81	16.72
Gladdedrif	NK 283	-	-	49.00	68.67	24.34	39.51	13.65
Gladdedrif	PAN 8564	-	-	67.00	82.33	38.19	35.43	13.6
Gladdedrif	PAN 8706W	-	-	57.00	76.33	24.59	37.69	14.28
Gladdedrif	PAN 8446	-	-	59.67	79.33	34.69	34.82	10.32
Potchefstroom	PAN 8564	5.50	8.75	-	-	-	-	-
Potchefstroom	SNK 3939	5.40	1.30	-	-	-	-	-
Potch.(Irr.)**	NK 283	-	-	47.33	54.67	22.3	45.86	8.8
Potch.(Irr.)	PAN 8564	-	-	71.67	84	30.31	38.56	10.21
Potch.(Irr.)	PAN 8706W	-	-	59.67	71	17.15	46.42	14.76
Potch.(Irr.)	PAN 8446	-	-	35.00	52	24.55	36.94	9.26
Platrand	NK 283	5.61	1.88	88.33	94.33	35.01	41.78	4.64
Platrand	PAN 8564	6.82	7.94	89.33	97.33	34.12	36.12	13.16
Platrand	PAN 8706W	6.41	0.08	81.00	93	31.85	39.33	16.58
Platrand	PAN 8446	5.37	13.11	74.67	90.67	31.02	36.85	14.49

-\* Data not available

Potch.(Irr.)\*\*=Potcheftsroom irrigated plots

Table 2.4 Correlation coefficients between the incidence of grain mould fungi and grain quality parameters (2003)

Fungal genera	Germination vigour	Germination energy	Diastatic power	Malt loss	Water uptake	Abrasive hardness	Dehulling Index
<i>A. alternata</i>	0.23	0.02	-0.37	-0.63*	0.39	-0.56*	0.32
<i>C. lunata</i>	0.39	0.21	-0.49*	-0.05	0.28	0.69*	-0.47
<i>Fusarium spp.</i>	0.01	-0.19	-0.63*	-0.28	0.48*	0.39	-0.34
<i>P. sorghina</i>	-0.01	-0.05	-0.31	-0.09	0.17	-0.61*	0.64*

\*Significantly ( $p \leq 0.05$ ) correlated

Table 2.5 Mean fungal re-isolation frequency recovered from sorghum grains inoculated in the glasshouse

Treatment	Cultivar	
	NK 283	PAN 8706W
Control	8.25 f	14.75 d
<i>Alternaria alternata</i>	53.00 e	42.75 c
<i>Curvularia lunata</i>	97.25 a	98.00 a
<i>F. graminearum</i>	72.67 d	67.75 b
<i>F. proliferatum</i>	93.25 b	95.50 a
<i>F. thapsinum</i>	92.00 b	93.25 a
<i>Phoma sorghina</i>	81.25 c	71.50 b
Mixed mould fungi	97.75 a	93.75 a

Means of cultivars in a column followed by the same letter do not differ significantly ( $P \leq 0.05$ ) according to Tukey-Kramer multiple comparison test

Table 2.6 Effect of glasshouse inoculated grain mould fungi on grain quality

Treatment	1000 kernel mass		Germination (%)		TGMS* (%)	
	NK 283	PAN 8706W	NK 283	PAN 8706 W	NK 283	PAN 8706W
Control	24.85 g	24.36 e	95.25 a	90.25 a	1.25 c	1.00 d
<i>A. alternata</i>	18.33 b	20.15 c	90.75 b	85.00 b	2.75 b	2.25 c
<i>C. lunata</i>	24.73 g	23.92 de	49.5 e	45.00 g	3.25 ab	3.75 a
<i>F. graminearum</i>	19.45 c	20.34 c	90.75 b	72.75 e	2.75 b	3.00 b
<i>F. proliferatum</i>	17.03 a	15.36 a	87.25 c	77.75 d	3.75 ab	4.00 a
<i>F. thapsinum</i>	23.89 f	23.48 d	86.00 c	79.25 cd	4.25 a	3.75 a
<i>P. sorghina</i>	22.23 d	19.09 b	84.25 c	80.75 c	3.25 ab	3.00 b
Mixed mould	23.00 e	24.03 e	68.75 d	63.00 f	4.25 a	3.75 a

\*TGMS = threshed grain mould score

Means of cultivars in a column followed by the same letter do not differ significantly ( $P \leq 0.05$ ) according to Tukey-Kramer multiple comparison test

Table 2.7 Correlation coefficient between grain quality parameters of glasshouse inoculated sorghum grains and grain mould fungi

	Fungal recovery	Kernel mass	Germination
Fungal recovery	1.00		
Kernel mass	0.06	1.00	
Germination	-0.69	-0.43	1.00
TGMS*	0.89**	-0.11	-0.41

\*Threshed grain mould score

\*\*Highly significant ( $P \leq 0.05$ )

Table 2.8 Mycotoxins isolated from sorghum grains inoculated with grain mould in the glasshouse

Isolate	Cultivar	<i>Fusarium</i> toxins (ppm)		
		Deoxynivalenol	Fumonisin	Zearalenone
<i>F. graminearum</i>	NK 283	0.27	-	-
	PAN 8706W	0.25	-	13.95
<i>F. proliferatum</i>	NK 283	-	0	-
	PAN 8706W	-	0.17	-
<i>F. thapsinum</i>	NK 283	-	0.55	-
	PAN 8706W	-	-	-
Mixed mould	NK 283	0.25	-	25.84
	PAN 8706W	0.09	0.05	-

## **CHAPTER 3**

**The role of weather on infection of sorghum by grain mould fungi and  
the effect on grain quality**

## Abstract

Five sorghum (*Sorghum bicolor*) cultivars were planted in three localities in South Africa on different planting dates and evaluated to determine the role of weather on the occurrence of grain mould. Fungal frequency varied across localities and flowering dates. In all cultivars, higher grain mould incidence was recorded from Potchefstroom. *Alternaria alternata* was the most dominant fungus at all localities and at all flowering dates. An increase in moisture and temperature was positively ( $P \leq 0.05$ ) correlated with fungal invasion. Generally, results indicated significant ( $P \leq 0.05$ ) correlations between grain mould incidence and certain grain quality parameters, such as the milling and malting quality. There was a negative correlation between germination energy (%) and incidence of specific fungi namely, *Fusarium* spp. ( $r = -0.85$ ), *Curvularia lunata* ( $r = -0.70$ ), *Phoma sorghina* ( $r = -0.68$ ) and *A. alternata* ( $r = -0.21$ ). Similar results were observed in correlations between grain mould and threshed grain mould score, kernel mass and germination. The effect of wetness duration on grain mould development under a controlled environment was determined. Panicles of a glasshouse grown sorghum cultivar (PAN 8706W) were spray inoculated with spores of *A. alternata*, *C. lunata*, *F. graminearum*, *F. proliferatum*, *F. thapsinum* and *P. sorghina* at soft dough stage and incubated in a dew chamber for 0, 24, 48, 72 and 96 hours. Plants were then kept for two weeks in the glasshouse to allow grain mould infection to develop and assessed for infection by plating grains on agar culture medium. Infection frequency varied significantly ( $P \leq 0.05$ ) between wetness durations. Increasing wetness duration resulted in an increase in grain infection by the respective grain mould fungi.

## **Introduction**

Sorghum is grown widely in the semi-arid tropics under hot, dry conditions and is a source of food, feed and forage (Reddy, Sharma & Stenhouse, 1995). Production can be limited by several abiotic and biotic factors and grain mould is one of the most important biotic constraints to sorghum production and improvement in many parts of the world (Menkir *et al.*, 1996). It is a condition resulting from infection of the developing floret by one or more parasitic fungal species (Esele, 1995). Grain mould involves a complex of three phenomena including (i) field infection of developing grains by parasitic and saprophytic fungi, (ii) grain discolouration and weathering and (iii) loss of grain quality (Murty, 2000).

In most sorghum growing countries the crop is grown under rain-fed conditions and many abiotic factors are conducive to the development of grain mould (Jambunathan, Kherdekar & Stenhouse, 1992). Thus, in addition to the relatively large array of fungal species that cause grain mould, the environment significantly influences the onset and severity of infection. High relative humidity during critical periods (early plant growth as well as between the end of flowering and harvesting periods) favours infection by enhancing sporulation or providing suitable conditions for infection (Ratnadass *et al.*, 2003). Time of harvesting and weather conditions during harvesting would also influence the incidence and severity of sorghum grain mould. Bandyopadhyay *et al.* (2002) reported that grain mould incidence and severity increases after physiological maturity when it coincides with delayed harvesting and/or when wet conditions persist.

Damage related to grain includes storage quality, food and feed processing quality as well as market value (Hall *et al.*, 2000). Fungal growth causes severe discolouration of the grain surface and breakdown of numerous grain components resulting in decreased

milling and processing yields, and quality for feed or food (Waniska *et al.*, 2002). Other detrimental effects on yield and quality caused by grain mould in sorghum include complete destruction of the grain, reduced seed viability, reduction in grain size and mass, and mycotoxin contamination (Williams & McDonald, 1983). Infection of cereal grains by fungi also influences the quality of end products such as beverages (Flannigan *et al.*, 1982, cited by Seetharaman, Waniska & Rooney, 1996).

The objectives of the present study were: (i) To determine the incidence of fungi in sorghum grains from plots planted on different dates and in different locations, (ii) to determine the effect of wetness duration on infection and, (iii) to evaluate the effect of fungi on sorghum grain quality.

## **Materials and Methods**

### ***Field trials***

Sorghum hybrids, NK 283, Buster, PAN 8564, SNK 3939 and PAN 8446 were planted at sites near Bethlehem (28°15'S 28°20'E), Cedara (29°32'S 30°17'E) and Potchefstroom (26°44'S 27°04'E), in South Africa. Locations were selected to represent the wide range of climatic conditions in which sorghum is cultivated in South Africa. Weather data were collected from each experimental site from class A weather stations within a 200 meter range. Plots consisted of 10 rows, 10 m in length with a 10 cm intra-row spacing and 0.85 and 1.2 inter-row spacing. Plots were replicated three times. Planting of replicates was split over three planting dates from mid-November to late December at Cedara and Bethlehem and Potchefstroom respectively to expose developing grains to varying weather conditions. Standard agronomic practices were applied as required in each area throughout the growing season. At flowering, ten sorghum heads were marked at weekly

intervals with the dates of anthesis. At maturity heads from each flowering date were pooled and threshed.

#### *Isolation of grain mould fungi*

The incidence of fungi was determined on 300 grains collected from each flowering date per hybrid. Grains were surface sterilized in 1% sodium hypochlorite for 3 min and rinsed in three changes of sterile distilled water, dried on sterile blotting paper and plated onto half-strength (1%) malt extract agar (MEA) (Biolab<sup>®</sup>) amended with streptomycin sulphate (0.3 ml/l). Petri dishes were incubated at 25°C and each grain was examined for grain mould fungi 7-14 days after incubation.

#### *100 kernel mass*

The effect of infection on grain density was determined by weighing 100 kernels from each hybrid/flowering date/locality combination on an electronic sensitive balance (Scout II) Ohatsu<sup>®</sup> Corporation, Pine Brook, NJ.).

#### *Threshed grain mould score (TGMS)*

A sample of 30 g of threshed grain from each flowering period was spread onto a 90-mm-diameter Petri dishes and scored visually using a stereo microscope (x30 magnification) for varying degrees discolouration (Audilakshmi *et al.*, 1999). Mould severity was assessed as a visual estimate of the percentage of grain surface discoloured. Grain samples were scored using a 1-5 scale, where 1 = no mould; 2 = slight superficial mould growth up to 10% of the grain surface covered by mould; 3 = moderate mould growth and 11-25% of the grain surface moulded; 4 = considerable mould growth with 26-50%

of the grain surface moulded and 5 = extensive mould growth with more than 50% of the grain surface covered by mould (Audilakshmi *et al.*, 1999).

#### *Effect of grain mould fungi on milling and malting qualities*

The effect of grain mould fungi on malting and milling qualities of sorghum that flowered at different times was determined using sorghum grains from five cultivars harvested from the three locations mentioned above.

#### *Germinative vigour (GV) and germinative energy (GE)*

Germination of grain samples was performed in triplicate according to the technique described by Dewar, Taylor & Joustra (1995). One hundred sorghum kernels were evenly spread on moistened filter paper in a Petri dish. Petri dishes were placed for 72 h in a germinating cabinet (Specht Scientific SG-330), at a temperature of 25°C and relative humidity of 95%. The number of germinated kernels was counted after 24, 48 and 72 h of germination. After 48 h, GV% was assessed and GE% after 72h. At 72 h of germination, both germinated and chatted kernels were counted. The latter are kernels that had started to germinate, but with only the tip of the root visible through the pericarp of the grain.

#### *Water absorption and malting loss*

Malting of the samples was done according to the technique described by Dewar *et al* (1995). The technique was modified by washing samples before steeping in a solution of 25 ml Biocide IO<sub>4</sub> (IO Dophor based reagent) (SABS 1081) (Lever Industrial) in 16 l of water and after steeping, in a solution of 0.16% NaOCl m/v (bleach) solution. Samples (50 g) of sorghum were weighed in nylon bags, then washed in the Biocide IO<sub>4</sub> solution,

by means of a 5 min scrubbing-10 min soaking process, followed by rinsing in sterile water three times. After rinsing, samples were spun for 3 min in a Miele WZ 259 Spinner. Steeping of the samples was done in a Labotec Model 101 Steeper at 25°C for 24 h. The steeping cycle of 24 h consisted of 3 h intervals of soaking and a 1 h period during which water flowed from the steeper, to allow aeration of the grain. The samples were washed but not scrubbed in the bleach solution after steeping, followed by soaking, rinsing and spinning. Water absorption was determined after the steeping period, by weighing the grain following washing and calculated as follows:

$$\text{WA (\%)} = \frac{\text{soaked mass of grain (g)} - \text{original mass (g)}}{\text{original mass (g)}} \times 100$$

Germination was done in a Specht Scientific SG-330 germination cabinet for 5 days, during which the samples were soaked in water (10 min) and spun (3 min) twice daily. The germinated grain was dried in a Memmert UL 80 drying oven at 50°C for 24 h. Samples were weighed after the drying process and the malting losses (ML) were determined as follows:

$$\text{ML (\%)} = \frac{\text{original mass of grain (g)} - \text{dried mass (g)}}{\text{original mass (g)}} \times 100$$

After malting, the samples were milled in a Janke and Kunkel mill to determine the diastatic power.

#### *Diastatic power (DP)*

The moisture content of malt was determined in a Memmert UL 80 drying oven set at 105°C for 3 h according to the method described by Dewar *et al* (1995). The DP assay was performed on the samples according to the micro-method described by Gomez *et al*

(1997) with peptone as extractant. A malt sample (prepared earlier) of 0.5 g was mixed with a peptone extract, by adding 10 ml of 2% peptone (bacteriological, Biolab®) solution and heating it for 150 min at 30°C, whilst covering the solution with Parafilm® and shaking it every 20 min. The samples were centrifuged at 3000 rpm for 2 min in a Heraeus Christ Labouge centrifuge. Supernatant (0.2 ml) from each sample was added to a buffered starch solution at 30 seconds intervals in a water-bath at 30°C and after 30 min the reaction in the water-bath was stopped by the addition of 4 ml 0.5 N sodium hydroxide (0.5 N ampule) (Merck reagent no. AC001252.500). The starch solution was prepared by dissolving 20 g starch (guaranteed reagent, Merck) in water over heat and adding 20 ml buffer before filling the solution up to the mark of a 1000 ml volumetric flask. The solution was maintained at 30°C and the starch buffer was prepared by diluting 68 g sodium acetate (guaranteed reagent, Merck) and 1 N acetic acid (guaranteed reagent, Merck) to 1 l with distilled water. In the case of the blank, sodium hydroxide was added to the buffered starch solution before the addition of the malt extract. One blank was prepared for each sample. A volume of 4 ml 0.05 N alkaline ferricyanide (guaranteed reagent, Merck) was pipetted into an Erlenmeyer flask and 2 ml of the digest starch solution was added. The flasks were kept in a bath of boiling water for 20 min. After cooling the solution, 10 ml 1 N acetic acid salt, prepared from glacial acetic acid (guaranteed reagent, Merck), and 0.4 ml potassium iodide (guaranteed reagent, Merck) were added before it was titrated against 0.05 N sodium thiosulphate (0.1 N ampule, Merck). The potassium iodide indicator was prepared by the addition of two drops of concentrated sodium hydroxide solution to 50 g of potassium iodide dissolved in 60 ml distilled water and diluting the solution to 100 ml. The DP was determined as follows:

$$DP = \frac{TB-TA}{100 - M} \times \frac{VE \times VD \times 2000 \times f}{W \times AE \times AD}$$

Where TA = titre of thiosulphate used for the samples;

TB = titre of thiosulphate used for the blank;

AD = aliquot of digest for sugar determination, i.e. 2.0;

AE = aliquot of extract for sugar determination, i.e. 0.5;

f = normality of thiosulphate, i.e. 0.05;

MC = % moisture content of malt;

VD = volume of digest for sugar determination, i.e. 20.5;

VE = volume of digest for sugar determination, i.e. 0.5, and

W = weight of malt extract

(Gomez *et al.*, 1997).

