EPIDEMIOLOGY OF GRAIN MOULD OF SORGHUM IN SOUTH AFRICA AND ETHIOPIA

By

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PREFACE

This dissertation consists of five chapters. The first chapter is a literature review that highlights the epidemiology and management of sorghum grain mould. Main topics discussed include variation in the causal pathogens, survival, dispersal mechanisms and range of damage caused by grain mould. Moreover, the role of host plants (including alternative hosts) on grain mould epidemiolgy, relationship of grain mould with weather variables and insects, control of grain mould by genetic resistance, chemical, cultural and biological methods are reviewed.

In chapter 2 the major grain mould causal fungi associated with endosperm and embryo of common sorghum cultivars from Ethiopia and South Africa are investigated. The importance of different mould pathogens with regard to damage they cause to seeds and seedlings is discussed.

The third and fourth chapters concern factors affecting grain mould epidemiology. So far, studies on sorghum grain mould have given little attention to epidemiological aspects of the disease. Chapter 3 discusses a three-season study on the relationship between post-flowering weather conditions and grain mould development under field conditions in South Africa. The fourth chapter presents results of field and greenhouse experiments intended to assess the effect of grain development stages on the incidence of mould fungi and on damage to grains.

Chapter 5 attempts to explain resistance factors associated with selected Ethiopian and South African sorghum cultivars and discusses chemical and physical characteristics of grains in relation to grain mould resistance.

Such comprehensive studies of grain mould have not been conducted previously both in Ethiopia and South Africa. It is therefore hoped that the present work provides useful information about grain mould in these two countries.

Since each chapter of this dissertation is compiled in the form of an independent manuscript, it has been impossible to avoid some redundancy mainly with regard to the introductory and reference sections of the chapters.

GENERAL INTRODUCTION

Grain sorghum (*Sorghum bicolor* (L.) Moench) is the fifth most important cereal in the world both in acreage and production (FAO and ICRISAT, 1996). In 2001, over 58.1 million tonnes of sorghum were harvested worldwide from about 42.6 million ha of land with an average yield of 1,364 kg/ha (FAO, 2001). In sub-Saharan Africa, sorghum is the second most important cereal following maize (*Zea mays* L.) (FAO, 1995). In Ethiopia, over 1.2 million ha of land was planted with sorghum in 2001, a larger area covered after wheat and maize. In the same year, the area under sorghum in South Africa was about 90, 300 ha (FAO, 2001).

Sorghum is used mainly for human consumption and as animal feed although its utilization in alcohol production and especially in the brewing industry is increasing (Hall *et al.*, 2000). In Africa and Asia, more than 70% of the crop is used as food while in developed countries such as the USA, it is used mainly for animal feed (FAO, 1995). The majority of sorghum produced in Ethiopia is used as food for humans with the rest utilized for home-made beverages. As an 'injera' (leavened traditional Ethiopian bread), sorghum ranks second to teff (*Eragrostis tef* (Zucc.) Trotter) in consumer preference in Ethiopia (Yilma and Abebe, 1984). In South Africa, sorghum is produced both by commercial and small-scale farmers mainly for traditional food and the production of opaque beer (Wenzel *et al.*, 1997). With the current alarming population growth and repeated drought occurrence in Ethiopia, cereals such as sorghum, which can adapt to relatively dry climate conditions, may help to curb the increasing demand for grain food.

Despite its importance in Ethiopia, the yield of sorghum in this country is lower (1,167 kg/ha) compared with the world average (1,364 kg/ha) and with that attained in the developed world (over 3762 kg/ha) (FAO, 2001). In addition to a lack of agricultural inputs for small-

holder farmers in developing countries, biotic factors such as diseases can significantly reduce sorghum grain yield and quality.

Grain mould is a major constraint to sorghum production worldwide. The predominant causal agents include fungal species *Fusarium moniliforme* Sheld., *Curvularia lunata* (Wakk.) Boedijn, *Fusarium semitectum* Berk. and Ravenel and *Phoma sorghina* (Sacc.) Boerema, Dorenbosch and van Kesteren (Singh and Agarwal, 1993). The disease reduces grain size (yield) and quality resulting in discoloured and less viable grains, which may also be contaminated by mycotoxins that can be harmful to animals and humans (Somani *et al.*, 1993).

The main fungal species that cause grain mould may differ based on geographical location (Menkir *et al.*, 1996a; Somani and Indira, 1999). Furthermore, species other than the major grain mould fungi mentioned above may be important in some regions (Menkir *et al.*, 1996b). In Ethiopia and South Africa, there is insufficient information on the prevalence and importance of sorghum grain mould pathogens.

Weather variables and host plant growth stages are major factors that influence disease dynamics. Understanding the effect of these factors may thus help reduce management costs and contamination of the environment by employing control methods such as fungicides at the right growth stage and only when optimum conditions for disease development are met. In major Ethiopian and South African sorghum cultivars, knowledge is lacking about mould progress in relation to grain development stages. Similarly, most information about influences of weather conditions on grain mould of sorghum is based largely on hypotheses lacking experimental evidence (Singh and Agarwal, 1993). Studies relating to quantitative relationships between weather variables and grain mould incidence are in particular very limited.

Since the use of genetic resistance is thought to be the most economical and effective method of grain mould control (Chandrashekar *et al.*, 2000), most studies on grain mould control have focused on development of resistant cultivars. A good knowledge of host plant resistance mechanisms is essential to breed durable mould resistant cultivars possessing a variety of resistance mechanisms (Audilakshmi, 1999; Chandrashekar *et al.*, 2000). Studies aimed at identifying resistance mechanisms in cultivars adapted to different geographical areas are therefore essential.

It is in the light of the above research requirements that the objectives presented in this dissertation were identified. Briefly, common fungi associated with seeds of major cultivars grown in Ethiopia and South Africa were determined first and then the importance of these fungi as grain mould pathogens was studied. Next, the effects of meteorological factors and grain development stages on grain mould were assessed and finally putative resistance mechanisms in certain cultivars adapted to both countries were determined.

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CHAPTER 1

REVIEW OF EPIDEMIOLOGY AND CONTROL OF SORGHUM GRAIN MOULD

INTRODUCTION

Epidemiology deals with the development of disease in plant populations. Plant disease dynamics are influenced by the host, pathogen as well as by other biotic and abiotic factors which interact with the former two entities. A knowledge of disease development as related to these factors may therefore aid in accurately predicting when disease may occur. Consequently, appropriate control options can be selected well in advance and applied at the right time (Maloy, 1993). In this way, effective and economical management practices can be employed.

The aim of this review is to present information related to the epidemiology and control of grain mould. The review is divided into five main sections. The first section examines the role of grain mould pathogens on disease development. Since the biology and genetic composition of pathogens have important influence on disease dynamics (Wolfe and Caten, 1987), this section emphasises on inter- and intra-species variations, survival and dispersal mechanisms of grain mould pathogens. The effects of these pathogens on grain damage including contamination with mycotoxins are also discussed. The second section deals with disease epidemics as influenced by the host plant. The overall susceptibility of sorghum and the effects of host growth stages on disease development are discussed. Furthermore, the possible role of alternative hosts on disease severity is also referred to. Thirdly, the effect of environmental factors such as moisture and temperature are presented. Moreover, the interactions of biotic factors (insects and other pathogens) with grain moulds are discussed in detail.

Various techniques presently employed for grain mould assessment and seed-borne pathogen detection are considered in the following section. The availability of such tools is vital in epidemiological studies of grain mould. Finally, different approaches to control grain mould are presented. Considerable attention has been given to the use of genetic resistance.

Techniques for screening, chemical and physical factors associated with resistance and the possible role of biotechnology in breeding for mould resistance are treated under this topic. Chemical, cultural and biological control methods are also briefly described. Relevant studies on cereals other than sorghum have also been considered since seed pathogens on these crops appear to be closely related to grain mould fungi of sorghum.

Presently, several species of *Fusarium* associated with grain sorghum have been identified. Many such species were designated in past literature as *Fusrium moniliforme*. It is difficult to determine which species the *F. moniliforme* in most literature is referring to. In many cases, *Fusarium thapsinum* and *Fusarium verticillioides* might have commonly been considered as *F. moniliforme*. The former is mostly isolated from sorghum while the latter is commonly encountered in maize. The tendency at present is thus to name *F. moniliforme* in sorghum grain mould literature as *F. thapsinum* (Frederiksen and Odvody, 2001; Leslie and Marasas, 2001). Accordingly, unless a clear distinction is found to designate it as *F. verticillioides*, *F. moniliforme* commonly reported on sorghum is considered *F. thapsinum* in this dissertation.