The DP was corrected to consider the moisture content of the malt and the titration volume of the blank was taken into consideration. The DP of the sample was measured in sorghum diastatic power (SDU)/g malt.

#### *Mycotoxin analysis*

Sorghum grain samples were ground in a laboratory blender so that 70% of the material would pass through a 200 mm mesh sieve.

#### *Zearalenone test*

A 5 g ground grain sample was added to 25 ml of 70% methanol/water and agitated vigorously for 3 min. The extract was filtered through a Whatman #1 filter and the filtrate was collected and analyzed using a Veratox® zearalenone quantitative test kit (Neogen® Corporation, 620 Lasher Place, Lansing, MI 48912, USA).

### *Deoxyievalenol (DON)*

A 10 g ground grain sample was blended with 50 ml of distilled water for 3 min in a high speed blender. The extract was filtered through a Whatman #1 filter and 5 ml of the filtrate was collected and analyzed using a Veratox® DON quantitative test kit (Neogen® Corporation, 620 Lasher Place, Lansing, MI 48912, USA).

### *Fumonisin test*

A 25 g ground grain sample was blended with 125 ml of 70% methanol/water solution for 2 min in a high speed blender. The extract was filtered through a Whatman #1. The filtrate was diluted by adding 100 µl of extract into a prefilled sample dilution bottle from the analytical kit. The sample was analyzed using a Veratox® fumonisin quantitative test kit (Neogen® Corporation, 620 Lasher Place, Lansing, MI 48912, USA).

### *Aflatoxin analysis*

A 25 g ground grain sample was blended with 125 ml of 70% methanol/water solution for 2 min. The extract was filtered through a Whatman #1. The filtrate (5 ml) was diluted by adding 100 µl of extract into a prefilled sample dilution bottle from the analytical kit. The sample was analyzed using a Veratox® aflatoxin quantitative test kit (Neogen® Corporation, 620 Lasher Place, Lansing, MI 48912, USA).

## ***Controlled environment trials***

### *Plants and fungal cultures*

An experiment was conducted to determine the effect of wetness duration on infection by grain mould fungi namely, *Alternaria alternata* (Fr.) Keissler, *Curvularia lunata* (Wakk.)

Boedijn, *Fusarium graminearum* (Schw.), *F. proliferatum* (Matsushima) Nirenberg, *F. thapsinum* (Klittich, Leslie, Nelson and Marasas) and *Phoma sorghina* (Sacc.) Sorghum panicles of cultivar PAN 8706W were inoculated at soft dough stage (Tarekegn *et al.*, 2004). A 10-day-old culture of each fungus growing on 1% malt extract agar (MEA) (Biolab®) was flooded with sterile water and agitated with a glass rod. The suspension was filtered through three layers of cheesecloth to remove mycelial fragments and the conidial concentration was determined with a haemocytometer and diluted to 10<sup>5</sup> spores/ml. A spore suspension of each isolate was sprayed onto panicles using a hand-operated sprayer and plants were immediately placed in a dew chamber for 24, 48, 72 and 96 h. Plants not requiring any wetness treatment were immediately moved to the glasshouse and maintained at 28/19°C day/night regime. Eight plants per time period were inoculated with the grain mould fungi. Half of the plants were kept for two weeks post-inoculation in the glasshouse after which grains were collected and assessed for infection and the other half were maintained in the glasshouse for grain quality assessment. Grains (400 grains/ fungal isolate/ per wetness-duration treatment) were surface sterilized using 1% NaOCl for 3 minutes rinsed in three series of sterile distilled water, dried on a sterile filter paper and plated onto 1% MEA amended with streptomycin (100 µl/l). *Fusarium* selective medium (Van Wyk, Scholtz & Los, 1986) was used for the isolation of *Fusarium* spp. Petri dishes were kept in a sporulation room at room temperature for seven to 10 days and fungal colonies were identified using light microscopy.

### *Threshed grain mould score (TGMS)*

Grain quality was assessed on plants maintained in the glasshouse until maturity and hand threshed. A 30 g sample of threshed grain from each treatment was spread onto a 90-mm-diameter Petri dish and scored visually using a stereo microscope (x30 magnification) for varying degrees of discolouration as outlined above (Audilakshmi *et al.*, 1999).

### *Germination test*

One hundred grain samples per replicate (10 grains per plate) obtained from each treatment were placed on moistened sterile germination paper in plates and incubated at room temperature (20-23°C) in the dark. Grains were regularly monitored and recorded as germinated when roots and shoots had emerged.

### ***Statistical analysis***

Data from each experiment were subjected to an analysis of variance (ANOVA), and Tukey-Kramer multiple comparison test was used to separate treatment means. All data analyses were performed using the statistical program NCSS 2000 (BMDP Statistical Software Inc., Los Angeles, CA). Correlation between data sets was performed using the statistical package Minitab (Minitab Inc. 1998).

## **Results**

### ***Field trials***

#### *Isolation of fungi*

The frequency of fungal species isolated varied across locations and flowering dates (Table 3.1). In all localities and at different flowering stages, *A. alternata* was the most

predominant fungus. The frequency of *A. alternata* in most cases was low on the earliest flowering dates but increased in sorghum that flowered 2-3 weeks later. Conversely, the incidence of most *Fusarium* spp. was high at the first flowering date but decreased 2-3 weeks later. A similar trend was observed in *C. lunata*.

A higher percentage of fungal species was recorded in kernels from Potchefstroom compared with Cedara and Bethlehem. In most cases, a positive ( $P \leq 0.05$ ) correlation was observed between weather variables and fungal frequency (Table 3.2). Maximum temperature and rainfall were positively correlated ( $P \leq 0.05$ ) with *A. alternata* and *C. lunata* isolation frequency, while relative humidity was closely correlated with the incidence of *Fusarium* spp. and *P. sorghina*.

#### *100 kernel mass*

Kernel mass within cultivar differed with locality and flowering date (Table 3.3). Most cultivars at Cedara showed a higher 100 kernel mass (maximum 2.55g) with earlier flowering (February and March). Plants that flowered during April had a relatively low 100 kernel mass, the highest being 1.61g. At Bethlehem, the 100 kernel mass was higher with flowering during February compared with April. In contrast, most cultivars at Potchefstroom exhibited an increased kernel mass with later flowering. A lower kernel mass was recorded during February but increased in plants that flowered during March with the exception of cultivar Buster that had a higher kernel mass during January. In most cultivars, *Fusarium* spp. had a negative effect on kernel mass and significant ( $P \leq 0.05$ ) negative correlations was observed between *Fusarium* spp. and 100 kernel mass in cultivar NK 283.

### *Threshed grain mould score (TGMS)*

TGMS varied between cultivars, flowering dates and localities (Table 3.4). At Cedara, a higher TGMS (up to 4.67) was recorded during early flowering (February) compared with early-March. TGMS started to increase again reaching 4.67 during the end of March and beginning of April. The same trend was evident at Bethlehem. A higher TGMS was recorded during early-February but declined towards mid-February and started to increase towards the beginning of March. All cultivars from Potchefstroom exhibited higher TGMS values than the same cultivars did in Cedara and Bethlehem. However, unlike Cedara and Bethlehem, the TGMS from Potchefstroom was higher during January and February and started to decline during March.

Grain mould fungi affected TGMS at different levels. In most cultivars *P. sorghina* isolation frequency was positively ( $P \leq 0.05$ ) correlated ( $r = 0.78$  in NK 283) with grain mould incidence followed closely by *C. lunata* (Table 3.6). Although not significant and sometimes slightly negatively correlated with mould fungal incidence, *A. alternata* and *Fusarium* spp. also had an effect on the discolouration of the grains.

### *Effect of grain mould fungi on milling and malting qualities*

Milling and malting quality traits are listed in Table 3.5. In most cases there was a negative correlation between infection by grain mould fungi and grain quality parameters. Milling quality, indicated by abrasive hardness and dehulling index of grains from Cedara and Potchefstroom, was negatively affected by fungal infection. In most cultivars, *C. lunata* and *Fusarium* spp. was negatively correlated with milling quality (Table 3.6). Although the correlation between mould fungi and milling quality was different for each cultivar tested, there was a strong negative correlation between abrasive

hardness index and *C. lunata* and *Fusarium* spp. Malting quality was also affected by fungal infection as indicated by a negative correlation (Table 3.6) between grain mould fungal incidence and germination.

Cultivars differed in their response to fungal infection. For example in NK 283, *Fusarium* spp. were more negatively (-0.85) correlated than *C. lunata* (-0.70) with germination energy while in Buster, *C. lunata* was more negatively (-0.73) correlated than *Fusarium* spp. (-0.69) with germination energy. The correlation between grain mould fungi with diastatic power, malt loss and water uptake differed among cultivars. In NK 283, the correlation between DP and individual fungi was: *A. alternata* (-0.76), *C. lunata* (-0.44), *Fusarium* spp. (-0.23) and *P. sorghina* (0.37). In Buster, DP and individual fungi were negatively correlated, *A. alternata* (-0.24), *C. lunata* (-0.57), *Fusarium* spp. (-0.07) and *P. sorghina* (-0.24). The same trend was exhibited when grain mould fungi were correlated with malt loss and water uptake (Table 3.6).

#### *Mycotoxin analysis*

Variation in mycotoxin production was observed across cultivars as well as between localities (Table 3.7). No fumonisin was found in all the cultivars sampled from Cedara but was detected from those planted in Bethlehem and Potchefstroom. Cultivars PAN 8564, SNK 3939, and PAN 8446 from Bethlehem as well as cultivars Buster, PAN 8564, SNK 3939 and PAN 8446 from Potchefstroom yielded fumonisins. In most cases, the production of fumonisin was observed at later flowering stages. Aflatoxin was found in grains from all locations. Higher concentrations were associated with earlier flowering, decreased towards the middle periods and increased again at later flowering stages. The level of zearalenone was higher in cultivars from Potchefstroom and Cedara than the

same cultivars from Bethlehem. The concentrations also differed between flowering dates and levels increased towards end of flowering stages.

### ***Controlled environment trials***

#### *Fungal recovery*

Significant ( $P \leq 0.05$ ) differences were observed between fungal re-isolation frequency and wetness periods of inoculated sorghum heads (Fig. 3.1). *A. alternata* required a long wetness period (96 h) whereas *C. lunata* and *P. sorghina* required 48 h for maximum infection. The mean infection frequency of panicles inoculated with *A. alternata* was 11% when panicles were not exposed to wetness compared to 65.25% with 96 h wetness duration. Panicles inoculated with *C. lunata* at 0 h wetness exposure showed 20.73% infection but increased after a wetness duration of 48 h reaching 71%. Although infection by *Fusarium* spp. took place even at 0 h wetness duration, infection also increased as the period of wetness increased. All *Fusarium* spp. resulted in an infection frequency of more than 50% in panicles that were not exposed to wetness. However, infection increased significantly ( $P \leq 0.05$ ) as wetness duration was extended beyond 48 h. *F. proliferatum*, *F. thapsinum* and *F. graminearum* had infection frequencies of 80.25, 87 and 79% respectively at a wetness duration of 72 h. *P. sorghina* also required longer period of wetness for infection. Although infection took place at 0 h wetness exposure (32.75%), it increased as the panicles were exposed for more than 48 h (96.5%).

#### *Threshed grain mould score (TGMS)*

The superficial discolouration of grains by mould fungi showed a significant ( $P \leq 0.05$ ) increase in the TGMS as the humidity increased (Table 3.8). *F. proliferatum* had the

highest TGMS at 96 h of wetness duration although a significant increase was evident after 72 h of wetness. An increase of TGMS with *F. graminearum* and *F. thapsinum* was evident at 48 h wetness. *A. alternata* had the lowest TGMS values followed by *P. sorghina*.

#### *Germination test*

In all cases, with the exception of *A. alternata* and *F. graminearum*, an extended period of wetness had a significant ( $P \leq 0.05$ ) influence on grain germination (Table 3.8). *C. lunata* had the greatest effect after 48 h of wetness. *F. proliferatum* and *F. thapsinum* required more than 48 h of wetness to influence grain germination significantly while *P. sorghina* decreased germination after only 24 h of wetness.

### **Discussion**

The present study indicated that the incidence of grain mould fungi varied within and between sorghum cultivars grown at different localities and having different flowering dates. This indicates an important role of environmental factors in the incidence of grain mould fungi and the development of the disease. More fungi were isolated from plants grown in Potchefstroom as plants were exposed to more moisture and relatively higher humidity during the critical growth stage. Thakur *et al.* (2003) also reported variation in grain mould infection within cultivars that were planted at different localities indicating the major role played by weather conditions.

Generally grain mould of sorghum is a major problem in areas where grain development i.e., flowering and maturity, coincide with warm weather and high humidity (Rodríguez-Herrera *et al.*, 2000; Singh & Bandyopadhyay, 2000 and Rooney *et al.*,

2002). In this study, the incidence of grain mould fungi generally decreased with later flowering dates, i.e., highest fungal frequencies were recorded during initial flowering stages and less at later flowering stages. Initially, high relative humidity and temperatures favoured fungal invasion at Cedara and Potchefstroom. Although rainfall recorded at Bethlehem was sufficient for the growth of the crop, reduction in temperatures after flowering and during the dough stage may have played a role in a relatively low grain mould fungal incidence.

According to Das *et al.* (2004), the variation in the severity and incidence of grain mould is determined mainly by relative humidity and maximum air temperature. In the present study there was a significant positive correlation between fungal incidence and weather variables, particularly relative humidity and temperature. Ratnadass *et al.* (2003) reported a high incidence of grain mould during high relative humidity at the end of flowering and during harvest in west and central African countries. An increase in grain mould usually occurs when relative humidity exceeds a threshold of about 95%.

Grain mould fungi reduce kernel mass as indicated by negative correlations between the incidence of grain mould fungi and 100 kernel mass in the present study. Symptoms associated with reduced kernel mass include grains having a powdery appearance, smaller grains that fail to mature and shrunken grains. This resulted in reduced kernel mass and consequently may also result in total yield loss. The powdery appearance of grains is also related to the brittleness of the grains. Such kernels may become soft and are easily disintegrated during the milling process. The softness of kernels resulting from grain mould infection may also contribute to deterioration of the grain during storage, which would consequently contribute to reduction of yield and quality. Although less grain mould was recorded during April in Bethlehem and Cedara,

this weight loss of the kernels could possibly be due to less rainfall at this critical growth stage. Reduction in biomass indicated by smaller panicles with fewer grains was previously reported on sorghum due to terminal drought (Montes-Belmont *et al.*, 2003).

The significant positive correlation between certain grain mould fungi and threshed grain mould score was an indication of their involvement in grain discolouration. Symptoms included black fluffy growth on the grain surface, a white to pink appearance and visible gray-black mycelial growth on the grain surface. Grain discolouration associated with mould results in reduced quality and the avoidance of discoloured grains by customers would lead to a loss in market value, which will result in loss of income to subsistence or small holding farmers.

Milling and malting of sorghum are two of the most important qualities in the food industry. Milling quality properties include traits such as sorghum hardness and colour of sorghum meal. In the present study it was found that the infection of sorghum grains by mould fungi resulted in lighter kernel mass and discoloured grains. Discolouration that affects the endosperm may result in dark sorghum meal while the lighter kernels will disintegrate easily during milling, which will negatively affect the quality of the grain. According to Singh & Bandyopdhyay (2000), the deterioration of grain quality related to grain mould infection can reduce yield significantly ranging from 30 to 100% depending on genotype and flowering time. The possible involvement of grain mould fungi in the grain hardness and their potential in deteriorating the quality of sorghum grains by making the grains soft and to easily disintegrate during milling must be considered despite a negative correlation

The diastatic power value was low in grains from early flowering plots while higher diastatic power values were associated with later flowering. In most cultivars and

localities, the highest fungal frequencies were observed in plants that flowered early. This may suggest that lower diastatic power values are related to fungal invasion. Infection by grain mould fungi may have affected alpha and beta-amylase activities, important enzymes in the malting process as indicated by the negative correlation between grain mould fungi and diastatic power unit. Abiodun (2002) similarly reported low diastatic activity in sorghum cultivars which were infected and weathered. In the present study, a similar trend was recorded through all flowering periods for water uptake and malt loss. There was also a negative correlation between grain mould fungi and malting loss and water uptake. Germination vigour and germination energy, which are important traits to determine malting quality of grains were affected negatively by grain mould infection. Among the mould fungi isolated in this study, *C. lunata* and *Fusarium* spp. had the most significant effect on decreased germination. Prom *et al.* (2003) reported a reduction of seed germination rate and increased level of grain mould severity in sorghum cultivars inoculated with two common grain mould fungi, *F. thapsinum* and *C. lunata*. Gaikwad *et al.* (2003) also found the highest seed germination in sorghum cultivars least infected by *F. moniliforme* (*sensu lato*) and *C. lunata*. They also noted increased grain mould infection and TGMS and decreased seed germination with delay in harvesting time.

The effect of weather was not only limited to influencing the incidence of grain mould fungi, but also to the production of mycotoxins. Fumonisin production was recorded in sorghum cultivars from Potchefstroom. The same cultivars from Cedara did not produce fumonisins although similar incidences of *Fusarium* spp. were recorded. Potchefstroom experienced continuous rain accompanied by high temperatures during flowering. Bhat *et al.* (2000) reported that sorghum harvested during unseasonal rain and

left heaped in the field became visibly mouldy and were contaminated with fumonisin B<sub>1</sub> due to high moisture levels inside the heap. They also noted that the incidence of fumonisin mycotoxins in sorghum samples was higher in rain-affected grains compared to normal samples. High relative humidity during anthesis in other cereals such as wheat has also been reported to be conducive to the development of grain mould fungi and the consequent increase in mycotoxin production (Turner, Jennings & Nicholson, 1999).

The zearalenone levels recorded in some samples were greater than set tolerances. The continuous exposure of sorghum heads to rain could also have played a role in the production of higher concentrations of zearalenone. Variation in the incidence of zearalenone and other mycotoxins between localities and flowering dates indicates that optimum conditions for mycotoxin production varies among commodities, weather conditions and regions. The acceptable concentration level of zearalenone is different for each country, commodity and consumer. For instance, the mycotoxin regulations in European Union states such as France set, the maximum permitted level of zearalenone in cereals and cereal products at 50 µg/kg (FAO, 2003). The maximum acceptable levels of zearalenone in South African animal feeds are 0.2 and 0.25 ppm for swine and cattle respectively (AFMA, 2001). Odhav & Naicker (2002) reported the contamination of South African sorghum malt grains by mould fungi where half of the samples tested exhibited positive results for the presence of zearalenone. Although no grain moulds have been detected in beer, as they would be destroyed by cooking during the brewing process, Odhav & Naicker (2002) noted the presence of toxins in commercial and home brewed South African beers. The presence of zearalenone in mouldy maize, maize porridge, malted sorghum and sorghum beer from Swaziland and Lesotho has also been reported (Sibanda, Marovatsanga & Pestka, 1997). This indicates that zearalenone is a

stable compound, both during storage, milling and processing or cooking of food, and does not degrade at high temperatures. The high concentration levels of zearalenone found in the present study should raise serious concerns. It may result in a significant economic impact on animal production and humans exposed to contaminated sorghum products. Thus, the cumulative effect of zearalenone should not be ignored and mycotoxins in sorghum grains warrant further investigation.

Aflatoxin was also found in grains from all localities, cultivars and different flowering dates. Concentrations increased with later flowering dates. According to Hamblin & White (1999) the greatest potential for disease development in maize kernels caused by *A. flavus* is attributed to dry environments, which consequently results in reduced grain quality and potential aflatoxin contamination. The acceptable level of total aflatoxin in South Africa is 10 ppb (Buitendag, 2002). In all cultivars and localities aflatoxin concentrations recorded in the present study were below the acceptable level. However, there were levels of aflatoxin ranging from 8 to 9 ppb in the late flowering samples which were probably due to dry weather conditions. Other mycotoxins such as ochratoxin and deoxynivalenol were detected in sampled sorghum grain at low concentrations. According to Lefyedi *et al.* (2005), ideally sorghum malt should be completely free of toxin producing grain mould fungi and mycotoxins and thus the presence of even traces of mycotoxins should not be ignored.

The results of the present study demonstrated that moisture is an important factor for certain fungi to infect sorghum heads. *A. alternata* and *C. lunata* required an increase in wetness period for infection, while *Fusarium* spp. and *P. sorghina* infected the plants with lesser wetness durations. Similarly, Bandyopadhyay *et al.* (2002) reported the occurrence of some infection in sorghum heads inoculated with *F. moniliforme* (*sensu*

*lato*) and *P. sorghina*, even with no wetness treatment. Wetness duration may favour grain mould either by providing favourable conditions for infection or by enhancing sporulation. Seitz *et al.* (1983) also reported an increase in percentage of kernels invaded by grain mould fungi in sorghum grains exposed to longer wet periods.

Increased wetness duration increased grain mould fungal infection and subsequently higher TGMS were also observed. This indicated that grain mould fungi required a longer wetness period to cause infection, and could also apply to grains that are exposed to longer periods of wetness in the field particularly during the susceptible period. Wet weather that occurs from the time of flowering onwards is necessary for grain mould development and longer wet periods provide favourable conditions for maximum infection (Williams & Rao, 1981). The exposure of sorghum kernels to wet periods may not only be restricted to the field, but also to storage. Sauer, Storey & Walker (1984) reported a positive correlation between moisture content and percentage of kernels of wheat and maize contaminated with mould fungi.

In the present study, the reduction in germination percentage with increasing humidity period was observed for almost all the fungi inoculated in the glasshouse. It was found that wet period requirements differed for individual species of fungi and that most needed long wet periods for maximum infection. Infection could thus occur in the field if sorghum heads are left for long periods after maturity. Manjusha *et al.* (2003) observed an increase in fungal incidence and higher threshed grain mould scores with subsequent decreased seed germination as the harvesting time was delayed.

The methods used in this study and information on the role of wetness duration on grain mould fungi and their relationships could be used as a technique to screen for resistance against grain mould fungi. In most cases wetness duration of more than 48 h

was optimal for maximum infection. Navi *et al.* (2005) also reported 40 h of wetness duration as optimum exposure for sorghum grain mould infection and recommended exposure of at least 40 hours of continuous wetness in the field for resistance screening.

Results of the present study convincingly demonstrated the role of environmental conditions in the development of grain mould and the effect that individual species of fungi have on quality and quantity of sorghum grain. Thus, in addition to the range of mould fungal species that cause grain mould, the environment significantly influences the development of the disease. The variation in grain mould incidence and the resulting effects on grain quality among the same cultivars at different localities and flowering dates indicates the role of environment in the incidence of the pathogens. The exposure of sorghum heads to high moisture and humidity especially during the critical growth stage results in higher fungal infection and thus sorghum should not be left in the field after maturity.

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Table 3.1 Incidence (%) of grain mould fungi on grains of sorghum cultivars from three localities planted on different planting dates

Locality	Flowering date (2004)	NK 283				Buster				PAN 8564			
		<i>A. alternata</i>	<i>C. lunata</i>	<i>Fusarium</i> spp.	<i>P. sorghina</i>	<i>A. alternata</i>	<i>C. lunata</i>	<i>Fusarium</i> spp.	<i>P. sorghina</i>	<i>A. alternata</i>	<i>C. lunata</i>	<i>Fusarium</i> spp.	<i>P. sorghina</i>
Bethlehem	4/2	82.0 a	4.3 a	6.0 a	0.7 a	72.7 a	0.0 a	8.3 a	0.0 a	73.3 a	1.3 a	6.0 a	1 a
	11/2	77.0 a	1.7 a	11.0 a	0.0 a	68.7 a	1.0 a	9.7 a	0.0 a	70.0 a	1.0 a	7.7 a	0 a
	18/2	74.3 a	2.3 a	10.7 a	0.0 a	76.0 a	0.3 a	3.7 a	0.0 a	63.3 a	1.0 a	10.0 a	0 a
	26/3	76.7 a	0.3 a	11.33 a	0.7 a	76.7 a	0.3 a	5.3 ab	0.0 a	69.3 a	0.3 a	8.0 a	0 a
	3/3	77.0 a	0.3 a	5.7 a	0.0 a	54.7 b	0.7 a	10.7 a	0.3 a	67.0 a	0.0 a	4.0 a	0 a
	10/3	29.3 b	0.0 a	7.0 a	0.0 a	33.3 c	0.3 a	1.0 b		26.3 b	0.0 a	4.0 a	0 a
Cedara	19/2					46.7 ab	7.7 a	17.0 a	1.3 a	54.0 abc	4.7 a	7.3 a	1 a
	25/2	42.7 c	3.7 a	26.3 a	3.3 a	61.3 a	11.0 a	14.3 a	2.7 a	63.0 a	5.0 a	6 a	1.33 a
	2/3	49.3 bc	2.3 a	19.7 ab	0.7 a	64.0 a	9.7 a	13.0 a	2.0 a	62.7 a	5.3 a	9 a	0.67 a
	9/3	61.0 ab	1.7 a	15.3 b	1.0 a	56.0 a	5.7 a	19.7 a	4.0 a	56.7 ab	2.7 a	11.3 a	1.33 a
	16/3	61.7 ab	2.3 a	21.7 ab	0.3 a	45.3 ab	6.0 a	11.7 a	1.7 a	44.7 c	1.3 a	6.7 a	1.33 a
	24/3	66.0 a	2.3 a	14.0 b	4.0 a	36.3 ab	0.0 b	10.0 a	0.3 a	51.3 bc	2.7 a	6.7 a	0.33 a
	1/4									18.3 d	1.3 a	7.3 a	0.33 a
6/4									26.3 d	2.7 a	6.7 a	1.33 a	
P'stroom	14/1					67.3 a	6.7 ab	6.3 c	6.7 a				
	24/1					63.7 ab	4.3 bcd	11.7 b	10.0 a				
	3/2	61 b	5.7 ab	12.3 a	6.7 a	65.0 ab	5.3 abc	8.3 c	4.3 a	67.7 a	3.0 a	4.3 a	4.33 b
	10/2	71.0 a	3.7 bc	5.3 b	5.3 ab	62.7 b	7.7 a	7.3 c	7.3 a	66.7 a	1.7 b	6.0 a	4.33 b
	17/2	71.7 a	7.7 a	2.7 c	3.7 ab	57.7 c	2.0 d	17.3 a	10.7 a	73.3 a	0.7 bc	7.0 a	2 b
	4/3	71.7 a	2.0 c	5.7 b	2.3 c	65.3 ab	3.0 cd	3.7 d	3.7 a	61.3 a	0.0 c	5.7 a	10.33 a
18/3	54.3 c	0.7 c	1.7 c	1.3 c					66.3 a	0.0 c	2.3 b	2.67 b	

Continued Table 3.1

Locality	Flowering date (2004)	SNK 3939				PAN 8446			
		<i>A. alternata</i>	<i>C. lunata</i>	<i>Fusarium</i> spp.	<i>P. sorghina</i>	<i>A. alternata</i>	<i>C. lunata</i>	<i>Fusarium</i> spp.	<i>P. sorghina</i>
Bethlehem	4/2	66.3 a	4.7 a	9.7 a	0.7 c	68.3 a	1.3 abc	9.0 a	1.3 d
	11/2	70.7 a	2.7 a	6.0 a	1.7 bc	68.76 a	2.0 abc	4.7 b	2.3 cd
	18/2	56.3 a	5.3 a	7.3 a	5.3 a	65.7 a	2.3 ab	7.3 ab	3.0 bc
	26/3	58.3 a	0.7 a	6.7 a	3.0 b	65.3 a	3.0 a	10.3 a	6.3 a
	3/3	40.7 b	0.3 a	6.3 a	1.3 bc	41.0 b	0.7 bc	3.3 b	4.0 bc
	10/3	24.7 c	0.7 a	5.7 a	0.3 c	29.3 c	0.3 c	4.0 b	1.3 d
Cedara	25/2					54.0 b	6.0 a	10.7 a	2.7 a
	2/3	65.0 a	4.0 a	7.7 b	1.7 ab	66.0 a	6.0 a	6.3 a	0.7 b
	9/3	52.3 b	4.0 a	14.7 a	2.3 a	75.3 a	2.7 ab	6.0 a	0.0 b
	16/3	42.7 c	2.7 b	7.7 b	0.7 bc	71.0 a	3.7 ab	9.0 a	1.3 ab
	24/3	28.0 d	1.3 c	7.3 b	0.3 c	45.3 a	2.3 ab	8.3 a	1.7 ab
	1/4	18.0 e	0.7 c	6.0 b	2.7 a	38.0 c	4.0 ab	6.3 a	1.7 ab
	6/4					10.7 d	0.0 b	6.0 a	0.0 b
Potchesftroom	3/2	62.0 ab	2.7 a	6.3 b	7.0 a	65.7 a	2.7 ab	9.0 a	4.7 a
	10/2	68.0 a	1.3 b	7.3 b	3.3 bc	58.7 a	0.0 b	6.3 ab	1.7 b
	17/2	67.3 ab	2.7 a	9.3 a	5.3 ab	63.3 a	4.3 a	7.7 a	5.3 a
	4/3	61.7 ab	0.0 b	4.0 c	3.7 ab	59.7 a	3.3 a	4.3 bc	4.3 a
	10/3	59.0 b	1.0 b	4.3 c	1.3 c	59.7 a	1.7 ab	2.7 c	3.7 a
	18/3	22.3 c	0.0 b	2.7 c	1.3 c				

Means of fungal incidence within columns for each locality followed by the same letter do not differ significantly ( $P \leq 0.05$ ) according to Tukey-Kramer multiple comparison test

Table 3.2 Pearson's correlation coefficients between incidence of grain mould pathogens (%) on grains at harvest and mean of 7 days post anthesis weather conditions on different sorghum hybrids.

		Hybrid								
		NK 283				SNK 3939				
Weather‡		<i>A. alternata</i>	<i>C. lunata</i>	<i>Fusarium</i> spp.	<i>Phoma</i>	Weather	<i>A. alternata</i>	<i>C. lunata</i>	<i>Fusarium</i> spp.	<i>Phoma</i>
Min. Temp.		0.19	0.81*	0.14	0.68*	Min. Temp.	0.59*	0.38	0.09	0.48*
Max. Temp.		0.53*	0.63*	0.14	0.49*	Max. Temp.	0.61*	0.47*	0.07	0.46
RH		0.40	0.56*	0.27	0.52*	RH	0.34	0.38	0.18	0.42
Rainfall		0.61*	0.61*	-0.08	0.28	Rainfall	0.68*	0.49*	0.15	0.14
		Buster				PAN 8446				
Weather		<i>A. alternata</i>	<i>C. lunata</i>	<i>Fusarium</i> spp.	<i>Phoma</i>	Weather	<i>A. alternata</i>	<i>C. lunata</i>	<i>Fusarium</i> spp.	<i>Phoma</i>
Min. Temp.		0.06	0.54*	0.45	0.74*	Min. Temp.	0.60*	0.62*	0.36	0.37
Max. Temp.		0.40	0.28	0.09	0.65*	Max. Temp.	0.41	0.20	0.49*	0.47*
RH.		0.38	0.59*	0.5*	0.47*	RH	0.39	0.40	.44	0.21
Rainfall		0.49*	-0.05	0.14	0.26	Rainfall	0.40	0.23	0.39	0.71*
		PAN 8564								
Weather		<i>A. alternata</i>	<i>C. lunata</i>	<i>Fusarium</i> spp.	<i>Phoma</i>					
Min. Temp.		0.59*	0.39	0.19	0.48*					
Max. Temp.		0.54*	0.47*	0.14	0.47*					
RH		0.20	0.52*	0.47*	0.36					
Rainfall		0.58*	0.49*	0.15	0.14					

‡Min. Temp. = Minimum temperature; Max. Temp. = Maximum temperature; RH = Relative humidity

\*Significantly correlated ( $P \leq 0.05$ )

Table 3.3 Mean 100 kernel weight of sorghum grain from three localities and different flowering dates

Location	Flowering dates (2004)	Cultivar				
		NK 283	Buster	PAN 8564	SNK 3939	PAN 8446
Bethlehem	4/2	2.46 c	3.0 a	2.42 cd	2.44 b	2.12 b
	11/2	2.88 a	2.84 b	2.6 a	2.53 a	2.16 b
	18/2	2.47 c	3.03 a	2.45 c	2.40 b	2.42 a
	26/2	2.57 bc	2.36 c	1.84 e	2.29 c	2.16 b
	3/3	2.26 c	1.69 e	2.36 d	1.99 d	1.50d
	10/3	1.35 d	1.87 d	2.53 b	1.46 d	1.60 c
Cedara	19/2	*	2.54 a	1.73 c	*	*
	25/2	0.92 c	2.14 b	1.73 c	*	1.53 a
	2/3	1.26 b	2.00 c	1.92 b	1.68 b	1.52 a
	9/3	1.30 b	2.22 b	1.99 a	1.91 a	1.34 b
	16/3	1.47 a	2.49 a	1.89 b	1.89 a	1.56 a
	24/3	1.47 a	1.47 d	1.73 c	1.69 b	1.24 c
	1/4	*	*	1.61 d	1.50 c	1.03 d
	6/4	*	*	1.38 e	*	0.98 d
Potcheftroom	14/1	*	2.98 a	*	*	*
	24/1	*	2.6 bc	*	*	*
	3/2	1.53 c	2.43 c	1.83 c	1.85 e	2.27 a
	10/2	1.31 d	2.85 a	1.81 bc	1.79 e	2.17 c
	17/2	2.11 b	2.07d	1.91 c	2.22 d	2.13 cd
	4/3	2.66 a	2.78 ab	2.52 a	2.43 c	2.09 d
	10/3	*	*	*	2.53 b	2.48 a
	18/3	2.54 a	*	2.36 b	2.77 a	*