INFLUENCE OF PATHOGEN

Causal organism. Even though fungi from over 40 genera may be associated with seeds of sorghum (*Sorghum bicolor* (L.) Moench (Bandyopadhyay, 1986), *Fusarium moniliforme* Sheld., *Curvularia lunata* (Wakk.) Boedijin (*C. lunata*), *Fusarium semitectum* Berk. and Ravenel and *Phoma sorghina* (Sacc.) Boerema, Dorenbosch and van Kesteren are the predominant species capable of infecting immature grains (Forbes *et al.*, 1992; Singh and Agarwal, 1993; Singh and Bandyopadhyay, 2000). Most other fungi encountered on sorghum seeds are not considered true pathogens but have been thought of as superficial colonizers at later development stages that cause relatively less damage (Forbes *et al.*, 1992; Singh and Agarwal, 1993). Late colonizers however may go deeper if favourable conditions extend to post maturity stages (Forbes *et al.*, 1992). Some of these late colonizers have been encountered on immature kernels, as are the true pathogens of grain mould. For instance, *Cladosporium* and *Epicoccum* spp., that are often considered saprophytes on sorghum, have been isolated from immature sorghum as early as seven days after anthesis with incidences of 14.5% and 4.8% respectively. In contrast, the incidence of *F. thapsinum* was 3.2% (Melake-Berhan *et al.*, 1996). Accordingly, Forbes *et al.* (1992) stressed the necessity of determining the importance of the most frequently observed species other than the "four" true pathogens referred to above. The genera *Alternaria, Helminthosporium, Drechslera, Bipolaris, Colletotrichum* and *Cladosporium* are most commonly isolated from sorghum seeds (Williams and Rao, 1981).

Although three or four major grain mould pathogens may be of worldwide importance, other species of local importance may prevail (Menkir *et al.*, 1996b). Based on observation at 40, 50 and 60 days after anthesis (Menkir *et al.*, 1996b), the incidence of *Gibberella zeae* (Schw.) Petch. was significantly related to grain discolouration at all grain development stages while *F. thapsinum* was linked to discolouration only at 60 days after

anthesis. This finding led to the suggestion that *G. zeae* was more important than *F. thapsinum* in causing late seed discolouration, at least in that locality.

Some commonly encountered fungi, for example *Alternaria* spp., although apparently unrelated to grain discolouration (Menkir, *et al.*, 1996b), can cause other forms of grain mould damage. *Alternaria alternata* (Fr.:Fr.) Keissl. is known to contribute to increased fat acidity and starch depletion in maize (Paul and Mishra, 1994). These fungi may also produce hazardous mycotoxins even if they are considered late invaders of sorghum grains. Therefore, the role of the frequently encountered species, other than the established grain mould pathogens, must be determined based on the various forms of damage they may cause. Although their significance remains to be established, *Colletotrichum graminicola* (Ces.) G.W. Wilson (*Cl. Graminicola*) and *A. alternata* have been listed important grain mould fungi (Singh and Bandyopadhyay, 2000).

Another point of debate regarding grain mould pathogens is the issue of grain mould in relation to sorghum head blight (Forbes *et al.*, 1992). It is unclear whether head blight caused by a strain of *F. thapsinum* is the same strain that causes grain mould (Forbes *et al.*, 1992). Williams and Rao (1981) tend to believe that the two pathogens are different. On the other hand, Frederiksen (2000) considers *F. thapsinum*, the cause of grain mould of sorghum, to also cause head blight. Singh and Bandyopadhyay (2000) support this view, reporting that the grain mould fungus can also cause head blight and kill developing spikes. Forbes *et al.* (1992) on the other hand reported that inoculation with *F. thapsinum* commonly caused grain mould but head blight symptoms did not always result. Mansuetus (1990) found that *F. thapsinum* isolates from peduncles and glumes could cause grain mould but were not as pathogenic as those from caryopsis. Others believe that the pathogens might not be different but that the two tissues might differ in resistance (Forbes *et al.*, 1992). Cultivars with

moderately high resistance to grain mould were reported to be susceptible to head blight (Frederiksen, 2000).

F. thapsinum has also been reported to cause root and stalk rot of sorghum (Nyvall, 1989). Isolates from sorghum seeds have been shown to cause stalk rot in the greenhouse following artificial inoculation (Jardine and Leslie, 1992) and no significant differences in resulting disease severity were found between these isolates and those obtained from stalks. Stalk and/or root colonization of sorghum by *F. monilifome* and *Alternaria* spp. have also been known to increase from anthesis to grain fill (Reed *et al.*, 1983) as commonly seen in grain moulds. Whether stalk colonization is important in grain mould epidemiology awaits investigation. Perhaps, spores from infected stalks or roots may serve as a source of inoculum for panicle infection and *vice-versa*. Red stalk rot infection caused by *Cl. graminicola* is known to occur when spores from heads were washed into leaf sheaths to infect stalk tissues (Vincelli and Hershman, 2001).

Pathogen variability. When physiological races are common in a given host-pathogen system, it is more likely for disease outbreaks to occur. The role of pathogen strains in grain mould epidemiology however seems to be negligible (Jardine and Leslie, 1992; Mansuetus *et al.*, 1997). *F. moniliforme* has been shown to consist of different biological species (*Gibberella fujikuroi* (Sawada) Ito mating populations). Mating population F (*Fusarium thapsinum* Klitich, Leslie, Nelson & Marasas) is common on sorghum while mating poulaion A (Fusarium verticillioides) is prevalent on maize (Jardine and Leslie, 1992). In some localities however, *F. verticillioides* has been found to be the most frequent isolate from sorghum seeds and is considered potentially more important than *F. thapsinum* (Mansuetus, 1997). Furthermore, differences in the pathogenicity of the two species on sorghum, is not well defined.

Mansuetus *et al.* (1997) reported that the composition and frequency of *G. fujikuroi* on sorghum from different geographical locations are relatively constant. They concluded that disease expression was related to environment and host genotype rather than to a biological species being present in a specific area. Moreover, Jardine and Leslie (1992) reported the absence of clear differences in aggressiveness among different vegetative compatible groups (VCGs) of *F. thapsinum* predominant on sorghum. In addition, the low number of VCGs in the group led to the conclusion that the chance for mutation in this pathogen would be very low (Jardine and Leslie, 1992).

Loss of resistance to grain mould has recently been observed in Tanzania where *F. thapsinum* is a major cause of grain mould (Mansuetus *et al.*, 1995). This may indicate that more pathogenic strains may occasionally arise within *F. thapsinum*. It was subsequently suggested that screening for grain mould resistance be carried out using all strains of the pathogen to obtain durable resistance (Mansuetus *et al.*, 1995). Cmplex of *Fusarium* spp. Are associated with grain sorghum (Leslie and Marasas, 2001) and new species are being identified from time to time. Thus, the detailed difference in the pathogenicity of such species is yet to be investigated it appears that *F. thapsinum* and *Fusarium andiyazi* Marasas, Rheeder, Lamprecht, Zeller and Leslie appeared more aggressive on sorghum (Leslie and Marasas, 2001).

Variations in genera other than *Fusarium* spp. have received considerably less attention. Somani *et al.* (1994) compared pathogenicity and cultural characteristics of *C. lunata* isolates from four locations in India. Although isolates from two locations were more pathogenic on sorghum than those from the remaining two locations, *in-vitro* growth and sporulation characteristics of all isolates were similar. It thus appears that variability within *C. lunata* may be of less epidemiological importance in sorghum grain mould.

Inter-species variation has been shown to have greater epidemiological significance in sorghum grain mould. The main species that cause grain mould tend to vary in the rate and degree of seed colonization as well as in the ability to invade different seed parts and developmental stages. *F. thapsinum* and *C. lunata* can infect flowers including lodicules, filaments, palea, glumes and lemma and both these pathogens can invade the ovary, endosperm and even embryo within five to ten days after anthesis (Singh and Bandyopadhyay, 2000). *F. thapsinum* also infects pedicels (Singh and Bandyopadhyay, 2000). In addition, infection by these two fungi is known to hasten seed maturity by up to 10-18 days (Bandyopadhyay, 1986) and such untimely-matured seeds are usually under-sized.