\* Dates with no flowering

Means of kernel mass along the column for each cultivar and locality followed by the same letter do not differ significantly ( $P \leq 0.05$ ) according to Tukey-Kramer multiple comparison test

Table 3.4 Threshed grain mould score of sorghum grains from three localities and different flowering dates

Location	Flowering dates (2004)	Cultivar				
		NK 283	Buster	PAN 8564	SNK 3939	PAN 8446
Bethlehem	4/2	2.00	1.67	2.67	3.00	1.67 bc
	11/2	2.33	1.67	2.67	2.67	1.33 c
	18/2	1.67	1.67	2.33	2.00	2.00 bc
	26/2	2.67	2.33	2.00	2.00	2.33 ab
	3/3	2.0	2.33	2.00	2.67	3.00a
	10/3	2.67	2.00	2.00	2.33	3.00a
Cedara	19/2	*	3.00 b	3.67 a	*	*
	25/2	4.67 a	3.67 b	3.00 ab	*	2.67
	2/3	3.33 b	2.67 b	2.00 b	2.00	2.67
	9/3	2.67 bc	2.33 b	2.33 b	2.67	2.00
	16/3	2.00 c	3.33 b	2.33 b	2.33	1.67
	24/3	2.00 c	4.67 a	2.00 b	1.67	2.33
	1/4	*	*	3.67 b	2.00	2.33
	6/4	*	*	3.67 a	*	2.33
Potchefstroom	14/1	*	4.33	*	*	*
	24/1	*	4.33	*	*	*
	3/2	4.33 a	4.33	4.67 a	4.33 a	4.33 a
	10/2	4.33 a	4.33	4.00 a	4.67 a	4.33 a
	17/2	3.67 ab	3.67	3.33 bc	3.00 b	4.00 a
	4/3	3.00 b	3.33	2.67 c	2.67 b	3.00 b
	10/3	*	*	*	2.33 b	2.33 b
	18/3	3.00 b	*	2.33 c	2.00 b	*

\* Dates with no flowering

Means of TGMS along the column for each cultivar and locality followed by the same letter do not differ significantly ( $P \leq 0.05$ ) according to Tukey-Kramer multiple comparison test

Table 3.5 Malting and milling quality traits of sorghum grains from three localities and different flowering dates

Location	Cultivar	Flowering date (2004)	Malting quality					Milling quality		
			Germinative vigour (GV)%	Germinative energy (GE)%	(DP) (SDU/g malt)	% Malting loss	% Water uptake	Abrasive hard.index	Dehulling index	
Bethlehem	NK 283	11/2	73.7	81.7	15.2	10.3	40.5	6.7	16.9	
		18/2	82.0	90.3	21.5	12.5	42.9	7.5	9.6	
		26/2	85.7	89.7	31.4	10.2	42.1	5.8	12.7	
		3/3	78.7	95.0	34.8	11.8	41.2	6.6	4.9	
		10/3	86.0	92.7	51.5	14.7	49.9	6.1	0.7	
	Buster	4/2	89.3	92.3	27.2	12.1	41.8	5.3	15.3	
		11/2	81.3	91.3	27.3	12.6	40.0	9.1	10.8	
		18/2	84.0	94.0	26.3	10.1	36.4	6.4	11.7	
		26/2	93.3	97.7	36.8	11.2	40.8	6.5	11.3	
		3/3	80.7	89.3	41.5	13.2	45.3	7.2	0.7	
	PAN 8564	10/3	89.0	94.0	39.7	13.1	43.4	6.2	2.2	
		4/2	90.0	97.3	36.8	8.4	33.2	13.3	10.9	
		11/2	88.7	96.0	24.1	11.1	37.5	8.6	13.2	
		18/2	88.7	96.0	30.3	10.1	34.7	8.0	4.6	
		26/2	94.3	97.3	45.5	11.0	37.5	9.5	6.2	
	SNK 3939	3/3	86.0	96.0	39.1	9.0	33.8	7.0	2.9	
		4/2	85.0	90.7	32.4	9.0	33.7	9.5	10.3	
		11/2	78.7	94.3	29.2	8.9	33.9	8.6	11.1	
		18/2	85.3	93.7	17.2	9.3	35.1	7.1	7.7	
		26/2	90.3	96.0	31.4	9.6	34.3	6.2	3.4	
	PAN 8446	3/3	84.3	95.7	40.3	12.3	42.7	5.1	5.5	
		10/3	80.7	97.3	52.1	10.8	40.8	4.0	0.1	
		4/2	88.7	94.3	38.0	11	36.3	8.8	15.9	
		11/2	85.0	95.3	38.8	9.2	32.3	7.1	16.2	
		18/2	85.3	92.0	34.3	10.9	36.3	5.8	10.7	
Cedara	NK 283	26/2	91.7	97.3	43.1	11.6	37.5	9.3	12.8	
		3/3	88.3	94.0	45.5	13.8	47.2	5.6	8.0	
		24/3	74.0	79.3	43.4	14.4	48.9	6.3	5.9	
		Buster	25/2	74.0	82.0	18.3	8.5	38.8	4.8	13.5
			2/3	83.3	91.0	22.0	9.9	38.9	6.2	10.4
	PAN 8564	9/3	79.3	89.0	42.1	8.9	40.1	5.7	9.6	
		16/3	83.0	89.0	18.7	9.9	40.3	7.4	10.8	
		19/2	90.3	92.0	35.8	9.4	37.6	5.7	13.8	
		25/2	92.7	97.3	51.8	11.3	40.6	5.1	10.8	
		2/3	92.0	96.0	42.8	8.6	36.9	7.5	4.8	
	SNK3939	9/3	90.7	97.3	49.3	9.6	36.7	7.7	5.8	
		16/3	90.3	93.3	46.4	9.8	36.7	8.8	4.2	
		2/3	86.3	96.7	35.1	8.8	36.9	5.6	6.0	
		9/3	85.0	93.3	25.9	9.4	36.8	5.4	5.5	
			16/3	82.3	92.3	35.3	10.3	38.5	7.8	4.6
24/3			78.3	87.7	42.2	10.4	41.6	6.1	6.6	

Continue Table 3.5

Location	Cultivar	Flowering date (2004)	Malting quality					Milling quality	
			Germinative vigour (GV)%	Germinative energy (GE)%	(DP) (SDU/g malt)	% Malting loss	% Water uptake	Abrasive hard.index	Dehulling index
Cedara	PAN 8446	2/3	85.3	95.0	40.1	9.0	35.9	5.5	14.9
		9/3	91.0	97.7	45.1	9.1	34.7	4.1	10.3
		24/3	78.7	82.7	38.8	11.4	41.6	6.3	13.2
Potchefstroom	NK 283	3/2	35.3	43.0	18.2	14.6	55.3	5.8	41.8
		10/2	26.7	30.7	24.3	11.4	48.7	5.3	30.6
	Buster	14/1	68.0	73.3	22.2	9.7	41.9	6.4	21.9
		24/1	43.0	47.0	15.7	10.8	45.1	6.4	36.0
		3/2	43.0	55.3	23.2	11.1	47.7	6.7	31.4
	PAN 8564	3/2	77.0	80.3	38.4	9.1	38.9	6.3	30.4
		10/2	75.0	79.7	37.0	9.9	41.6	7.3	30.1
		4/3	90.0	94.3	34.3	7.7	39.7	9.2	12.7
		10/3	81.3	97.0	40.5	10.3	37.4	8.1	4.5
	SNK 3939	3/2	35.3	43.0	20.3	13	39.9	6.5	21.8
		10/2	62.7	70.0	33.7	10.3	40.0	6.1	14.5
		10/3	75.0	92.3	37.4	7.1	37.6	11.1	8.2
PAN 8446	3/2	43.7	51.7	28.0	10.5	43.1	6.9	36.9	
	10/2	48.7	60.0	19.4	8.4	40.5	6.3	39.2	

Table 3.6 Pearson's correlation coefficients between incidence of grain mould pathogens (%) and grain quality parameters

Quality trait	Cultivar								
	NK 283				Buster				
	<i>A. alternata</i>	<i>C. lunata</i>	<i>Fusarium</i> spp.	<i>Phoma</i>	Quality trait	<i>A. alternata</i>	<i>C. lunata</i>	<i>Fusarium</i> spp.	<i>Phoma</i>
TGMS.	-0.31	0.58*	0.23	0.78*	TGMS.	-0.17	0.43	0.14	0.69*
100k. mass	0.67*	-0.17	-0.60*	-0.38	100k. mass	0.66*	0.7	-0.28	0.15
AHI	0.48	-0.28	-0.65	-0.33	AHI	-0.30	-0.45	-0.07	-0.13
DI	0.73	0.56	0.83	0.32	DI	0.47	0.34	-0.04	0.71
GV	-0.44	-0.63	-0.40	-0.46	GV	-0.07	-0.46	-0.09	-0.32
GE	-0.21	-0.70	-0.85*	-0.68	GE	0.25	-0.73*	-0.69*	-0.65*
DP	-0.76	-0.44	-0.23	0.37	DP	-0.24	-0.57	-0.07	-0.24
ML	-0.74	0.10	-0.04	0.44	ML	-0.15	-0.75*	-0.59	-0.47
WU	-0.98*	-0.44	-0.38	-0.18	WU	-0.26	-0.13	-0.22	0.39
Quality trait	PAN 8564				SNK 3939				
	<i>A. alternata</i>	<i>C. lunata</i>	<i>Fusarium</i> spp.	<i>Phoma</i>	Quality trait	<i>A. alternata</i>	<i>C. lunata</i>	<i>Fusarium</i> spp.	<i>Phoma</i>
TGMS.	0.19	0.24	-0.18	0.58*	TGMS.	0.44	-0.13	0.15	0.55*
100k. mass	0.35	-0.58	-0.20	0.12	100k. mass	0.36	-0.37	-0.27	0.08
AHI	0.35	-0.54	-0.09	-0.04	AHI	0.65*	-0.08	0.14	0.41
DI	0.12	0.32	-0.17	0.32	DI	0.7	0.11	0.17	0.21
GV	-0.31	0.44	0.24	-0.12	GV	0.13	0.08	0.08	-0.01
GE	0.64	-0.21	0.17	-0.24	GE	0.09	-0.37	-0.25	0.04
DP	-0.36	0.34	0.05	0.10	DP	-0.62	-0.38	-0.37	-0.58
ML	0.20	-0.06	0.18	-0.49	ML	-0.75*	-0.18	-0.35	-0.58
WU	-0.14	0.46	0.01	0.51	WU	-0.82*	-0.09	-0.21	-0.37

Continued Table 3.6

Quality trait	PAN 8446			
	<i>A. alternata</i>	<i>C. lunata</i>	<i>Fusarium</i> spp.	<i>Phoma</i>
TGMS.	0.07	-0.07	0.08	0.32
100k. mass	0.59*	-0.09	-0.01	0.61*
AHI	0.07	-0.19	0.65	0.56
DI	0.74	0.53	0.52	0.01
GV	-0.14	-0.32	-0.01	0.33
GE	0.63	0.13	-0.15	0.13
DP	-0.17	-0.07	-0.31	0.15
ML	-0.79*	-0.61	-0.02	0.63
WU	-0.92*	-0.39	-0.22	0.34

TGMS = Threshed grain mould sorghum; 100. mass = 100 kernel mass; AHI = Abrasive hardness index;

DI = Dehulling index; GV = Germination vigour;

GE = Germination energy; DP = Diastatic power; ML = Malt loss; WU = water uptake

\*Significantly correlated ( $P \leq 0.05$ )

Table 3.7 Mycotoxins isolated from sorghum grains from three localities and different flowering dates

Locality & Cultivar	Flowering date (2004)	Fumonisin (ppm)	Aflatoxin (ppb)	Zearalenone (ppm)	Ochratoxin (ppb)	DON (ppm)
<b>Bethlehem</b>						
NK 283	4/2	0.00	7.18	22.99	3.11	0.25
	11/2	0.00	2.97	0.00	2.42	0.15
	18/2	0.00	3.65	14.38	1.82	0.12
	26/2	0.00	5.11	33.82	4.08	0.12
	3/3	0.00	5.38	25.69	2.16	0.20
	10/3	0.00	6.97	46.20	1.65	0.28
Buster	4/2	0.00	3.72	2.99	1.83	0.14
	11/2	0.00	5.04	40.98	2.02	0.12
	18/2	0.00	4.32	56.97	1.99	0.12
	3/3	0.00	6.59	30.78	1.75	0.16
	10/3	0.00	9.00	44637	1.98	0.21
PAN 8564	4/2	0.00	1.65	31.29	1.75	0.10
	18/2	0.00	3.09	0.00	2.37	0.11
	26/2	0.03	4.96	24.54	2.46	0.12
	3/3	0.143	5.09	37.37	1.43	0.14
	10/3	0.00	4.64	0.00	2.08	0.14
SNK 3939	4/2	0.00	5.92	27.43	2.24	0.07
	11/2	0.098	1.64	8.15	1.85	0.10
	18/2	0.00	5.75	14.04	2.58	0.08
	26/2	0.023	4.46	0.00	2.58	0.15
	3/3	0.089	2.84	12.93	2.05	0.09
	10/3	0.00	4.98	37.23	1.88	0.11
PAN 8446	4/2	0.189	3.88	44.82	1.55	0.16
	11/2	0.00	4.17	20.36	2.27	0.14
	18/2	0.00	6.00	59.20	1.52	0.16
	26/2	0.202	4.748	0.00	1.90	0.15
	3/3	0.00	2.75	37.41	3.39	0.31
	10/3	0.00	5.03	20.22	2.62	0.18
<b>Cedara</b>						
NK 283	2/3	0.00	6.87	64.95	2.55	0.37
	9/3	0.00	6.43	88.74	2.95	0.39
	16/3	0.00	5.99	117.60	2.68	0.33
	19/2	0.00	2.81	91.24	2.48	0.30
Buster	25/2	0.00	2.38	88.14	2.33	0.43
	9/3	0.00	3.05	67.52	2.49	0.25
PAN 8564	25/2	0.00	2.50	33.14	2.68	0.32
	2/3	0.00	1.19	102.48	2.78	0.19
	16/3	0.00	2.78	72.96	1.69	0.24
	24/3	0.00	2.56	88.96	2.23	0.23
SNK 3939	2/3	0.00	4.88	71.55	0.98	0.36
	9/3	0.00	7.67	17.56	2.90	0.14
	16/3	0.00	6.30	3.64	2.83	0.14
	24/3	0.00	7.60	27.77	1.90	0.21
	1/4	0.00	8.33	47.97	1.78	0.14

Continued Table 3.7

<b>Cedara</b>	Flowering	Fumonisin	Aflatoxin	Zearalenone	Ochratoxin	DON
PAN 8446	25/2	0.00	7.93	36.15	2.16	0.24
	2/3	0.00	8.36	34.72	0.00	0.40
	24/3	0.00	7.42	51.49	0.47	0.28
<b>Potchefstroom</b>						
NK 283	3/2	0.00	3.85	94.11	2.49	0.19
	10/2	0.00	8.89	76.44	2.05	0.18
	17/2	0.00	4.35	97.43	2.31	0.22
	4/3	0.00	5.14	72.25	2.41	0.18
Buster	14/1	0.0	4.53	40.31	1.86	0.20
	24/1	0.15	4.07	69.32	0.00	0.00
	3/2	0.00	3.92	81.29	1.51	0.17
	10/2	0.02	5.04	10.75	0.00	0.18
	17/2	0.00	6.23	57.68	1.59	0.18
	4/3	0.00	5.18	52.05	2.70	0.19
PAN 8564	3/2	0.00	3.04	70.74	2.15	0.15
	17/2	0.00	3.78	65.71	0.00	0.18
	4/3	0.02	2.83	0.00	1.58	0.11
	18/3	0.05	2.97	64.63	2.02	0.09
SNK 3939	3/2	0.12	1.00	88.501	2.25	0.11
	10/2	0.08	3.25	62.582	1.83	0.11
	17/2	0.14	3.57	80.216	2.08	0.11
	4/3	0.13	5.37	85.834	3.09	0.10
	10/3	0.05	3.88	40.64	2.21	0.06
PAN 8446	3/2	0.00	4.36	88.22	1.15	0.19
PAN 8446	10/2	0.07	1.17	114.27	1.06	0.19
PAN 8446	17/2	0.09	2.94	92.66	1.81	0.15
PAN 8446	4/3	0.02	2.98	112.33	1.01	0.12
PAN 8446	10/3	0.00	0.94	82.74	1.79	0.15