Bsed on inoculation at 50% flowering under glasshouse conditions, Singh *et al.* (1988) found infection by *C. lunata* and *F.* ***thapsinum*** to become established within three days and *P. sorghina* within eight days after anthesis. *C. lunata* progressed to the endosperm and embryo within 10 days while *F.* ***thapsinum*** did so within five to ten days after anthesis. *P. sorghina* colonized the ovary wall, aleurone layer or the pericarp but not the other seed parts (Forbes *et al.*, 1988; Singh *et al.*, 1988; Singh and Agarwal, 1989). Forbes *et al.* (1988) further observed that *F.* ***thapsinum*** was more important in embryo infection than *C. lunata* in that its recovery from embryos was not affected by the degree of seed infection whereas with *C. lunata*, invasion of the embryos was affected by the overall degree of seed infection.

Differences in pathogenicity of grain mould fungi can be viewed from the perspective of resulting damage. *C. lunata*, *F.* ***thapsinum*** and *P. sorghina* reduced the 1000 seed mass of sorghum by 67, 43 and 40% respectively (Singh and Agarwal, 1989). Moreover, *F. thapsinum* caused the maximum loss of electrolytes. Gopinath and Shetty (1992) reported that *F.* ***thapsinum*** resulted in severe seed rot and seedling blight followed by *F. semitectum, Fusarium oxysporum,* and *Fusarium solani* indicating the more aggressive nature of the former. *C. lunata* and *F.* ***thapsinum*** infection of grains changed the amino acid spectrum

and also caused a reduction in crude fat, starch, crude protein and ash (Somani *et al.*, 1993). On maize, *F. verticillioides* is known to contribute to increased fat acidity and starch depletion (Paul and Mishra, 1994).

Survival. Few studies on the survival of sorghum grain mould pathogens are documented. However, much information can be obtained from related crops. Nyvall (1989) stated that *F. thapsinum*, as the cause of sorghum head blight, might survive as mycelium in infected sorghum or maize residues. *F. verticillioides* on maize has been known to survive in the laboratory as microconidia for 900 days under different humidity and temperature regimes (Liddell and Burgess, 1985). In the field, hyphae and conidia also survived winter temperatures for two seasons without loss of pathogenicity and viability (Manzo and Claflin, 1984).

F. thapsinum and *Fusarium proliferatum* constituted some of the major *Fusarium* spp. isolated from soil debris in sorghum fields (Leslie *et al.*, 1990). McGee (1995) reported *Fusarium, Alternaria* and *Cladosporium* spp. to be common inhabitants of soil and crop residues from where they can infect seeds of many crops during maturation. Likewise, infested debris is known as an important source of *F. verticillioides* for maize stalk rot disease (Skoglund and Brown, 1988).

Being facultative parasites, grain mould pathogens can readily reproduce on plant debris and decaying organic matter in soil as well as on the lower senescent leaves of sorghum (Bandyopadhyay, 1986; Bandyopadhyay *et al.*, 1991). It may thus be assumed that infested debris in the field can aid the survival of grain mould pathogens of sorghum and possibly become the source of inoculum for panicle infection. After observing a logarithmic decrease in the spore concentration of *Fusarium* and other fungal species from within and above sorghum fields, Reddi and Ramakrishna (1978) suggested that the source of inoculum for infection of panicles should arise mainly from within the host canopy. Such patterns in

spore concentrations from ground level upwards, are known to occur when inoculum source is mainly from within the host canopy (Eversmeyer and Kramer, 1987). Moreover, increased amounts of inoculum near ground level as opposed to higher levels may imply that crop residues are important inoculum sources (Reddi and Ramakrishna (1978).

Cultural practices have been shown to affect the survival of *F. **verticillioides*** in residues. Cotton and Munkvold (1998) studied the survival of *F. **verticillioides***, *F. proliferatum* and *Fusarium subglutinans* in maize residue under different cropping systems and tillage practices. After 630 days, the pathogens were recovered from residues with more inoculum obtained from continuous maize and less from maize/soybean (*Glycine max* (L.) Merr)/ oat (*Avena sativa* L.) rotations. Survival was greater from surface residues than from those buried to 15 or 30 cm. They concluded that these fungi could survive for at least 630 days and that residues could serve as long-term sources of inoculum for maize ear infection.

Grain mould pathogens can also survive in seed and *F. **verticillioides*** was shown to survive in maize seed for up to five years (Nyvall and Kommedahl, 1968). Bandyopadhyay (1986) also reported *F. **verticillioides*** on sorghum to be seed-borne but doubted the role of seed-borne inoculum as a direct cause of grain mould. Munkvold *et al.* (1997) compared the paths of infection of maize kernel by F. ***verticillioides*** under field conditions. They observed transmission from inoculated seeds to the developing kernel. Infection through silks was most effective in increasing kernel infection. The pathogen also infected kernels from inoculated crowns and stalks, although not as effectively as infection through silks (Munkvold *et al.*, 1997).

Dispersal. Fungi that commonly infect grains appear to be air-borne. *Fusarium, Alternaria, Curvularia, Helminthosporium, Cladosporium* and *Epicoccum* spp. have been reported to form large numbers of air-borne spores (Halwagy, 1994; Li and Kendrick 1994). Similarly, *Alternaria* and *Curvularia* spp. were commonly found in air over wheat fields at

Peshwar, Pakistan (Safdar *et al.*, 1992) and wind was known to be important in the dissemination of *Fusarium* head blight of wheat (Fernando *et al.*, 1997). In sorghum grain mould, Bandyopadhyay (1986) and Bandyopadhyay *et al.* (1991) reported that sorghum grain mould pathogens might be disseminated by wind and rain splash. Furthermore, Bandyopadhyay *et al.* (1991) observed spores of *Fusarium*, *Curvularia* and *Alternaria* spp. in the air above sorghum field during all grain developmental stages starting at flowering. They also isolated *F. thapsinum*, *C. lunata, Alternaria tenuissima* (Kunze: Fr.) Wiltshire and *P. sorghina* from seeds of sorghum grown in the same field. In maize, Ooka and Kommedahl (1977) suggested that *F. verticillioides* may multiply rapidly on residues, leaf surfaces and water trapped in leaf sheaths from where insects, rain and wind could disseminate them to different parts of the crop.

Bandyopadhyay *et al.* (1991) demonstrated that wet conditions and high humidity favour spore production and dispersal. They reported that spore concentrations of grain mould pathogens in the air above sorghum fields increased in wetter seasons and decreased in dry seasons. They also showed that rain contributed to the dispersal of *Fusarium* spp. but heavy rain-washed spores from the air. Similarly, populations of *Fusarium* and *Curvularia* spp. increased in soil during wet seasons in cereal fields (Fakir *et al.*, 1989).

Whether secondary infections occur and/or play an important role in the development of sorghum grain mould remains to be investigated. Based on the significant increase in the numbers of spores of grain mould pathogens in the air after hard dough stage in sorghum seeds, it has been suggested that in addition to other sources, moulded grains in the field may also contribute to such increments (Bandyopadhyay *et al.*, 1991). Experimental evidence that sporulation on infected grain may serve as a source of secondary infection in the field is limited. However, Bandyopadhyay *et al.* (1991) cited unpublished sources, which recognize that sporulation occurs on grain surfaces after the hard dough stage. They also found that

mature infected grains released spores of *Curvularia, Fusarium* and *Alternaria* spp. into the air upon being shaked. In contrast, *Fusarium* head blight of wheat has yielded no evidence of secondary infection (Fernando *et al.*, 1997). The pattern of maize ear infection by *F. verticillioides* has also been shown to be monocyclic (King, 1981). In sorghum, the contribution of secondary spores to grain mould severity requires further study.

Mycotoxin production. Grain mould pathogens differ in their ability to produce harmful toxins. In a review on grain mould, Forbes *et al.* (1992) reported aflatoxin and zearalenone contamination of sorghum grain. They also concluded that evidence for toxicity to animals was limited and was based on speculation. A few cases of suspected mycotoxicosis to swine due to aflatoxin, ochratoxin and zearalenone were cited (Forbes *et al.*, 1992).

Mycotoxin production by *Fusarium* spp. on sorghum has been studied and a strong and positive correlation has been observed between visible grain mould on sorghum caused by *Fusarium* spp. and zearalenone and vomitoxin (Bowman and Hagler, 1991). In millet (*Pennisetum glaucum* (L.) R. Br. or *Pennisetum americanum* (L.) Leeke), seeds harvested 33 days after anthesis, were found infected principally with *F. semitectum* (26%), *Alternaria* spp. (19%) and *Curvularia* spp. (13%) with total isolation frequencies of *Fusarium* spp. averaging 46% (Wilson *et al.*, 1993). Aflatoxin, deoxynivalenol (vomitoxin), nivalenol, zearalenone and acetylscirpentriol were extracted from grains infected with these pathogens. Concentrations of tricothecene and zearalenone were related to the incidence of *Fusarium chlamydosporum* Wollenw. & Reinking (R = 0.66). Deoxynivalenol has been known to inhibit protein synthesis and its contamination of wheat and barley caused losses exceeding three billion dollars in the USA from 1991 to 1996 (Trail, 2000). Recently, the need for investigations into the distribution of trichothecenes in sorghum growing areas has been strongly advocated (Forbes *et al.*, 1992).

Carcinogenic potential (on fore-stomach and liver) of the mycotoxin produced by *F. verticillioides* (N-3-methybutyl-N-1-methylacetonyl nitrosamine) has been demonstrated on rats. Moreover, this and other mycotoxins were extracted from cooked food or processed products (flour) inoculated with *F. verticillioides* (Li *et al.*, 1986). This fungus is also known to produce other carcinogenic toxins including fumonisin B1 and B2 in maize as does *F. proliferatum.* Recently, studies in sorghum samples obtained from various sources of sorghum growing regions in India indicated higher levels of fumonisin contamination in mouldy grains than normal ones (Bhat *et al.*, 2000). Some isolates of *F. verticillioides* from these mouldy grains produced 5.8 to 27.4 µg/g fumonisin in the laboratory. Furthermore, people who consumed moulded grains (colonised predominantly by *Fusarium* spp., *Aspergilus* spp. and *Alternaria* spp.) were diseased and higher levels of Fumonisin B1 were found in these grains (Bhat *et al.*, 2000)

Fusarium verticillioides and *F. proliferatum* are known to be major fumonisin producers even though these species were encountered less commonly on sorghum (Leslie and Marasas, 2001). On maize and maize products (animal feed and human foods), fumonisin concentrations ranging from 0.3 to 330 µg/gram of product were recorded (Bacon and Nelson, 1994). Munimbazi and Bullerman (1996) also obtained 12.2 to 75.2 µg fumonisin B1/gram of maize and sorghum meal in Burundi. Rheeder *et al.* (1992) and Keyser *et al.* (1999) reported the association of fumonisins with oesophageal cancer in humans.

F. verticillioides and *F. proliferatum* also produce moniliformin, fusarin c and fusaric acid (Bacon and Nelson, 1994). The effect of moniliformin on humans and animals after consuming contaminated sorghum is not well understood although it is produced by the major species associated with sorghum (Leslie and Marasas, 2001). Maize colonized with cultures of *F. verticillioides*, caused leg weakness in chicken and reduced immune response (reduced antibody responses to SRBC) in chicks (Marijanovic *et al.*, 1991). The consumption of

mouldy sorghum grains is known to cause body aches, fever, eye burning and loss of appetite in humans (Singh and Bandyopadhyay, 2000).

High frequencies of *A. alternata,* that were tumorigenic, have been isolated from wheat, maize and millets in areas with a high incidence of oesophageal cancer (Liu *et al.*, 1988). Alternariol extracted from these isolates were shown to be involved in the tumourgenicity. In contrast, alternariol contaminated sorghum grain fed to chicks and rats failed to show any sign of toxicity (Forbes *et al.*, 1992). In addition, maize, rice and tomato infected with *A. alternata* were shown to have altertoxins (Visconti *et al.*, 1991).

All isolates of *A. alternata* associated with cereal seeds (wheat, barley, maize, oat and rye) obtained from eight different Mediterranean countries, produced tenuazonic acid (Logrieco *et al.*, 1990). No reports of tenuazonic acid contamination of sorghum are known despite the common occurrence of *A. alternata* on sorghum seeds (Forbes *et al.*, 1992). Furthermore, Forbes *et al.* (1992) indicated the production of tenuazonic acid by *P. sorghina* and presumed the possible involvement of this toxin in onyalai disease (haemorrhagic vesicles in the mouth) of humans in Africa.

From an epidemiological point of view, Forbes *et al.* (1992) reported that short-season sorghum varieties, that develop seeds under wet conditions, had high aflatoxin levels (10-80 µg/g) while long-season varieties that were not exposed to rain subsequent to milk stage had no mycotoxins. However, long-duration varieties grown in moist conditions did also show mycotoxin contamination (100 µg/g). In addition, aflatoxin and zearalenone contamination appeared to start at the hard dough stage and mycotoxin production by grain mould pathogens was known to continue in storage (Forbes *et al.*, 1992). Bhat *et al.* (2000) reported that moist conditions during sorghum grain development to harvesting were conducive to mould and fumonisin production.

INFLUENCE OF HOST

Genetic susceptibility. The susceptibility of a host plant to infection is an important factor in disease development. Generally, grain sorghum seed, including the various floret parts, are readily infected by grain mould pathogens. According to Forbes *et al.* (1988), infection of sorghum florets by some grain mould pathogens may occur as early as three days after anthesis. They observed 44% infection incidence of *F. thapsinum* five days after flowering. Seeds can therefore be colonized very rapidly and infection can be established within five to ten days in all grain parts including the pericarp, endosperm and embryo (Singh and Bandyopadhyay, 2000). Complete immunity of sorghum to grain mould has rarely been reported (Williams and Rao, 1981; Mukuru, 1992).

Sorghum varieties may vary in resistance and traditional varieties in Africa and Asia tend to escape grain mould. Flowering in these varieties in countries such as India, is related to day length (photoperiod-sensitive) and hence they develop to maturity when the rainy season favouring disease development has ended (Forbes *et al.* 1992; Mukuru, 1992; Hall *et al.*, 2000). However, improved, short-to medium-duration photoperiod-insensitive cultivars are mostly prone to grain mould since they usually develop during the rainy period and are thus exposed to moist conditions (Singh and Bandyopadhyay, 2000). Grain mould appears to have increased in importance with the increased cultivation of such susceptible, short-duration photoperiod-insensitive cultivars (Singh and Agarwal, 1993). Despite the ability to escape grain mould, the traditional and/or photoperiod-sensitive cultivars have low yields because of exposure to drought stress during maturity (Mukuru, 1992; Singh and Bandyopadhyay, 2000). It is, therefore, likely that the incidence of grain mould could become devastating where susceptible varieties are widely grown under conditions suitable for disease. In 1976, grain mould destroyed over 400,000 ha sorghum in Texas resulting in losses in exceeding $46

million (Castor and Frederiksen, 1980). This confirms that grain mould may develop to epidemic levels following the large-scale cultivation of susceptible varieties.

The general susceptibility of grain sorghum to this disease is reflected by the response involving both quantitative and qualitative damage (loss). Detailed yield loss studies due to grain mould have not been conducted but yield losses of 30 –100% have been proposed (Williams and Rao, 1981; Singh and Bandyopadhyay, 2000). In Asia and Africa alone, economic loss due to grain mould has been estimated to be more than US$ 130 million (Chandrashekar *et al.*, 2000).

Susceptibility to early infection may lead to the abortion of the ovary resulting in fewer grains per panicle. Moreover, such infections at the outset of flowering may cause a reduction in seed size and mass (Bandyopadhyay, 1986; Esele, 1995) through interference with grain filling and/or premature black layer formation. Severe infection also results in softening of the caryopsis that easily disintegrates thus being liable to destruction during harvesting and threshing (Bandyopadhya, 1986; Singh and Agarwal, 1989; Esele, 1995).

Infected grains commonly show surface discolourations and consequently fetch lower prices (Williams and Rao, 1981). Grain mould could also negatively affect the chemical composition of seeds, including proteins and starches (Singh and Agarwal, 1987; Singh and Bandyopadhyay, 2000). Consumption of grains contaminated by mycotoxins produced by some grain mould pathogens can impose severe health hazards to animals and humans (Munimbazi and Bullerman, 1996; Singh and Bandyopadhyay, 2000). Grain mould also causes a loss of viability whereby germination or the emergence of seedlings is reduced (Williams and Rao, 1981; Singh and Agarwal, 1989). Even though definitive evidence is lacking, the transmission of pathogens to seedlings thereby resulting in seedling blight may occur and this is also assumed to be an aspect of grain mould damage (Williams and Rao, 1981).