0 = invalid concentration

Concentrations

Fumonisin = ppm (parts per million)

Aflatoxin = ppb (parts per billion)

Zearalenone = ppm

Ochratoxin = ppb

DON (Deoxynivalenol) = ppm

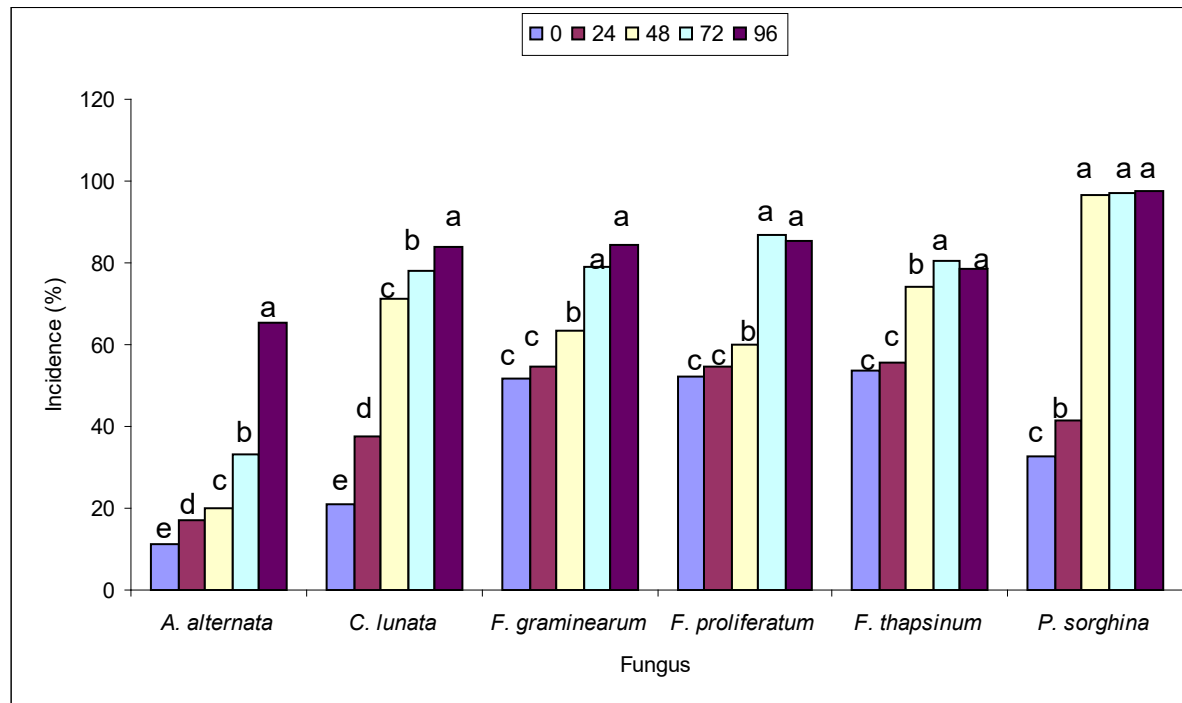
Table 3.8 The effect of grain mould fungi on grain germination and threshed grain mould score (TGMS) at different wetness durations

WD* (h)	Germination (%)						TGMS					
	<i>Alternaria alternata</i>	<i>Curvularia lunata</i>	<i>Fusarium graminearum</i>	<i>Fusarium proliferatum</i>	<i>Fusarium thapsinum</i>	<i>Phoma sorghina</i>	<i>Alternaria alternata</i>	<i>Curvularia lunata</i>	<i>Fusarium graminearum</i>	<i>Fusarium proliferatum</i>	<i>Fusarium thapsinum</i>	<i>Phoma sorghina</i>
0	84.25 a	71.75 a	72 a	75.75 a	79 a	77 a	1 b	1 c	1.75 b	2.5 b	2.25 b	1.25 c
24	84.25 a	70.75 a	73.75 a	74.75 a	72.75 a	69.5 b	1 b	1.25 c	2 b	2.5 b	2.25 b	1.75 b
48	85.25 a	48.25 b	70.75 a	72 ab	71.25 ab	66 b	1.5 b	2.25 b	3a	2.5 b	3.25 a	2.5 ab
72	82 a	49.5 b	69.75 a	71 ab	69.25 ab	67.25 b	2.25 a	2.5 b	3.25 a	3.75 a	3.75 a	2.75 ab
96	83.5 a	46.5 b	69.25 a	67.75 b	65.25 b	69.25 b	2.5 a	3.25 a	3.5 a	4a	3.75 a	3a

Means along the column for each fungus followed by the same letter do not differ significantly ( $P \leq 0.05$ ) according to Duncan's multiple range test

\*WD (h) = wetness duration (hour)

Fig. 3.1 Effect of wetness duration on infection (%) of grain mould fungi. The grains collected for isolation were from the panicles of sorghum cultivar PAN 8706W inoculated at soft dough stage. Bars denoted by the same letter are not significantly ( $P \leq 0.05$ ) different according to Tukey-Kramer multiple comparison test.



## **CHAPTER 4**

### **Influence of insects on sorghum grain mould**

## Abstract

The effect of insect damage to sorghum grains on mould fungi and subsequent grain quality was studied. Head-bugs were collected from sorghum heads at two localities in South Africa and fungi were isolated from all specimens collected. *Alternaria alternata* was isolated at the highest frequency from specimens collected from Cedara while the incidence of *Fusarium* spp. was highest in insect samples from Bethlehem. Insecticide sprayed and unsprayed grains were used to quantify the relationship between insects and grain mould fungi and subsequent grain quality. Fungal frequencies differed in insecticide treated and untreated plots with lower fungal incidences in the former. Significant differences ( $P \leq 0.05$ ) were observed between treatments in 100 kernel mass of sorghum grains. Mycotoxin production in grains from the insecticide treated and untreated plots was assessed but with the exception of deoxynivalenol, results were not consistent. The effects of puncture marks created by insect damage on grain mould fungi infection were determined. Sorghum lines differed significantly ( $P \leq 0.05$ ) in grain mould score which ranged from 2.33 and 4.83 on a 0 to 5 rating scale. A significant positive correlation ( $r = 0.52$ ,  $P \leq 0.05$ ) was recorded between the incidence of *Fusarium* spp. and puncture marks. These results indicated an association between insect infestation and grain mould fungi which can result in qualitative and quantitative losses of sorghum grains.

## Introduction

Grains are the main source of food and a breeding ground for many insects (Agrios, 1980). In most cases the direct damage caused by insects is aggravated by fungi that invade injured grains and cause infection (Agrios, 1980). Grain mould severity is thus increased by concurrent attacks of panicle feeding insects (Murty, 2000). Both feeding and oviposition punctures by head bugs in maturing sorghum caryopses result in severe quantitative and qualitative losses, and favour secondary infection by grain mould fungi (Ratnadass *et al.*, 1995). In addition to providing a site through which mould fungi can enter and colonize the grain, sap exuded from head-bug injuries provide a substrate for fungal growth on the grain surface (Bandyopadhyay *et al.*, 2002).

An insect-fungus interaction occurs when either or both populations exert a definite influence on each other. The classification of these interactions is based on the type of insect involvement, i.e. whether the insect transports the fungal pathogen to the host, damages the host by feeding and thus provides an entrance for the fungus, or participates in symbiotic relations (Mills, 1983). Marley & Malgwi (1999) observed an increase in the incidence and severity of grain mould on sorghum that previously had a very low incidence and noticed that grain with insect punctures had a higher fungal incidence than the same grain without punctures.

Sorghum head structures may influence the degree of infestation by head-bugs. According to Teetes & Pendleton (2004), open panicles are less affected by head-bugs than compact panicles. Head-bug damage causes shrivelling of the panicles which then become infected by grain mould fungi, resulting in yield loss and deterioration in grain quality. The influence of insects on infection by grain mould fungi is not only limited to

the field but may also occur during storage where they predispose grain to fungal infection and subsequently to mycotoxin production (D'Mello & Macdonald, 1997).

The objectives of this study were: (i) To isolate grain mould fungi from head-bugs collected from sorghum heads at experimental plots; (ii) determine the incidence of grain mould fungi in sorghum grains from experimental plots; and (iii) to determine the association of insect punctures of the grains and grain mould incidence; and (iv) to assess the effect of grain mould on sorghum grain quality.

## **Materials and Methods**

### ***Collection of insects for fungal isolation.***

Head-bugs (Hemiptera : Miridae) were collected from experimental plots at Bethlehem and Cedara to determine the presence of grain mould fungi associated with insects. An aspirator (pooter) was used to sample specimens from sorghum heads and the insects were subsequently deposited into individual Polytop® bottles. Bottles containing live insects were taken to the laboratory and placed in a freezer at -75°C for 5 min to kill the insects without harming the fungal propagules. A total of 178 and 89 head-bugs were captured from experimental plots at Cedara and Bethlehem respectively, and screened for the presence of fungi. Each insect was transferred to a Petri-dish (65 mm diameter) containing malt extract agar (MEA) (Biolab®) (1%) amended with streptomycin sulphate (100 µl/l) and incubated at 25°C in a light-dark cycle of 12 h each. After seven to 14 days of incubation, fungi associated with the insects were identified and quantified.

### *Insecticide trial*

A trial was conducted at Cedara during the 2004/05 cropping season. A randomized block design with three replications containing selections from a white sorghum evaluation trial was used. The trial consisted of single row plots, 8 m in length with a 10 cm intra-row and 85 cm inter-row spacing. The trial was repeated twice to accommodate insecticide sprayed and unsprayed plots. Eleven entries were selected from the Southern African Breeding Nursery obtained from Texas A & M Agricultural experiment station, at Lubbock, Texas (courtesy of Dr. G. C. Peterson). Standard agronomic practices, typical of the region were applied throughout the season. There were two treatments: sorghum heads were first sprayed with Deltamethrin (sc 50 a.i./l) at a rate of 250 ml/ha at soft dough stage and then a follow-up spray 21 days after the first application at the same rate. The control treatment remained insecticide free.

### *Isolation of grain mould fungi*

Heads were harvested at maturity and hand threshed. The incidence of grain mould fungi was determined from a sample of 100 grains collected from each of the treatment replications. Grains were surface sterilized in 1% sodium hypochlorite for 3 minutes and rinsed in three changes of sterile distilled water, dried on sterile blotting paper and plated on half-strength (1%) malt extract agar (MEA) (Biolab<sup>®</sup>) amended with streptomycin sulphate (100 µl/l). Petri dishes were incubated at 25°C and each grain was examined for emerging fungi 7-14 days after incubation.

### *Germination percentage*

One hundred grain samples from each of the three treatment-replications were plated on moistened sterile filter paper (Copeland & McDonald, 2001) in Petri dishes. Grains were incubated at room temperature in the dark. Grains were regularly monitored and scored as germinated when roots and shoots had emerged from the grains.

### *100 kernel mass*

The effect of infection on grain density was determined by weighing 100 kernels from each treatment-replicate on an electronic sensitive balance (Scout II Ohatsu® Corporation, Pine Brook, NJ).

### *Mycotoxin analysis*

Sorghum grain samples were ground in a laboratory blender so that 75% of the material would pass through a 200 mm mesh sieve. A ground sample (5 g) was then blended with 25 ml of 70% methanol/water and shaken vigorously for 3 minutes. The extract was filtered through a Whatman #1 filter and the filtrate was collected and analysed for aflatoxin and zearalenone using respective Veratox® quantitative test kits (Neogen® Corporation, 620 Lesher Place, Lansing, MI 48912, USA).

A further ground sample (10 g) was vigorously mixed with 50 ml of distilled water for 3 min in a high speed blender. The extract was filtered through a Whatman #1 filter and the filtrate was collected and analyzed for deoxynivalenol (DON) using a Veratox® DON quantitative test kit (Neogen® Corporation, 620 Lesher Place, Lansing, MI 48912, USA).

Fumonisin analysis entailed blending a ground sample (5 g) with 50 ml of 70% methanol/water solution for 3 min in a high speed blender. The extract was filtered through a Whatman #1 filter and the filtrate was collected and diluted by adding 100 µl of extract into a prefilled sample dilution bottle from the test kit. The sample was then analyzed for fumonisin using a Veratox® fumonisin quantitative test kit (Neogen® Corporation, 620 Lesher Place, Lansing, MI 48912, USA).

### ***Insect injury and grain mould assessment***

A trial was established at Potchefstroom to determine the effect of insect feeding damage on grain mould development. Plots consisted of single row plots 6 m in length with a 10 cm intra-row and 1.2 m inter-row spacing with three replications. Standard agronomic practices pertinent to the region were applied throughout the season. Fifteen sorghum entries from the Sugarcane Aphid Nursery obtained from Texas A & M Agricultural experiment station, at Lubbock, Texas (courtesy of Dr. G. C. Peterson) were used in the assessment. Feeding punctures by insects were evaluated based on the number of visible feeding lesions per grain when observed under a stereo microscope (x30 magnification).

### ***Field grain mould rating (FGMR)***

At maturity, five panicles from each entry were scored visually for grain mould severity on the panicle surface. Scoring was based on a 1-5 scale following the methods of Audilakshmi *et al.* (1999), where 1 = no mould visible on the panicle; 2 = scant superficial mould growth up to 10% of the panicle surface covered by mould; 3 = moderate mould growth and 11-25% of the panicle surface moulded; 4 = considerable

mould growth with 26-50% of the panicle surface moulded; and 5 = extensive mould growth with more than 50% of the surface moulded.

#### *Grain quality parameters*

At maturity panicles were harvested and threshed and grains were assessed for mould severity. The number and type of fungi, percent grain germination and 100 kernel mass were assessed as outlined for the insecticide trial.

#### *Threshed grain mould score (TGMS)*

A 30 g sample of threshed grain from each entry was spread in a 90-mm-diameter Petri dish plate and scored visually for degree of discolouration (Audilakshmi *et al.*,1999). Using a stereo microscope (x30 magnification), grain mould severity was assessed as a visual estimate of the percentage of grain surface discoloured.

## **Results**

### *Isolation of fungi from insects*

Fungi were isolated from all insect specimens collected from sorghum trials at Bethlehem and Cedara (Table 4.1). More than one fungus was also isolated from some specimens. *A. alternata* followed by *Fusarium* spp. had the highest frequency from specimens collected from Cedara. In insect samples from Bethlehem, the incidence of *Fusarium* spp. were highest followed by *A. alternata*. Other fungal genera that were isolated included *Curvularia*, *Epicoccum*, *Helminthosporium*, *Mucor* and *Penicillium* spp.

### ***Insecticide trial***

#### *Fungal frequency*

There was a significant ( $P \leq 0.05$ ) difference in fungal infection of grain between sorghum lines (Table 4.2). Although not significant, a lower frequency of fungi was observed on grains collected from insecticide sprayed plots than unsprayed plots. *A. alternata* was the most predominant fungus isolated from all lines followed by *Fusarium* spp., *Phoma sorghina* and *C. lunata* in order of priority.

#### *100 kernel mass*

There was a significant difference ( $P \leq 0.05$ ) in 100 kernel mass between sorghum lines of insecticide treated and untreated grains (Table 4.3). In all cases, with the exception of cultivars 86ED361\*MACIA and 88BE2668, the insecticide treated grains had a higher 100 kernel mass.

#### *Grain germination*

There was no significant ( $P \leq 0.05$ ) difference in grain germination between insecticide treated and untreated plots (Table 4.3). However, the mean germination of the insecticide treated grains showed a slight improvement ( $\pm 3\%$ ) over untreated grains.

#### *Mycotoxin analysis*

The concentration of DON was lower in insecticide treated grains compared with untreated grains with the exception of line 86ED361\*MACIA which had the highest concentration (2.34 ppm) of DON (Table 4.4). Although the concentration of fumonisin

did not show much variation between the insecticide treated and untreated grains, a concentration as high as 0.99 ppm was recorded in untreated line BLD6(wxy) compared to 0.38 ppm in the treated grains of the same lines. Different levels of zearalenone were recorded from all the grains tested. The highest concentration of zearalenone was 590 ppb from grains of untreated line MACIA\* DORADO-1. Aflatoxin was found in both treated and untreated grains with the highest concentration (8.56 ppb) occurring in treated grains of line SURENO\* KUYUMA.

### ***Insect injury and grain mould assessment***

#### *Isolation of grain mould fungi*

There was a significant ( $P \leq 0.05$ ) difference in the incidence of grain mould fungi between lines (Table 4.5). The most frequently isolated fungus from all lines was *A. alternata* followed by *Fusarium* spp. Lines 53, 10 and 70 had the highest frequency of *A. alternata* (more than 73%) while the highest frequency of *Fusarium* spp. were isolated from line 4.