Temporal susceptibility. The application of disease control practices when a host plant is most susceptible to diseases provides for effective and economical control (Maloy, 1993). For example, based on knowledge about host susceptible stage, the use of fungicides at the appropriate time can maximize profitability. It is therefore important to accurately identify the relevant growth stage. Maloy (1993) considered the identification of the susceptible stage as one of the essential criteria for the development of a disease forecasting system.

Tarr (1962) stated that infection of sorghum grains by grain mould pathogens may occur at any stage from young inflorescence to maturity as long as moist conditions prevail. Whether the degree of infection, at different development stages varies or not, should favourable conditions occur, was not established. Subsequent studies have indicated that the incidence of grain mould pathogens increases subsequent to anthesis with a major increase prior to maturity (Singh and Agarwal, 1993; Melake-Berhan *et al.*, 1996; Menkir *et al.*, 1996b).

Observations at different growth stages in field trials indicated that the highest incidence of grain mould on sorghum was recorded between 25 and 35 days after anthesis (Melake-Berhan *et al.,* 1996). This increase was noted in both resistant and susceptible varieties. Increased infection started at soft dough stage (three weeks after anthesis). Singh and Agarwal (1993) reported a similar increase in infection rate after the dough stage of grain development. Colonization by *F. thapsinum* also increased towards the hard dough stage (Forbes *et al.*, 1988). Furthermore, Narendrappa *et al.* (1988) observed the highest percentage of seed infection (95.6%) when sorghum heads were inoculated at soft dough stages with *Gonatobotrys ramose,* while incidences of 81.6, 89.9 and 42% were recorded from inoculations at anthesis, grain fill and fully formed grains respectively. No infection was recorded when newly emerging heads were inoculated. Similar trends have been observed in

the susceptibility of other host plants to seed pathogens. *A. alternata*, *C. lunata*, *F. moniliforme*, *Phoma* spp., *F. semitectum* and other seed borne fungi occur in the glumes and seeds of pearl millet (*Pennisetum glaucum* (L) R. Br.) at all seed development stages, but, incidence tends to increase starting from grain filling to physiological maturity (Ingle and Raut, 1993).

The susceptibility of sorghum to grain mould is related to changes in the chemical properties of grains. Concentrations of flavan-4-ols known to be important in grain mould resistance, were found to decrease significantly in susceptible varieties starting three weeks after anthesis (soft dough stage) while in resistant varieties concentrations remained higher throughout seed development (Melake-Berhan *et al.*, 1996). Similarly, Jambunathan *et al.* (1991) found detectable differences in concentrations of flavan-4-ols in susceptible and resistant varieties at or after 30 days post-flowering. In a related study, Doherty *et al.* (1987) found the maximum level of free phenolic compounds (FPC) in immature grains (5-22 days after anthesis) and glumes indicating the expression of resistance at early growth stages. The level of FPC was significantly lower in the mature caryopsis. Kumari and Chandrashekar (1994) observed that the resistance of hard grains compared to soft (immature) ones was related to grain protein and proline levels. Proteins and prolines that inhibit the growth of *F. thapsinum* (*G. fujikuroi*) occurred at higher concentrations in the endosperm of hard grains than in softer ones.

Mills (1983) stated that most biotic interactions related to seed deterioration occur during seed enlargement since at this growth stage, seeds have sufficient moisture, increased nutrient levels and ideal temperatures for the development of microorganisms. This author further stated that at anthesis and ripening, food and moisture become limiting, suggesting that seeds may be more susceptible between anthesis and physiological maturity. Nevertheless, recent findings have indicated that sorghum inoculated with *C. lunata* and *F.*

thapsinum at anthesis did not set seed and that the mass of seed increased linearly according to growth stage at the time of inoculation (Somani and Indira, 1999). This result indicates that sorghum may also be susceptible earlier than the dough development stage.

In other instances, susceptibility seemed to extend beyond the hard dough stage. Bandyopadhyay *et al.* (1988) found that in susceptible varieties, severity of grain mould increases after maturity and can reach serious proportions when harvesting is delayed in hot and moist conditions. It has also been demonstrated that electric conductivity of grain leachates and water absorption capacity (related to mould susceptibility) of grains increases as sorghum grains mature (Maiti *et al.*, 1985). However, Singh and Agarwal (1993) believe that late infections are generally limited to the outer surface of seed coats. Thus, an increase in severity of grain mould after grains are nearly matured may not have a significant influence on yield or other grain quality aspects. Similarly in wheat, infection after kernels were filled resulted in a lower yield loss. Toxin production by *F. graminearum* however, proved to be dependent on head wetness periods rather than on stage of kernel development (Hart *et al.*, 1984). Inoculated ripened seeds, without symptoms, produced significant amounts of deoxynivalenol in less than six hours after inoculation. Hence, late colonization may be important from a toxicity perspective. Sorghum seed samples with no visible mould have been shown to contain zearalenone and/or vomitoxin (Bowman and Hagler, 1991).

Alternative hosts. Alternative hosts may affect disease severity by acting as a source of inoculum. *F. verticillioides* and *F. proliferatum* are known to occur worldwide on maize, sorghum, rice, millet, and many fruits and vegetables (Bacon and Nelson, 1994). Barley is also commonly infected by *A. alternata* (Wilcoxson and Miles, 1995) and *C. lunata* has been isolated from *Striga hermonthica* (Del.) Benth. in sorghum fields in Nigeria (Czerwenka *et al.*, 1997). In addition, strains of *F. thapsinum* and F. verticillioides have been isolated from banana and fig with the latter also having been isolated from rice (Leslie, 1995). It is

therefore possible that these alternative hosts may act as sources of inoculum for grain mould of sorghum.

Although grain mould pathogens seem to associate with diverse groups of plants, only cereals could really be considered important alternative hosts. For example, AAL-toxin produced by *A. alternata* f.sp. *lycoprersici* is effective as a herbicide against many broad leaf plants but monocotyledons such as maize and wheat tolerate it (Abbas *et al.*, 1995) suggesting the inability of *A. alternata* isolated from tomato to attack cereals. In cereals however, alternative host infection even at strain level has been demonstrated. For example, *F. thapsinum* and *F. vertcillioides* can infect both maize and sorghum (Jardine and Leslie, 1999). Thus, maize plants can affect grain mould development by acting as inoculum sources when grown in proximity to sorghum fields.

INFLUENCE OF ENVIRONMENT

Weather. Relationships between environmental factors and sorghum grain mould are not well understood. Most reports on the influence of environment are very general and simply indicate the conduciveness of moist or hot conditions for disease development, without quantifying the degree of influence. Nevertheless, understanding the mechanism and effect of environment on infection of seeds is considered important in the formulation of disease management strategies (McGee, 1995). It is essential to determine factors that affect disease progress over time in order to limit development to an acceptable level. The importance of information on grain mould-weather relations in improving disease management must therefore be stressed (Forbes *et al.*, 1992).

Moisture and temperature. Forbes *et al.* (1992) stated that the degree to which signs of disease are expressed depends on weather. Visible fungal growth may extend over most of the seed surface if the weather is favourable. Singh and Agarwal (1993) reported that post-

flowering temperature and rain can influence grain mould development in the field. According to Tarr (1962), the presence of moist conditions favour infection any time after flowering. Esele (1995) stated that extended rainfall, high temperature and relative humidity favour mould development. *Fusarium* head mould of sorghum was reported to occur after humid and hot weather at, or shortly after bloom (Vincelli and Hershman, 2001) while mould due to *Alternaria, Curvularia* and *Cladosporium* spp. was common if it rained after maturity (Wrather and Hershman, 1999). Williams and Rao (1981) also reported that extended wet periods during flowering increase mould severity. The same variety planted at two locations with different climatic conditions suffered from greater sorghum grain mould severity in areas with higher rainfall and relative humidity (Mansuetus *et al.*, 1997).

Above reports do not quantify the weather variables or their relationship with grain mould development. Melake-Berhan *et al.* (1996) reported that temperatures between 70 and 85°F (21 and 29°C) and relative humidity between 75 and 100% were conducive to grain mould development. An average temperature of 24.4°C and precipitation of 100 mm/month was also regarded as ideal for the development of *Fusarium* spp. on sorghum heads (Gray *et al.*, 1971). Nyvall (1989) reported that rain, high relative humidity and temperatures between 24 and 30°C favoured infection by *C. lunata.* Padma and Reddy (1996) reported that high temperatures (36.4°C) and low relative humidity (68.4%) during pearl millet heading stages, reduced the incidence of grain mould compared to the maximum incidence recorded where maturity of plants coincided with a temperature of 32.1°C and high relative humidity of 81.2%. They stated that 20 days of continuous rain may be conducive to infection and grain mould development. Rainfall intensity also appears to influence severity with higher rain intensities being associated with increased mould incidence (Padma and Reddy, 1996)

Moisture seems to affect grain mould severity by influencing initial infection rather than by affecting development in seed. Sorghum inoculated with *F. thapsinum,* incubated for

24 hours in moist conditions and then kept under unfavourable conditions in the greenhouse resulted in severe mould development (Forbes *et al.*, 1992). Weather conditions may also affect disease development indirectly by affecting the host. Cool wet weather following hot dry conditions near maturity was believed to increase head blight severity of sorghum caused by *F. thapsinum* (Nyvall, 1989). Drought stress is known to predispose sorghum to seed diseases such as *Fusarium* head mould and anthracnose (Anonymous, 1998).

Biotic factors: *Insects.* Disease incidence may be severely affected by the interaction between plant pathogens and other biotic factors (Fry, 1982). Marley and Malgwi (1999) demonstrated that the incidence of grain mould fungi (*F. thapsinum*, *P. sorghina* and *C. lunata*) increased in grain damaged by head bugs (*Eurystylus oldi* Poppius). This interaction was reflected by reduced seed yield and germination where pathogen and insects occurred together. A change in the incidence of the grain mould fungi following insect damage was observed where *F. thapsinum* became the most abundant pathogen in contrast to the dominance of *P. sorghina* under normal conditions. The incidence of *C. lunata* was not affected by insect damage. Infestation of sorghum panicles by *Eurystylus immaculatus* Odhiambo is also known to increase the incidence of grain mould (Sharma *et al.*, 1992). Maximum grain damage (loss of hardness, germination and grain mass) was observed when panicles were infested midway to complete anthesis (Sharma *et al.*, 1992). Sharma *et al.* (2000) also reported that damage by sorghum head bugs (*Calocoris angustatus* Lethiery) increases grain mould severity.

The way in which insects affect grain mould development has yet to be determined. Insects may aid in dissemination of inoculum and/or in penetration through their oviposition and feeding wounds as has been observed for *Aspergillus flavus* Link: Fries and mandibulate insects such as Lygus and stinkbugs (Mills, 1983) and for *A. tenuissima* and bean leaf beetles on soybean (Shortt *et al.*, 1982). It has been known that sorghum head bugs, which are

important panicle pests (Marley and Malgwi, 1999), commonly feed on developing grains causing oviposition punctures (Sharma *et al.*, 1994; Teetes, 2000).

A. alternata and *Cladosporium cladosporoides* (Fres.) de Vries constituted the predominant fungal species isolated from pollen pellets collected by bees (*Apis melifera* (L.) from different crops including maize and sunflower (Maghazy *et al.*, 1987). Shortt *et al.* (1982) also reported that injury to pods of soybean by leaf beetles was related to loss of seed germination and incidence of seed infection by *A. tenuissima.* It was suggested that the insect may stress the host through feeding injury thereby predisposing it to the weak pathogen. It is therefore possible that grain mould pathogens such as *A. alternata* might act in the same way.

Head bugs may also change the water uptake pattern of grains. Injury to seeds may result in increased water absorption and the outflow of seed leachates, both of which may provide suitable conditions for grain mould fungi (Maiti *et al.*, 1985; Waniska *et al.*, 1992). Williams and Rao (1981) recognize reports in which grains with some deterioration and cellular disruption can absorb water readily and hence enhance deterioration by grain moulds. Similarly, Waniska *et al.* (1992) reported that sorghum seed with breakages or unbroken seeds that absorb water rapidly have lower resistance to weathering. Moreover, where birds left broken kernels at different growth stages, moulds commonly flourished, causing heads to appear black (Wrather and Hershman, 1999).

Other biotic factors. Grain mould pathogens may also interact with other biotic factors (pathogens) and one another. The major grain mould pathogens are known to interact with each other *in-vivo* as well as *in-vitro. F. verticillioides* appears to be inhibitory to *P. sorghina* and *C. lunata* (Singh and Agarwal, 1993). The toxin-producing ability of *F. verticillioides* may play a role in this regard. Fumonisin B1 inhibited mycelial growth of *A. alternata, F. graminearum, Penicillium expansum* (Link) Thom. and *Botrytis cinerea* Pers.: Fr. but *F. verticillioides* and *F. globosum,* which produced the toxin, were insensitive (Keyser

et al., 1999). On the other hand, mixtures of *C. lunata* and *F. thapsinum* inoculated to sorghum panicles caused greater seed mass loss than each separately (Somani and Indira, 1999) indicating the presence of synergistic interaction among grain mould pathogens. Disease injury caused by other pathogens has also been considered a predisposing factor for sorghum head blight caused by *F. thapsinum* (Nyvall, 1989).

DETECTION AND MONITORING OF GRAIN MOULD

Numerous methods of grain mould assessment and/or seed-borne pathogen detection have been described. Visual assessment of grain mould severity, or determination of incidence of grain mould fungi and reduction in seed size are some of the techniques available (Forbes *et al.*, 1992). In the field, early infection by grain mould pathogens can occur at the tip of spikelet tissues such as the lemma, palea and glume, gradually progressing towards the base (Forbes *et al.*, 1992). Pigmentation of these structures appears as the first symptom of the disease. Grain infection occurs at the base (near pedicel) with early infection leading to a reduction of seed size (Forbes *et al.*, 1992; Singh and Agarwal, 1993; Singh and Bandyopadhyay, 2000). Sometimes, small portions of the seeds show limited surface discolouration, the internal parts looking normal (Williams and Rao, 1981; Singh and Bandyopadhyay, 2000).

The first sign of the fungus is seen at the hilar end (base of glume) extending to the uncovered portions depending on weather conditions and host resistance (Singh and Agarwal, 1993; Singh and Bandyopadhyay, 2000). Based on the fungal species involved, commonly encountered symptoms of grain mould consist of pink, orange, grey, white or black discolouration on seed surface (Esele *et al.*, 1993; Singh and Agarwal, 1993; Esele, 1995; Singh and Bandyopadhyay, 2000). *Phoma* infection shows up as small round black pycnidia which when infection is severe, become over-grown by *Fusarium* and *Curvularia* spp. giving

rise to thick, rough dusty crusts on the pericarp surface (Singh and Bandyopadhyay, 2000). Sometimes, *F. thapsinum* is known to rupture the pericarp erupting as scattered tufts on the surface (Singh and Bandyopadhyay, 2000). Colonization of seeds after maturity produces symptoms with colour variations depending on fungi involved (Forbes *et al.*, 1992). Generally, late invaders cause a mouldy appearance of the seed and primarily occur on the exposed surface of the seed with the glume-covered part being protected. These late colonizers may mask signs of early infections (Forbes *et al.*, 1992; Singh and Bandyopadhyay, 2000).

Invasion of grain tissue may take place before externally visible symptoms are expressed in the field. Williams and Rao (1981) reported that grain mould pathogens have been isolated as early as ten days after flowering but infection only becomes visible 30 days after flowering. Bandyopadhyay *et al.* (1991) also reported that infection started at flowering but remained internal until the hard dough growth stage when sporulation occurred on the seed surface. Similarly, Nyvall (1989) reported that symptoms of grain mould induced by *C. lunata* were observed at maturity on spikelets. Thus, not all infected grains show visible mould growth (Singh and Bandyopadhyay, 2000).

Despite the simplicity and speed of visual appraisal methods when evaluating large numbers of varieties (Forbes *et al.* (1992), they lack sensitivity in detecting the degree of colonization. Furthermore, it has been shown that significant losses may occur before mould growth becomes visible on the seed surface (Magan, 1993). Accordingly, it would be essential to develop methods that may quantify the degree of invasion as well as determine the identity of fungi associated with the disease. Incubation of infected seeds at 25°C on wet blotting paper at alternating 12 hours exposure to light and darkness encouraged mould development and enabled the differentiation of cultivars with low infection severity, which could otherwise not be identified by means of visual examination (Williams and Rao, 1981).