#### *Grain quality parameters*

There were significant ( $P \leq 0.05$ ) differences between sorghum lines in terms of field grain mould score (FGMS), grain germination, 100 kernel mass and threshed grain mould score (Table 4.6). The FGMS ranged from 2.33 and 4.83 in lines 40 and 90 respectively. Lines 40, 45, 70, 83, 88 and 90 had less than 80% grain germination. The TGMS score of lines 80, 83, 88 and 90 was higher than 50% indicating that grain mould infection resulted in more than 50% of the grains surface discolouration. *Fusarium* spp. exhibited

significant ( $P \leq 0.05$ ) positive correlation with insect puncture marks (Table 4.7). No significant correlation was observed between insect puncture marks and the other mould fungi isolated. The occurrence of *Fusarium* spp. and *P. sorghina* correlated negatively with grain germination and 100 kernel mass while *C. lunata* and *P. sorghina* frequencies were positively correlated to TGMS.

## **Discussion**

In the present study grain mould fungi were isolated from all head-bug specimens collected from Bethlehem and Cedara. The isolation of fungi from the exoskeleton of insects indicates their possible role in transporting fungal propagules from infected sorghum panicles to uninfected panicles or from infected grain to healthy grain within a panicle. Insect activity would also carry conidia of grain mould fungi from soil, decaying material or from infected stored grains. Insects may also be involved in providing an easy entry for the fungi by damaging the grain surface. Fennell *et al.* (1977) isolated *Aspergillus flavus* from insects from maize heads and suggested that insect related damage to the pericarp of developing kernels would provide the fungal mycelium with easy entry into the seed. Faecal matter as well as the exudates from the sap provide a suitable media for fungal growth and may subsequently increase the fungal incidence (Bandyopadhyay *et al.*, 2002).

In the present study, fungi were isolated more frequently from insecticide untreated plots than treated plots. This suggests the involvement of insects in the development of grain mould fungal infection. Thus the grain mould infection may have been exacerbated when there was a concurrent attack by panicle feeding insects

particularly in the plots with no insecticide treatments. Maximum head-bug infestation and grain mould infection are generally observed during the dough stage (Ajayi *et al.*, 2001; Tarekegn, McLaren & Swart, 2004). The coincidence of head-bug abundance in the panicle and mould damage leading to yield and grain quality losses warrants further attention. Ratnadass *et al.* (2001) reported a reduction in head-bug infestation in insecticide treated sorghum plants and a consequent reduction in grain mould infection confirming the role played by head-bugs as an aggravating factor for fungal infection. Marley & Ajayi (1999) recommended a number of methods for controlling head-bugs including synchronizing planting such that serious damage by head-bugs is escaped, planting head-bug resistant varieties and the use of insecticides. The application of appropriate insecticides at the correct time and rate, could therefore, reduce the severity of sorghum grain moulds and the subsequent reduction of grain quality associated with fungal infections.

Kernel mass is an indicator of grain quality and yield and in the present study the mass of grains from insecticide sprayed plots was higher than those from unsprayed plots. This variation in the kernel mass observed between insecticide treated and untreated grains could be attributed to differences in levels of infection by grain mould fungi. Marley & Malgwi (1999) reported an interaction between insects and grain moulds that resulted in a significant increase in mould incidence and a concomitant reduction in kernel mass. No significant effect of insecticides on germination of grains was recorded in the present study. This is contrary to a previous study in which a significant reduction in germination resulted from insect aided grain mould infection (Marley & Malgwi, 1999).

Although not evident in all lines tested, there was a definite indication that insect infestation of panicles prompted mycotoxin contamination. Insect infestation, together with weather conditions during the crop growing season has been reported to be a significant factor affecting fungal infection and mycotoxin synthesis (Doyle, 1997). Positive results for DON were exhibited from grains that were not treated with insecticides. In most parts of the world the acceptance level of DON in commodities for human consumption is 1 ppm (Buitendag, 2002). Within the lines used in this study, four of the unsprayed plots yielded more than 1 ppm DON. With the exception of 86ED361\*MACIA, all the lines in insecticide sprayed plots yielded less than 1 ppm, indicating the role of insects in promoting DON producing *Fusarium* spp. In contrast, the concentrations of zearalenone, fumonisin and aflatoxin did not differ between insecticide treated and untreated plots.

Puncture marks created by insects were positively correlated with the incidence of infection by *Fusarium* spp. in the present study which suggests that the damage caused by insects may have created a point of entry for the fungi to colonize kernels. Feeding by insects breaks the testa of grains, the natural barrier to fungal growth (Sétamou *et al.*, 1998). Dowd *et al.* (1999) also reported that rupturing of the kernel pericarp, either by insect damage or other means, breaks down kernel defence against mould fungi. They noted a significant correlation between *Fusarium* mould of maize at harvest and insect incidence.

Although not statistically significant, 100 kernel mass and grain germination were negatively correlated with grain mould infection. These reductions of kernel mass and percent germination could be as a result of insect feeding and the concurrent grain mould

infection. Marley & Malgwi (1999) associated decreased kernel mass and seed germination percentage with the simultaneous attack by insects and grain mould fungi. Puncture marks created by head-bugs favour secondary infection by grain mould fungi and thus reduce the grain quality (Ratnadass *et al.*, 1995; Marley & Ajayi, 1999). In the present study, *C. lunata* and *P. sorghina* were the most frequent pathogens that affect grain discolouration. Discolouration of sorghum may affect the processing quality of grain and its end products as reported by Seetharaman, Waniska, & Rooney (1996) who observed that the blackening of milled products resulted from discolouration of sorghum grains caused by grain mould fungi.

It was evident from the present study that insects are involved either in transporting the fungal propagule or damaging the sorghum kernels which results in higher fungal incidence, leading to reduced grain quality. To minimize insect aided fungal infection and consequent reduction of grain quality, effective insect management strategies should be implemented. Possible control methods include deployment of cultivars resistant to insects and grain mould, weed control particularly those that harbour sorghum insect pests as well as frequent monitoring of sorghum plants. Insects continue to infest grains in storage and create favourable conditions for fungal infection. Thus implementation of sanitation, frequent monitoring, control grain moisture and integration of the possible control methods may control insects and reduce the risk of fungal infection in stored grain.

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Table 4.1 Grain mould fungi isolated from insects collected from Bethlehem and Cedara

Fungus	Total fungi isolated		Fungi isolated (%)	
	Bethlehem	Cedara	Bethlehem	Cedara
<i>Alternaria alternata</i>	37.0	69.0	41.57	38.76
<i>Fusarium</i> spp.	39.0	47.0	43.82	26.4
<i>Epicoccum</i> spp.	1.0	17.0	1.12	9.55
<i>Penicillium</i> spp.	5.0	16.0	5.61	8.9
<i>Alternaria</i> and <i>Fusarium</i> mix	3.0	15.0	3.37	8.43
<i>Alternaria</i> and <i>Epicoccum</i> mix	0.0	11.0	0.00	6.18
<i>Helminthosporium</i> sp.	1.0	1.0	1.12	0.56
<i>Curvularia lunata</i>	1.0	0.0	1.12	0.00
<i>Mucor</i> spp.	2.0	2.0	2.25	1.12
Total	89.0	178.0		

Table 4.2 Grain mould fungal frequency (%) in sorghum grains collected from insecticide treated and untreated plots

Treatment	Line*	Grain mould fungus			
		<i>A. alternata</i>	<i>C. lunata</i>	<i>Fusarium spp.</i>	<i>P. sorghina</i>
Sprayed	MACIA*DORADO-1	41.3 def	1.3 de	8.7 cd	11.3 abcd
	MACIA*DORADO-2	48.7 bcdef	1.3 de	15.3 abc	9.0 abcdefg
	86ED361*MACIA	61.0 ab	2.0 bcde	10.7 abc	3.3 gh
	SURENO* KUYUMA	52.7 abcde	0.7 e	11.0 abcd	4.7 efgh
	ISCV				
	1089BF*MACIA	61.3 ab	1.3 de	16.3 ab	1.7 h
	SC56-14E*86EO361	42.7 def	2.3 bcde	6.7 d	11.0 abcde
	88BE2668	44.0 cdef	1.0 d	10.3 abcd	6.3 bcdefgh
	BLD6(WXY)	43.0 def	3.3 abcde	12.7 abcd	15.0 a
	87EO361*MACIA	37.0 f	6.0 a	9.3 bcd	1.3 h
	02CA5053	57.0 abc	4.3 abc	12.0 abcd	2.3 h
	MACIA*DORADO	48.0 bcdef	2.3 bcde	9.3 bcd	4.7 efgh
Unsprayed	MACIA*DORADO-1	43.3 def	1.7 cde	11.7 abcd	12.0 abc
	MACIA*DORADO-2	46.3 cdef	1.7 cde	12.0 abcd	10.0 abcdef
	86ED361*MACIA	61.0 ab	2.3 bcde	8.3 cd	5.3 defgh
	SURENO* KUYUMA	50.3 abcdef	1.3 de	9.7 bcd	3.0 gh
	ISCV				
	1089BF*MACIA	62.3 a	1.7 cde	17.3 a	2.3 h
	SC56-14E*86EO361	39.0 ef	2.7 bcde	8.3 cd	12.7 ab
	88BE2668	47.0 cdef	3.7 abcd	9.3 bcd	5.7 cdefgh
	BLD6(WXY)	39.7 def	3.7 abcd	13.0 abcd	13.3 a
	87EO361*MACIA	40.0 def	4.7 ab	8.3 cd	2.7 gh
	02CA5053	53.0 abcd	3.7 abcd	12.3 abcd	3.3 gh
	MACIA*DORADO	48.3 bcdef	2.0 bcde	10.0 bcd	3.7 fgh

Means of fungal incidence along the column followed by the same letter do not differ significantly ( $P \leq 0.5$ ) according to Tukey-Kramer multiple comparison test

Table 4.3 100 kernel mass and germination (%) of sorghum grains collected from insecticide treated and untreated plots

Line	100 Kernel mass (g)		Grain germination (%)	
	Sprayed	Unsprayed	Sprayed	Unsprayed
MACIA*DORADO-1	0.94 ef	0.68 gh	46.00 efg	42.00 g
MACIA*DORADO-2	0.90 f	0.65 h	43.75 g	44.50 fg
86ED361*MACIA	0.71 gh	0.73 gh	58.00 bc	56.50 bc
SURENO* KUYUMA	1.55 a	1.43 b	81.50 a	77.25 a
ISCV 1089BF*MACIA	1.00 def	0.64 h	54.00 bcd	56.50 bc
SC56-14E*86EO361	0.98 def	0.93 ef	52.25 bcde	49.00 defg
88BE2668	0.79 g	0.91 ef	58.75 b	57.75 bc
BLD6(WXY)	1.09 d	0.92 ef	55.00 bcd	56.25 bc
87EO361*MACIA	0.95 ef	0.65 h	43.00 g	44.75 efg
02CA5053	1.25 c	1.05 de	58.50 bc	55.50 bcd
MACIA*DORADO	0.81 g	0.59 h	54.75 bcd	51.50 cdef

Means along the column followed by the same letter do not differ significantly ( $P \leq 0.05$ ) according to Tukey-Kramer multiple comparison test

Table 4.4 Mycotoxins isolated from insecticide treated and untreated sorghum grains

Line	Aflatoxin (ppb)		DON (ppm)		Fumonisin (ppm)		Zearalenone (ppb)	
	sprayed	unsprayed	sprayed	unsprayed	sprayed	unsprayed	sprayed	unsprayed
MACIA*DORADO-1	3.38	2.88	0.06	1.45	0.44	0.41	451	590
MACIA*DORADO-2	4.34	3.87	0.34	1.02	0.35	0.54	508	558
86ED361*MACIA	0.13	2.35	2.34	1.21	0.4	0.48	466	375
SURENO* KUYUMA	8.56	1.78	N/A	N/A	0.53	0.42	535	356
ISCV 1089BF*MACIA	4.75	2.53	0.76	0.71	0.43	0.36	533	282
SC56-14E*86EO361	3.67	4.29	0.11	0.33	0.28	0.51	135	339
88BE2668	4.86	2.65	0.14	0.11	0.53	0.38	310	179
BLD6(wxy)	4.95	2.56	0.45	0.28	0.38	0.99	575	124
87EO361*MACIA	2.43	3.03	0.11	0.33	0.54	0.54	494	358
02CA5053	5.21	4.85	0.22	1.37	0.36	0.36	517	346
MACIA*DORADO	3.88	4.04	0.8	0.46	0.38	0.42	529	530

\*ppb= parts per billion; ppm=parts per million; DON=Deoxynivalenol

Table 4.5 Mean fungal frequency isolated from sorghum grains harvested from Potchefstroom insect trial

Line*	<i>A. alternata</i>	<i>C. lunata</i>	<i>Fusarium</i> spp.	<i>P. sorghum</i>
4	67.7 cde	2.3 ab	19.0 a	1.0
10	73.7 abcd	2.7 ab	6.7 bc	1.3
17	62.7 de	2.7 ab	10.7 abc	1.0
27	60.0 e	1.7 b	8.3 bc	2.3
31	65.3 cde	4.7 a	4.7 c	1.7
39	69.7 abcde	3.3 ab	4.7 c	1.3
40	70.7 abcde	2.7 ab	6.7 bc	1.7
41	68.7 bcde	2.0 b	5.3 c	3.0
45	70.7 abcde	1.3 c	10.0 bc	1.7
53	80.7 a	3.7 ab	5.0 c	1.7
70	80.0 ab	2.3 ab	7.3 bc	1.7
80	71.7 abcd	3.7 ab	14.3 ab	1.3
83	71.0 abcde	2.0 ab	5.5 bc	2.0
88	70.0 abcde	3.0 ab	10.7 abc	2.0
90	75.3 abc	2.3 a	7.0 bc	2.3

Means of fungal incidence along the column followed by the same letter do not differ significantly ( $P \leq 0.05$ ) according to Tukey-Kramer multiple comparison test.

\*List of lines refer appendix 4.1

Table 4.6 Grain quality parameters of sorghum grains from Potchefstroom insect trial

Line	Germination (%)	TGMS (%)*	100 kernel mass (g)	FGMS**	Insect puncture
4	81.0 bc	48.3 bc	2.2 def	3.5 ab	6.0
10	80.3 bc	31.7 cdef	3.5 a	3.5 ab	0.8
17	87.0 abc	36.7 cdef	2.3 cd	3.5 ab	0.7
27	87.7 abc	68.3 a	2.5 cd	2.5 ab	4.4
31	81.3 abc	71.7 a	2.3 cd	4.7 ab	0.3
39	92.7 a	41.7 cde	3.1 b	2.5 ab	3.0
40	79.0 c	18.3 f	1.9 ef	2.3 b	2.5
41	80.3 bc3	66.7 b	2.4 cd	4.5 ab	1.8
45	79.7 c	28.3 def	2.3 cd	4.5 ab	2.0
53	91.7 ab	45.0 cd	2.5 cd	3.5 ab	1.4
70	79.7 c	38.3 cde	2.5 c	4.3 ab	2.2
80	81.7 abc	46.7 cd	1.8 f	2.5 ab	1.2
83	78.0 c	25.0 ef	2.2 def	3.5 ab	2.0
88	77.0 c	38.3 cde	1.9 def	2.7 ab	2.7
90	77.0 c	43.3 cde	2.2 cde	4.3 a	1.0

Means along the column followed by the same letter do not differ significantly ( $P \leq 0.05$ ) according to Tukey-Kramer multiple comparison test.