Singh and Agarwal (1993) presented the selective isolation on specific media as one option to detect the incidence of seed-borne grain mould pathogens such as *Fusarium spp.* and *C. lunata.* The idea of using a whole seed plating method to detect the incidence of different species has also been favoured by other workers (Bosman, 1991; Menkir *et al.*, 1996b). Some researchers, however, consider the whole seed plating technique to be laborious and time consuming (Magan, 1993). Besides, Forbes *et al.* (1992) commented that whether infected early or late, the incidence of fungi (derived from isolation or visual assessment) might be the same while severity, and hence the resulting, damage could be different. Plating finely crushed seeds onto culture media may be considered to improve detection. However, this technique is also laborious and time consuming and detects only viable spores (Forbes *et al.*, 1992; Singh and Agarwal, 1993).

Quantification of fungal biomass using specific biochemical criteria such as ergosterol for fungi, has been considered sensitive to measure disease severity even before mould growth is visible to the unaided eye (Forbes *et al.*, 1992; Magan 1993; Singh and Agarwal, 1993). Ergosterol assays detect total fungus biomass (viable and nonviable) in grains and thus have the potential to determine the amount of mould damage (Seitz, *et al.*, 1983b; Jambunathan *et al.*, 1991; Forbes *et al.*, 1992; Singh and Agarwal, 1993). Grain discolouration was significantly and positively correlated with ergosterol content though the former did not give sufficient indication of the extent of colonization (Seitz *et al.*, 1983b). Wheat grains with no mould, microscopically visible mycelial growth and visible mould respectively gave 4-6, 7.5-10 and >10 μg ergosterol/gram of seed (Tothill *et al.*, 1992).

Recently a microwave assisted ergosterol extraction technique was reported as an improvement over previous methods (Young, 1995). The technique is believed to be simple, rapid, reliable and economical regarding quantity of reagents needed compared to the traditional solvent extraction and supercritical fluid extraction methods. Other more accurate

techniques (e.g., the use of DNA probes and development of monoclonal antibodies for specific fungi), which may help in species differentiation (Magan, 1993), can also be explored. These methods are assumed important in disease diagnosis mainly in seed production fields where diseases are less tolerated (Magan, 1993).

CONTROL OF GRAIN MOULD/GRAIN WEATHERING COMPLEX

Cultural methods. Avoidance has been considered as one of the most practical and economical control methods for grain mould (Mukuru, 1992; Singh and Agarwal, 1993). Farmers use this practice by commonly growing traditional varieties that flower and mature during unfavourable conditions for mould development (Forbes *et al.*, 1992; Singh and Bandyopadhyay, 2000). Avoidance is also being used in commercial seed production in which planting is done in relatively dry areas to obtain mould free seed (Forbes *et al.*, 1992; Singh and Bandyopadhyay, 2000). Bandyopadhyay *et al.* (1986) and Singh and Agarwal (1993) recommended adjustments in plating date such that grain-filling stages do not coincide with frequent rainy periods.

Harvesting immediately after sufficient drying was reported as one option in controlling seed diseases caused by species of *Fusarium, Alternaria* and *Cladosporium* (McGee, 1995). This principle may also apply to grain moulds of sorghum. Seitz *et al.* (1983a) reported that invasion of sorghum seeds may continue for a few weeks after physiological maturity. They further found that seed invasion after physiological maturity depended on harvest date. Bandyopadhyay *et al.* (1988) found that severity of grain mould may increase to a serious level even after maturity if harvesting was delayed under hot and moist conditions. Similarly, Garud *et al.* (1998) recommended harvesting of sorghum at physiological maturity to avoid reduced grain quality due to grain mould caused by an increase in *F. thapsinum* and *C. lunata* should harvest be delayed. Similarly, in pearl millet,

harvesting at 30, 40 and 50 days after anthesis increased the incidence of *F. semitectum* and *F. chlamydosporum* when harvest was delayed (Wilson *et al.*, 1995). Harvesting after maturity at average moisture contents of 20-22% and drying subsequently to 12-14% moisture is recommended to reduce damage from moulds and to limit shattering and harvest losses resulting from drying in the field (Donald and Ogburn, 1982; Anonymous, 1998).

However, some researchers feel that on early maturing sorghum varieties, avoidance by harvesting immediately after physiological maturity helps to reduce colonization by saprophytes during wet conditions (Williams and Rao, 1981). Furthermore, Bandyopadhyay and Mughogho (1988) doubt the practicality of avoidance since late sowing to avoid moist conditions during seed development may result in lower yields than early sowing.

Removal of infected debris as an inoculum source by incorporating crop residues into the soil before planting reduces disease pressure. Cotton and Munkvold (1998) suggested that tillage might reduce inoculum in the field for maize ear infection by negatively influencing the survival of *Fusarium* spp. including, *F. verticillioides*, provided that nearby fields are inoculum free.

Fungicides. Chemical control of sorghum grain mould has not been encouraged mainly for economic reasons (Williams and Rao, 1981; Mukuru, 1992; Singh and Bandyopadhyay, 2000). However, where mould pressure is high and a history of disease development is well understood (McGee, 1995), chemicals might provide an efficient and economical control strategy. In addition, until satisfactory resistant varieties are available, chemical control may be the only option to protect susceptible but high yielding varieties. Furthermore, in the present concept of integrated plant disease management, combinations of fungicide application with other options such as host resistance, could significantly improve the validity of fungicides to control grain mould in the field. Spraying plants while in the field has been considered important in seed production (Singh and Agarwal, 1993).

Lukade (1986) evaluated the effects of fungicide sprays at dough growth stage and found Dithane M-45 (mancozeb) and captan to be more effective in suppressing grain mould in the field than an unsprayed control treatment. However, there were no significant differences among treatments regarding grain yield and 100-seed mass. On millet (*P. glaucum)*, sprays of Bavistan alone or in combination with thiram, provided the most effective reduction of *A. alternata*, *C. lunata, F. moniliforme* and *Phoma* spp. (Ingle and Raut, 1994).

Somani *et al.* (1995) compared the effects of pre-harvest spray and seed treatment on grain mould pathogens. Thiram + aueriofungin, captan + carbendazim, captan, thiram + carbendazim were effective in controlling seed-borne *C. lunata*. Carbendazim was also the most effective against *F. thapsinum*. Although there was no significant increase in germination following a pre-harvest spray of these chemicals, post-harvest seed treatment by a carbendazim + thiram slurry increased germination over the spray treatment by 22-24%. Post-harvest treatment with carbendazim + thiram and pre-harvest spray with captan + aureofungin, captan and thiram + carbendazim improved seed germination by 42.37, 42.3 and 40.74%, respectively.

There are several reports of using fungicides as seed treatments to control seed-borne grain mould pathogens on sorghum and related crops. These sources may aid as guidelines in selecting fungicides in field trials for sorghum grain mould control. Effective fungicides tested include Bavistan, Delsan (Gopinath and Shetty, 1992), Emisan (Singh *et al.*, 1988; Mahling and Anahosur, 1998), carbendazim, thiram (Singh and Agarwal, 1988; Paul and Mishra, 1993; Mahling and Anahosur, 1998), benomyl (Senapati and Narain, 1990; Amal *et al.*, 1993; Mahling and Anahosur, 1998), Rizolex, vitavax (Amal *et al.*, 1993), captafol (Senapati and Narain, 1990), captan (Paul and Mishra, 1993), thiram + captan (Paul and Mishra, 1994) and mancozeb (Paul and Mishra, 1994).

Biological control and plant extracts. In the long-term, the combination of biocontrol agents and plant extracts as a strategy to combat grain mould may lead to alternative control methods that are more affordable. Saprophytes have been found to reduce the *in vitro* growth of pathogens by producing volatile and non-volatile antibiotics. Artificial inoculation of winter wheat ears with saprophytic microflora including *A. alternata, B. cinerea* and *C. herbarum* followed by inoculation with *F. culmorum* resulted in a reduction of Fusarium ear blight (Liggitt *et al.*, 1997). Average disease incidence was 5% in the presence of *B. cinerea* and *C. herbarum* and 4.6% when *A. alternata* was inoculated prior to the pathogen. Inoculation with the pathogen alone gave a 19% disease incidence.