TGMS\* = Threshed grain mould score

FGMS\*\* = Field grain mould score

Table 4.7 Correlation between grain mould fungi and grain quality parameters of sorghum grains from Potchefstroom insect trial

	<i>A. alternata</i>	<i>C. lunata</i>	<i>Fusarium</i> spp.	<i>P. sorghina</i>	Germination	TGMS	Kernel mass	FGMS
Germination	-0.13	0.28	-0.19	-0.28				
TGMS	-0.39	0.27	-0.03	0.38	0.25			
Kernel mass	0.13	0.01	-0.041	-0.15	0.41	0.04		
FGMS	0.25	-0.12	-0.21	-0.21	-0.35	0.23	0.08	
Insect puncture	-0.28	-0.40	0.52*	-0.06	0.10	0.08	-0.13	-0.34

TGMS = Threshed grain mould score

FGMS = Field grain mould score

\*Significantly ( $P \leq 0.05$ ) correlated

Appendix 4.1 List of lines used in the effect of feeding puncture mark on grain mould incidence

Entry number	Line
4	WM#177
10	PRGC/E#222878
17	(MACIA*TAM428)
27	SEGAOLANE*WM#322
31	(6OB128/(Tx2862*6EO361)*CE151)
39	(SV1*SIMA/IS23250)
40	(6OBS14/94CE81-3/GR134B-LG56-*WM#177)
41	(6OBS128/94CE88-3/(Tx2862*6EO361)*EPSON2-40/E#15/SADC)
45	(6BRON161/(7EO366*Tx2783)*EPSON2040/E#15/SADC)
53	(6BRON126/5BRON154/(87BH8606-14*GR107-90M46)*EPSON2-40/E#14/SADC)
70	(CE151*A964)
80	(6BRON161/(7EO366*Tx2783)*HG54-*CE151)
83	(96ADC4/6BRON116/5BRON131/(80C2241*GR108-90M30)-HG46-*WM#177)
88	(MACIA*GR128-92M12)-1
90	(MACIA*GR128-92M12)-2

## **CHAPTER 5**

**The effect of grain mould fungi on the quality of sorghum products and  
its management during malting**

## **Abstract**

To determine the effect of mould fungi on malting quality, samples were taken from different stages of malting. The highest concentration of aflatoxin (11.52 ppb) was found in samples from the steeping stage while lower concentrations were found in subsequent malting stages. Zearalenone and fumonisin were also isolated from the malt samples. Mycotoxin contamination of sorghum products, sorghum brewing grains and other cereal products from supermarket shelves were assayed. Higher concentrations of afltoxin were found in sorghum products followed by maize samples, while aflatoxins were only recorded in two of six wheat samples. Whole grain sorghum for brewing had the highest aflatoxin (16.21 ppb) concentration. No fumonisin was found in all the samples tested. The effect of NaOCl during malting in reducing grain mould fungi and improving malt quality was tested. Significant differences ( $P \leq 0.05$ ) in grain mould fungal frequency, percentage germination and root length were observed between different concentrations of NaOCl. Fungal frequency was reduced by up to 98% at 1.75 and 3.5% NaOCl. However, the germination and root length of grains were negatively affected. Highest germination was recorded at 0.11, 0.21 and 0.43% NaOCl (85, 81.75 and 84.25% respectively) and the longest root length (32.25 mm) was recorded in seedlings treated with 0.43% NaOCl. It was concluded that grain mould fungi are potential mycotoxin producers in sorghum products and may reduce the quality of sorghum products. Treating of sorghum grains with appropriate concentrations of NaOCl may reduce the contamination of grains by mould fungi and improve the quality of malt.

## Introduction

In most parts of Africa sorghum malt provides an important raw material in brewing (Dewar, Taylor & Perjak, 1997). According to Dewar & Taylor (1993), sorghum malt is widely used in South Africa and up to 200 000 t of malted sorghum is used in traditional sorghum beer. However, the quality of grains for malting may be affected by grain mould fungi. Sorghum grains infected with mould fungi such as *Curvularia lunata*, *Fusarium* spp. and *Phoma sorghina* are more prone to breakage than healthy seeds. Grain mould fungi can cause the loss of electrolytes from seeds in leachate, with a resulting loss in seed viability and germination. Seedlings from infected seeds then may be less vigorous than those from healthy seeds (Singh & Agarwal, 1989). Aspects of reduced grain quality therefore, include storage quality, food and feed processing quality as well as market value (Hall *et al.*, 2000). Fungal infection of grains also influences the quality of end products such as beverages. Flannigan *et al.* (1982, cited by Seetharaman, Waniska & Rooney, 1996) reported that moulding of cereals for brewing significantly reduces malt quality. Infection with grain mould also causes lower test weight, endosperm density and decortication yield, increased amylase, protease and lipase activities as well as darker colour of milled products (Seetharaman, Waniska & Rooney, 1996). This results in decreased milling and processing yields, and the quality of sorghum for feed or food (Waniska *et al.*, 2002).

Mould fungi account for the contamination and spoilage of food more than any other group of micro-organisms. They render contaminated food unpalatable and unsafe for consumption due to the production of mycotoxins (Munimbazi & Bullerman, 1996). The main sources of mycotoxins in the human and animal food chains are seedborne

fungi and contaminated agricultural products such as cereals and oil seeds (Tseng, Tu & Tzean, 1995).

The objectives of this study were: (i) To determine the presence of mycotoxins in sorghum grains at different malting stages (ii) to compare the concentrations of mycotoxins in sorghum and other cereal products, and (iii) to evaluate the effect of treating sorghum grains with different NaOCl concentrations during steeping on fungal colonization, germination and root length during the malting process.

## **Materials and Methods**

### ***Mycotoxin and ergosterol analyses during malting***

Samples were collected from different stages of the malting process namely: steeping, malting floor, drying bins and from readily available home brewing malt packs. Mycotoxin levels of the samples and ergosterol concentrations were assayed as follows.

#### *Mycotoxin analysis*

Sorghum grain samples were ground in a laboratory blender so that at least 75% of the material would pass through a 20 mesh sieve.

#### *Aflatoxin and zearalenone tests*

A 5 g ground grain sample was added into 25 ml of 70% methanol/water and agitated vigorously for 3 min. The extract was filtered through a Whatman #1 filter and the filtrate was collected for analysis. The samples were analyzed using respective Veratox®

aflatoxin and zearalenone quantitative test kits (Neogen® Corporation, 620 Lasher Place, Lansing, MI 48912, USA).

#### *Fumonisin test*

A 5 g ground sample was blended with 50 ml of 70% methanol/water solution for 3 min in a high speed blender. The extract was filtered through a Whatman #1 filter and the filtrate was collected. The sample was diluted by adding 100 µl of extract into a prefilled sample dilution bottle from the test kit. The sample was then analyzed for fumonisin using a Veratox® fumonisin quantitative test kit (Neogen® Corporation, 620 Lasher Place, Lansing, MI 48912, USA).

#### *Ergosterol analysis*

Ergosterol was analysed using the method of Sietze *et al.* (1977) as modified by Jambunathan *et al.* (1991). Samples from the different malting stages were ground using a laboratory mill. Twenty grams of flour was added to 50 ml methanol and mixed vigorously in a 100 ml beaker for 30 min using magnetic stirrer. The mixture was centrifuged at 5000 rpm for 5 min. When the suspension had settled, 25 ml of supernatant was added to a thick-walled, screw-capped test tube containing 3 g KOH. This was agitated vigorously on a vortex mixer to dissolve the KOH. Ten ml of n-hexane was added to the mixture after which it was incubated for 30 min in a water bath at 75°C. The mixture was then cooled to room temperature. After adding 5 ml distilled water and mixing thoroughly, the upper hexane layer was transferred to a clean beaker and 10 ml hexane was added to the remaining suspension and thoroughly mixed. The hexane layer

was again removed and added to the previous aliquot and this procedure was repeated again. Finally the pooled hexane extracts were evaporated to dryness in a hot water bath. The residue was re-dissolved in 5 ml high performance liquid chromatography (HPLC) grade ethanol and filtered through a 0.45 µm filter (Millex, Millipore Corporation, Bedford, USA).

Ergosterol content in the filtrate was determined using a Shimadzu CLASS-VP™ HPLC. The extract was loaded on a reverse-phase column (C18 125A 10 µm particle size, 3.9 mm x 300 mm). The mobile phase was methanol-water (96:4, v/v) at a flow rate of 1.2 ml/min. The column temperature was maintained at 50°C, and the absorbance of eluted ergosterol was detected at 282 nm. The ergosterol standard had a retention time of 7.9 min.

### ***Mycotoxin analysis of sorghum and other cereal products***

Grain mould fungi as a possible source of mycotoxin contamination of commercial sorghum products was studied. Samples were collected from supermarket shelves in Bloemfontein, South Africa. Mycotoxin contamination of sorghum products was compared with maize and wheat products. Aflatoxin, fumonisin, and zearalenone levels were determined as outlined above.

### ***NaOCl treatment during steeping***

Steeping was simulated for 15 hours in NaOCl solutions of different concentrations. Sorghum (75 g) were placed in 250 ml beaker containing concentrations of NaOCl of 0.11, 0.21, 0.42, 0.84, 1.75 and 3.5%. The control treatment was water without NaOCl.

### *Grain mould incidence*

Grain mould fungi, were determined from 100 grains with four replications for each concentration after 15 h of steeping. Grains were blot dried using sterile filter paper and plated onto Petri dishes containing malt extract agar (1%) (Biolab<sup>®</sup>) amended with streptomycin sulphate (0.3 ml/L). One week after incubation, the percentage of fungal infected grains was determined.

### *Grain germination and root length*

The effect of NaOCl treatment on grain germination after steeping was evaluated by plating 100 seed samples with four replications of each treatment onto moistened germination paper in Petri dishes (Copeland & McDonald, 2001) and kept at 25°C in the dark. Seeds were regularly monitored and counted as germinated when roots and shoots emerged from the seeds. Germination and root length were recorded 72 h post-incubation.

## **Results**

### ***Mycotoxin and ergosterol analyses during malting***

#### *Mycotoxin analysis*

Mycotoxins were isolated from sorghum samples at different stages of the malting process (Table 5.1). The highest concentration of aflatoxin (11.52 ppb) was found in sorghum samples after steeping. Significantly lower but similar concentrations (3.79-4.97 ppb) of aflatoxin were isolated from the other three stages namely malt floors, drying before milling and home brewing malt. The concentration of zearalenone was

highest in both steeping and malt floor samples with 453 and 454 ppb, respectively. Fumonisin concentrations were similar at all stages.

#### *Ergosterol analysis*

Ergosterol levels differed at various stages of malting (Table 5.1). The highest (31.91 µg/g) level was found at steeping followed by samples from the malt floor. Ergosterol level decreased with each stage of malting with the lowest (10.79 µg/g) ergosterol level recorded in the sorghum malt.

#### *Mycotoxin analysis of sorghum and other cereal products*

Higher concentrations of aflatoxin were found in sorghum products followed by maize samples (Table 5.2). No aflatoxin was recorded in wheat products with the exception of WHT15 that had 3.96 ppb. The whole grain sorghum brewing base exhibited the highest aflatoxin concentration (16.21 ppb). No fumonisin was found in all the samples tested. Zearalenone was found in all products with the highest concentration (910 ppb) in the sorghum product SRG6 followed by the wheat sample WHT19 (594.07 ppb) and the maize sample MZ13 (549.52 ppb).

#### *NaOCl treatment during steeping process*

##### *Grain mould incidence*

NaOCl concentrations had a significant ( $P \leq 0.05$ ) effect on the frequency of grain mould fungal colonies (Table 5.3). The highest fungal frequency (100%) was recorded in the control grains that were steeped in sterile water (Fig. 5.1). Fungal frequency declined

with increasing NaOCl concentrations. At 0.11 and 0.21% NaOCl, the reductions were 16 and 25%, respectively. A notable decline was recorded when the concentration was doubled (0.43 % NaOCl) with 90.75% reduction in grain mould incidence. At concentrations of 1.75 and 3.5% reductions in fungal frequency of 98 and 98.75% were observed respectively.

#### *Grain germination and root length*

NaOCl concentrations had a significant ( $P \leq 0.05$ ) effect on grain germination (Table 5.3). Germination increased as the concentration of NaOCl increased to 0.43% but decreased at higher concentrations. The lowest grain germination was 8.25 % in seeds treated with 3.5% NaOCl.

Similar significant ( $P \leq 0.05$ ) effects were observed on root length (Table 5.4). The longest roots (32.25 mm) were recorded in seedlings treated with 0.43% NaOCl with similar lengths at 0.11 and 0.21% NaOCl. Reduced root lengths were recorded at higher concentrations.

#### **Discussion**

Aflatoxin levels declined from 11.52 ppb during steeping to 3.79 – 4.96 ppb at other stages. The maximum allowable level of aflatoxin in South Africa is 10 ppb (Buitendag, 2002) and most foreign importers reject shipment of commodities with aflatoxin levels higher than 4-15 ppb (Bandyopadhyay *et al.*, 2000). The levels of aflatoxin found in the present study indicates the potential for aflatoxin contamination of grains and malt, that should be closely monitored.

A similar trend was observed for zearalenone although the decline in concentration was not as pronounced with each malt stage. Indications are that zearalenone is a more stable compound. Odhav & Naicker (2002) noted the presence of toxins including zearalenone in commercial and home brewed South African beers although no grain mould pathogens have been detected in beer, as they are destroyed by cooking during the brewing process. In other countries in southern Africa such as Swaziland and Lesotho, zearalenone in mouldy maize, maize porridge, malted sorghum and sorghum beer has also been reported (Sibanda, Marovatsanga & Pestka, 1997).

Concentrations of aflatoxin and zearalenone declined as malting progressed. During steeping, sorghum grain is submerged in water for an extended period. Thus the reduction in mycotoxins subsequent to steeping may be attributed to the slight water solubility of, in particular, aflatoxins which were leached from the grains. During malting, sorghum grains are exposed to heat during drying and then milled. These too may have played role in reducing mycotoxins at later stages of the process. According to Bandyopadhyay *et al.* (2000), drying of sorghum grains by solarization can reduce grain mould incidence.

Fumonisin levels remained constant throughout all malting stages, ranging from 0.44 - 0.56 ppm. The maximum limit for fumonisins in cereals and cereal products for human consumption in European Union countries is 3 ppm (FAO, 2003) while the interim tolerable level in South Africa is 0.3 ppm (Buitendag, 2002). The concentration of fumonisin found in the present study borders on the latter limits. Sorghum malt should be free of toxin producing grain mould fungi and mycotoxins to be accepted. Failing this may render the product unpalatable resulting in reduced demand and therefore losses to

the producer (Lefyedi *et al.*, 2005). Hence, measures should be implemented to minimize the contamination of grains by mycotoxins during malting process.

The concentration of ergosterol was low in all stages of the malting process. The decline observed in ergosterol concentrations as the process proceeded suggests that mould fungi present during steeping do not spread to the plant tissues during germination. As a result, fungal biomass relative to total plant tissue declines, which may account for the decline in mycotoxin concentrations during malting. Ergosterol concentration was positively correlated with aflatoxin levels ( $r = 0.9$ ;  $P \leq 0.05$ ), and although not significant, ergosterol concentration tended to be positively correlated with zearalenone ( $r = 0.7$ ). Despite the low fungal biomass in the present study, the mycotoxin levels recorded indicate the potential of grain mould fungi to contaminate grains during malting.

Mycotoxins varied between cereal products assayed. Aflatoxin was higher in sorghum products followed by maize products while the level of zearalenone in maize and sorghum products was higher than in wheat samples. Compared to maize, groundnut and other oil-rich seeds, sorghum is considered as a poor substrate for the production of aflatoxin (Bandyopadhyay *et al.*, 2000). This apparent anomaly could be due to infection of sorghum grains by *Aspergillus* spp. due to more favourable field conditions (sorghum generally being produced in the drier areas of the country), poor handling in the processing plants, or improper storage. According to Sauer *et al.* (1984), if environmental conditions favour the growth and multiplication of fungi, grain mould fungi can continue to infect grains in storage. D'Mello & Macdonald (1997) reported aflatoxin levels of 152 ppb in sorghum from Uganda, which is unacceptably high. In the present study, the highest concentration (16.21 ppb) of aflatoxin was isolated from whole

sorghum brewing grain. This could be due to the high concentration of aflatoxin in the bran rather than the internal grain parts. Ilang (1984) found higher levels of aflatoxin in rice bran layers compared with polished rice. The removal of the bran layers of the grains during processing could account for reduced aflatoxin levels in flour compared with whole grains.

Zearalenone toxin has been detected in natural sources as well as laboratory substrates and cereal grains (Shotwell, 1977; Palti, 1978). It is found at particularly high concentrations in agricultural commodities notably maize (Logrieco *et al.*, 2003). High concentrations were found in maize and sorghum products collected from supermarket shelves, in some samples beyond acceptable levels. According to Herman (2002), zearalenone occurs particularly in the bran and thus the toxin bearing portion of the grain is removed during milling resulting in lower toxin concentrations in flour. However, when the kernels are heavily infected, toxin levels can be similar in all milled fractions. These results suggest that the grains used to develop the test products may have been highly contaminated before milling.

Some of the cereal products were contaminated by more than one mycotoxin. This co-occurrence of mycotoxins could be due to the simultaneous contamination from substrates by two or more grain moulds of different species. According to Hussein & Brasel (2001), substrates are commonly contaminated by more than one mycotoxin. The contamination of grains by more than one mycotoxin can affect the toxicity of the contaminated grain as synergistic effects have been reported in laboratory animals (FAO, 1994). Swamy *et al.* (2002) reported reduced growth, altered brain neurochemistry and increased serum immunoglobulin concentration in pigs fed with *Fusarium* contaminated

grain. They also noted synergistic toxicity of *Fusarium* mycotoxins such as deoxynivalenol, acetyldeoxynivalenon, fusaric acid and zearalenone. The presence of different mycotoxins in a product may exacerbate the effect of the toxins and reduce the quality of the products.

Treating sorghum grains with appropriate concentration of NaOCl during steeping reduced the grain mould incidence by more than 90%. At the highest concentration of NaOCl (3.5%), grain mould incidence was reduced by 98.75% but percentage germination and root length were also reduced. NaOCl concentration during steeping is critical if toxic effects on grain are to be avoided. There were no significant differences in germination and root length in grains treated with 0.21 and 0.43% NaOCl. However grain mould incidence was significantly reduced when NaOCl was administered at a concentration of 0.43% and this appeared to be the critical concentration to minimize fungal contamination of grains on the malt floor. It should be cautioned, however, the optimal concentrations of NaOCl should also be tested for other malt qualities such as diastatic power and its effect on the aroma.

Grain mould fungi cause losses in seed viability and a subsequent increase in seedling mortality (Williams and McDonald, 1983; Singh & Agarwal, 1989). Reduced germination and root lengths in this study were associated with grain mould fungi. Germination and root length were improved when the concentration of NaOCl increased during steeping. Navi, Bandyopadhyay & Hall (2002) reported a similar improvement in grains that were surface sterilized with NaOCl. Diastatic activity, which results from the combined activity of alpha- and beta-amylase in sorghum malt, increases during germination (Abiodun, 2002), and is an important aspect of malting. Reduced

germination due to fungal infection in the untreated control would therefore affect the enzyme activity of the grain and its quality for malt would thus be compromised.

In the present study it was evident that grain mould results in the production of mycotoxins both during malting and in sorghum end products. The concentrations of mycotoxins produced in certain samples were beyond acceptable levels. This indicates that harvest and post-harvest grain handling, shipping and storage require attention. Grain mould fungi may also affect the malting quality of grains by reducing germination and root length. The results of the present study suggest that treating of grains during steeping with an appropriate concentration of NaOCl can reduce grain mould development on the malt floor and improve malt quality. In addition, the treatment can reduce the contamination of sorghum malt by mycotoxins and minimize the devastating losses that can follow contamination.

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Table 5.1 Mycotoxins isolated from sorghum grains at different malting stages `

Malting stage	Fumonisin (ppm)	Aflatoxin (ppb)	Zearalenone (ppb)	Ergosterol ( $\mu\text{g/g}$ )
Steeping	0.47	11.52	453	31.91
Malt floor	0.49	4.96	454	25.18
Drying	0.44	4.64	367	20.76
Sorghum malt	0.56	3.79	336	10.79

Table 5.2 Mycotoxins isolated from commercial cereal products collected from supermarket shelves

Sample	Aflatoxin (ppb)	Zearalenone (ppb)
SRG1	6.07	37.24
SRG2	9.17	185.39
SRG3	5.21	504.00
SRG4	8.53	522.00
SRG5	4.61	200.65
SRG6	6.78	910.00
SRG7	4.75	626.00
SRG8	16.21	205.00
MZ9	2.47	5.93
MZ10	4.63	11.37
MZ11	0.00	529.33
MZ12	6.76	57.84
MZ13	5.90	549.516
WHT14	5.64	185.95
WHT15	3.96	15.52
WHT16	0.00	4.22
WHT17	0.00	11.46
WHT18	0.00	12.39
WHT19	0.00	594.07

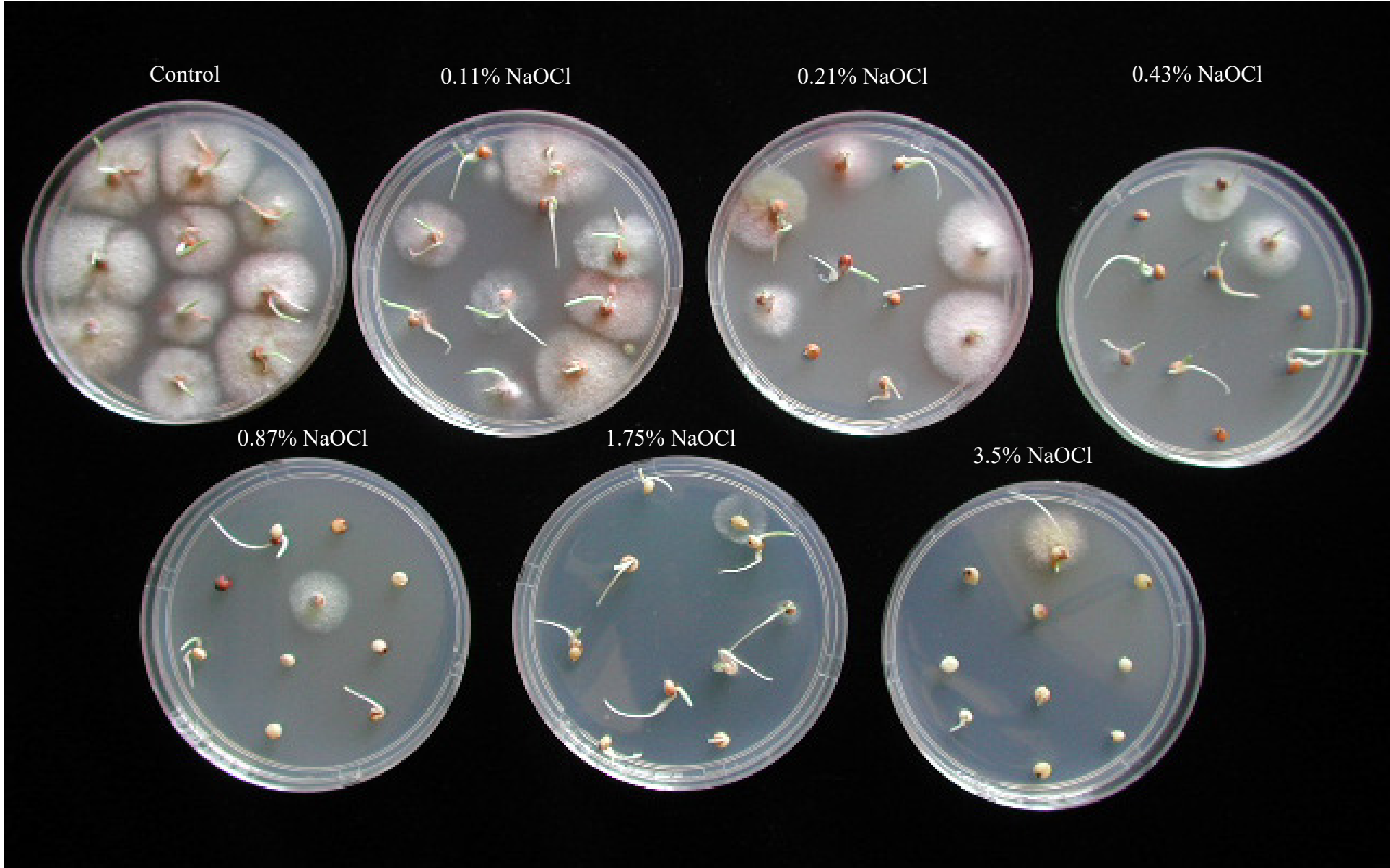
Samples 1-7= sorghum products  
 8 = sorghum brewing base (whole grain)  
 Samples 9-14= maize products  
 Samples 15-19= wheat products

Table 5.3 Frequency of fungal infected grains, grain germination and root length subsequent to steeping

NaOCl concentration	Grain mould incidence (%)	Germination (%)	Root length (mm) of germinated seeds	Mean overall root length (mm)
Control	100.00 a	77.75	26.67 ab	20.93 ab
0.11%	84.00 b	85.00 a	30.67 ab	26.19 ab
0.21%	75.00 c	81.75 c	30.03 ab	24.55 ab
0.43%	9.25 d	84.75 a	32.25 a	27.15 a
0.87%	9.25 d	68.75 b	28.92 ab	19.91 b
1.75%	2.00 e	38.00 c	23.53 bc	8.44 c
3.50%	1.25 e	8.25 d	16.51 c	1.18 d

Means along the column followed by the same letter do not differ significantly ( $P \leq 0.05$ ) according to Tukey-Kramer multiple comparison test

Fig. 5.1 Incidence of fungal infected sorghum grains after steeping in NaOCl at different concentrations.





## SUMMARY

The major sorghum grain mould fungi in South Africa were determined by assaying five sorghum cultivars from three localities in 2002 and eight localities in 2003. The predominant fungal isolates from all the cultivars and localities were *Alternaria*, *Curvularia*, *Fusarium* and *Phoma* spp. The relative frequency of grain mould fungal isolates differed with locality. Grain mould fungi were least frequently encountered at Bethlehem followed by Heilbron. The incidence of grain mould fungi negatively affects the milling and malting quality of sorghum grains. The effect of fungal isolates on various grain quality parameters was examined by inoculating sorghum panicles under glasshouse conditions. All inoculated fungi reduced seed germination with *C. lunata* showing the highest reduction, followed by a mixed population of fungi. *F. proliferatum*, *A. alternata*, *F. graminearum*, *P. sorghina* and a fungal mixture were important pathogens in terms of reducing the 1000 kernel mass. *F. thapsinum*, *F. proliferatum* and the mixed fungal population followed by *P. sorghina*, *C. lunata* and *A. alternata* resulted in higher levels of grain discolouration in glasshouse studies. Various levels of mycotoxin were produced in grains inoculated in the glasshouse and sorghum grains from experimental plots.

The role of weather on the grain mould fungal incidence was determined by planting five cultivars on different planting dates at three localities. Fungal frequency varied across localities and flowering dates. In all cultivars, highest grain mould incidence was recorded at Potchefstroom. *A. alternata* was the most dominant fungus at all localities and at all flowering dates. An increase in moisture and temperature was positively correlated with fungal invasion. Results indicated significant correlations

between grain mould incidence and certain grain quality parameters, such as the milling and malting quality. Wetness duration significantly affected grain mould development and subsequent grain quality under controlled conditions. Increased wetness duration resulted in an increase in grain infection by the respective grain mould fungi.

Sorghum grain infection and subsequent grain quality deterioration as affected by insect damage was assessed in experimental plots at Bethlehem, Cedara and Potchefstroom. Insects were collected from sorghum heads and grain mould fungi were isolated from all specimens collected. *A. alternata* were most frequently isolated from specimens collected from Cedara while the incidence of *Fusarium* spp. were highest in insect samples from Bethlehem. Insecticides significantly increased 100 kernel mass compared with unsprayed grains. Mycotoxin production of grains from the insecticide treated and untreated plots were assessed and some of them produced significant levels of mycotoxins, but the effect was not consistent. Puncture marks created by insect damage were positively correlated with the incidence of *Fusarium* spp. It was evident that insects are involved either in transporting the fungal propagules or damaging the sorghum kernels resulting in higher fungal incidence, which consequently reduced the quality of the grains.

Mycotoxins in samples from different stages of malting were assayed. The highest concentration of aflatoxin was found in samples from the steeping stage. Different concentration levels zearalenone and fumonisin were also isolated from the malting samples. Mycotoxin contamination of commercial sorghum products and other cereal products were assayed. Higher concentrations of aflatoxin were found in sorghum products followed by maize samples while aflatoxin was only recorded in two of six

wheat samples. No fumonisin was found in all the samples tested. NaOCl in water used for steeping resulted in reduced isolation frequency of grain mould fungi with significant improvement in the percentage germination and root length. It was concluded that grain mould fungi are potential mycotoxin producers in sorghum products and may reduce its quality.

The results of the current study confirmed the involvement of various fungi in the disease complex known as grain mould and elucidate their possible role in both qualitative and quantitative losses in sorghum. The results also demonstrated the role of favourable weather conditions and insects in the development of grain mould and the effect that the pathogens have on quality and quantity of sorghum grain. The involvement of insects in the development of grain mould warrants further attention. It is hoped that the findings of this study may serve as motivation for the development of a holistic strategy to manage grain moulds and to maintain the quality of sorghum product.

## OPSOMMING

Die vernaamste fungi wat met skimmel van graansorghum geassosieer word, is deur middel van 'n opname van vyf cultivars by drie lokaliteite in 2002, en agt lokaliteite in 2003 vasgestel. Die mees algemene isolate afkomstig vanaf alle cultivars en lokaliteite het behoort aan die genera *Alternaria*, *Curvularia*, *Fusarium* en *Phoma*. Die relatiewe frekwensie van graanskimmel-isolate het tussen lokaliteite verskil. Die minste graanskimmel-isolate was vanaf graanmonsters uit Bethlehem geïsoleer, gevolg deur Helibron met die tweede meeste. Die voorkoms van graanskimmel het 'n negatiewe invloed op die maal- en moutkwaliteit van graansorghum gehad. Die effek van swamisolate op verskeie kwaliteits parameters van graan was ondersoek deur die inokulasie van sorghumare onder glashuistoestande. Alle geïnokuleerde swamme het saadontkieming verminder. *C. lunata* het die grootste verlaging veroorsaak, gevolg deur 'n gemengde swampopulasie. *F. proliferatum*, *A. alternata*, *F. graminearum*, *P. sorghina* en die gemengde swampopulasie was belangrike patogene in terme van hulle invloed op die verlaging van 1000 pit massa. *F. thapsinum*, *F. proliferatum* en 'n gemengde swampopulasie, gevolg deur *P. sorghina*, *C. lunata* en *A. alternata* het hoë vlakke van graanverkleuring in glashuis-studies veroorsaak. Verskeie vlakke van mikotoksiene was in grane wat in die glashuis geïnokuleer was, asook in die wat vanaf veldproewe afkomstig was, waargeneem.

Die rol van weerfaktore op die voorkoms van graanskimmel is deur die aanplanting van vyf cultivars op verskillende plantdatums by drie lokaliteite bepaal. Swam-frekwensie het oor lokaliteite en met blomdatums gevarieer. Die hoogste voorkoms van graanskimmel is in alle cultivars uit Potchefstroom waargeneem. *A. alternata* was die mees algemene swamsort by alle lokaliteite en met alle blomdatums. 'n Toename in vog en temperatuur is positief gekorreleer met

swamkolonisasie van graan. Resultate het beduidende korrelasies tussen graanskimmelvoorkoms en sekere graankwaliteitsparameters soos maal- en moutkwaliteit, getoon. Die benatingstydperk van are onder beheerde toestande het die ontwikkeling van graanskimmel en graankwaliteit beduidend beïnvloed. 'n Toename in die benatingstydperk het 'n toename in graaninfeksie deur die onderskeie graanskimmelfungi meegebring.

Graansorghum-infeksie en die daaropvolgende afname in graankwaliteit soos deur insekskade beïnvloed, was in eksperimentele persele by Bethlehem, Cedara en Potchefstroom, ondersoek. Insekte was vanaf are versamel en graanskimmelfungi was vanaf alle insekmonsters geïsoleer. *A. alternata* was die mees algemene swamsoort afkomstig van insekte wat in Cedara versamel is, terwyl *Fusarium* spp. die hoogste voorkoms getoon het op insekte wat in Bethlehem versamel is. Insekdoders het 'n beduidende positiewe invloed op 100 pit massa getoon in vergelyking met onbehandelde grane. Mikotoksienproduksie was op grane wat met insekdoder behandel is, asook op onbehandelde grane, bepaal. Sekere behandelings het beduidende vlakke mikotoksiene getoon maar die voorkoms daarvan was nie konsekwent nie. Penetrasiëpunte wat deur insekte veroorsaak is, is positief gekorreleer met die voorkoms van *Fusarium* spp. Dit was duidelik dat insekte óf in die vervoer van swampropagules betrokke is, óf dat hulle skade aan grane veroorsaak wat swamkolonisasie vergemaklik en sodoende graankwaliteit verlaag.

Mikotoksiene in graanmonsters in verskillende stadia van vermoeding is gemeet en die hoogste konsentrasie aflatoksien was in monsters vanuit die “steeping” stadium waargeneem. Verskillende vlakke van zearalenone and fumonisin was ook uit moutmonsters waargeneem. Moontlike mikotoksien kontaminasie van kommersiële sorghumprodukte en ander graanprodukte was bepaal. Hoër

konsentrasies aflatoksien is in sorghumprodukte as in mielie-produkte waargeneem, terwyl dit slegs in twee uit ses koringmonsters waargeneem is. Geen fumonisin was in enige van die monsters waargeneem nie. Die toevoeging van NaOCl tot die water wat vir “steeping” gebruik is, het ‘n verlaagde voorkoms van graanskimmelfungi meegebring, met ‘n gepaardgaande en beduidende verbetering in ontkiemingspersentasie en wortel-lengte. Die algemene afleiding was dat graanskimmelfungi potensieële mikotoksienproduseerders in sorghumprodukte is wat kwaliteit kan verminder.

Die resultate van die huidige studie het die betrokkeheid van verskeie fungi in die siektekompleks bekend as graanskimmel bevestig. Die studie het ook fungi se moontlike rol in beide kwalitatiewe en kwantitatiewe verliese in sorghum verduidelik. Die resultate het ook die rol van gunstige weerfaktore en insekte in die ontwikkeling van graanskimmel en die effek daarvan op kwaliteit en kwantiteit demonstreer. Die betrokkeheid van insekte in die ontwikkeling van graanskimmel verdien dus verdere aandag. Daar word gehoop dat die bevindings uit hierdie studie as motivering sal dien vir die ontwikkeling van ‘n holistiese strategie om graanskimmels te bestuur tot die voordeel van geassosieerde produkte.