Potential biocontrol agents may be sought on plant parts other than seed. Saprophytic fungi (*Penicillium chrysogenum* Thom., *Penicillium thomi(i)* Marie and *Stachybotrys atra* Corda) isolated from the phylloplane of maize, wheat and rice were found to be antagonistic to *F. moniliforme* and *A. alternata in-vitro* while *C. Cladosporoides* also inhibited *Bipolaris oryzae* (Breda de Haan) Shoemaker (Mangiarotti *et al.*, 1987). Some biocontrol agents can degrade toxins produced by some pathogens. Faraj *et al.* (1993), for example, demonstrated that microorganisms isolated from naturally contaminated maize have the ability to degrade aflatoxin. Other biocontrol agents and plant extracts were also found useful in controlling seed-borne fungal pathogens such as *F. thapsinum, F. semitectum* and *C. lunata* in sorghum and maize (Utikar and Shinde, 1989; Chatterjee 1990; Ekpo, 1991; Bjornberg and Schnurer, 1993; Ekpo and Banjoko, 1994; Gupta and Raut, 1997).

Resistance. The search for sorghum varieties with resistance to grain mould started in the late 1970s (Williams and Rao, 1981). The use of genetic resistance to grain mould of sorghum has been considered the most economical and effective control method (Bandyopadhyay and Mughogho, 1988; Forbes *et al.*, 1992; Mukuru, 1992; Singh and Agarwal, 1993). As a result, most research on sorghum grain mould management has

concentrated on resistance including screening techniques and the determination of resistance factors (mechanisms of resistance).

Screening techniques. In their review of sorghum grain mould, Forbes *et al.* (1992) and Singh and Agarwal (1993) noted the importance of moisture during seed development in the field for effective screening. Traditionally, field screening has been carried out under natural conditions, where rainfall occurs frequently from flowering to grain fill (Williams and Rao, 1981). Planting earlier in the season (using irrigation for crop establishment) so that the rainy period coincided with flowering, facilitated screening (Williams and Rao, 1981).

Bandyopadhyay and Mughogho (1988) compared the efficacy of some commonly used methods of screening namely, overhead sprinkler irrigation, inoculation with pathogens and bagging of panicles in the field. They found no significant difference in threshed grain mould rating (TGMR) between irrigated and unirrigated plots when rainfall was abundant and frequent from flowering to harvest. Germination however, was significantly lower in the irrigated plots compared with unirrigated plots. Whether it rained frequently or not, inoculation and/or bagging susceptible varieties did not result in a significant difference in TGMR compared to non-inoculated and non-bagged treatments. Accordingly, they suggested that screening could be conducted without inoculation and bagging of panicles if overhead sprinkler irrigation is used from flowering to harvest.

In screening for resistance, it was suggested that evaluations be conducted at physiological maturity for each grain mould pathogen in order to minimize errors arising from late colonizers (Forbes *et al.*, 1992) thereby ensuring clearer host reactions. Other researchers also demonstrated the reliability of this method (Menkir *et al.*, 1996b). Based on evaluation at different growth stages, Menkir *et al.* (1996b) found that highly susceptible varieties could be identified based on visual mould rating (discolouration) as early as 40 days after flowering and resistant varieties, a few weeks after physiological maturity (50 days after flowering).

Disease assessment in the field must be followed by threshed seed evaluation after incubation (Williams and Rao, 1981) since not all infections are visible without incubation (Singh and Agarwal, 1993). Moreover, in the visual assessment method, Forbes *et al.* (1992) suggested the need for comparisons with germplasm of known reactions since the colour of varieties may influence precision of results.

Due to the diversity of damage resulting from grain mould, many workers have considered different parameters instead of relying only on discolouration or visible damage (Castor and Frederiksen, 1980; Forbes *et al.*, 1992). Owing to the difficulty of using such complex evaluation systems on large numbers of plants however, these methods are reserved for use on small numbers of accessions that have already passed through simpler screening techniques. The ergosterol method is believed to be relatively sensitive and fast in determining the degree of infection (Seitz *et al.*, 1983b; Forbes *et al.*, 1992; Singh and Agarwal, 1993). Ergosterol measurements at different seed growth stages could differentiate resistant and susceptible varieties (Jambunathan *et al.*, 1991) (See Detection and monitoring).

Recently, the potential of analysing host chemicals involved in resistance to predict reactions of sorghum plants to grain mould has been demonstrated. Jambunathan *et al.* (1990) carried out methanol and acidified methanol extract analysis for levels of seed flavan-4-ols at different development stages. They found the concentrations in mould resistant varieties to be at least twice that of susceptible varieties 30 days post-flowering. They concluded that flavan-4-ol concentrations in mature grains could indicate reactions to grain mould and suggested that the method was a good tool in screening varieties. The possibility of rapidly screening large number of accessions using antibodies against anti-fungal proteins in seeds was also suggested (Seetharaman *et al.*, 1996).

Measurement of leaf phenols has been found to be promising in determining variety reactions to mould. Jambunathan and Kherdekar (1991) found that concentrations of flavan-

4-ols in leaves were at least three times higher in resistant varieties than in susceptible varieties as observed at different growth stages (between 56 and 70 days after emergence). Other workers also demonstrated the possibility of using host phenol content to predict grain mould reactions of sorghum (Jambunathan *et al.*, 1986; Waniska *et al.,* 1989).

Screening of late-maturing sorghum varieties for resistance to grain mould was found to be a major problem because such varieties do not flower during the long day rainy season that favours mould development, and consequently escape disease pressure. Sprinkler irrigation applied for long periods until plants matured appeared impractical (Williams and Rao, 1981). In response to this problem, an *in vitro* screening technique of inducing flowering has been developed (Singh and Prasada Rao, 1993). Flowering was induced by shortening day-length by covering plants after eight hours exposure to daylight. Using this technique, it was possible to screen a large numbers of sorghum accessions originating from various geographical areas (Prasada Rao *et al.*, 1995) thus ensuring the utilization of photosensitive germplasm as a source of resistance.

Resistance mechanisms. Singh and Agarwal (1993) summarized research related to grain mould resistance factors in sorghum. They reported that germplasm with coloured pericarps, including yellow seeds, showed increased resistance. They also pointed out the association of a pigmented testa with increased resistance. Hiremath *et al.* (1993) also found that among 48 sorghum genotypes evaluated, red seeded accessions had higher resistance with some white seeded genotypes showing moderate resistance to grain mould (*Fusarium*, *Phoma* and *Curvularia* spp.).

Resistance of seeds with coloured pericarp appears to be due to their phenol contents, mainly, flavan-4-ols (Martinez *et al.*, 1994). From their observations at different seed developmental stages, Melake-Berhan *et al.* (1996) found that as seed flavan-4-ols and proanthocyanidins declined, seed infection increased proportionally. They concluded that

both these chemicals could play an important role in resistance. Moreover, significant and negative correlations have been found between ergosterol and flavan-4-ol contents in coloured mould resistant and mould susceptible varieties without a testa but not in white mould resistant and susceptible accessions (Jambunathan *et al.*, 1991). This latter finding indicated that flavan-4-ols were not involved in the resistance of white seeded varieties.

Coloured pericarp and/or high flavan-4-ol concentration may not always be related to resistance. Williams and Rao (1981) observed severe mould development on varieties with dark red, white or yellow grains. Similarly, the relationship between grain mould (ergosterol content, mouldiness of threshed grains and panicles in the field, percentage germination) and seed colour and seed flavan-4-ol content was not highly significant (Audilakshmi *et al.*, 1999) suggesting that these traits had relatively little effect on grain mould response. Waniska *et al.* (1989) also found that pericarp colour was not important in grain mould resistance.

Susceptibility of seeds with high flavan contents could partially be related to the stage of seed development at which these chemicals reach significant levels (Melake-Berhan *et al.* (1996). In susceptible varieties, flavan-4-ols declined significantly starting three weeks after anthesis. However, these chemicals remained high throughout the season in resistant varieties (Melake-Berhan *et al.*, 1996). Consequently, they concluded that the susceptibility of varieties with relatively high flavan-4-ols could be due to a decline in the flavan-4-ol concentration during high disease pressure.

Seeds with pigmented testa were usually resistant to grain mould (Bandyopadhyay *et al.*, 1988; Mukuru, 1992; Singh and Agarwal, 1993; Garud *et al.*, 1994). Testa pigmentation thus appears to be important in conferring resistance to white seeds even though resistance has also been observed in grains with white pericarp and without a pigmented testa (Bandyopadhyay *et al.*, 1988; Jambunathan *et al.*, 1991). It was thus presumed that factors other than tannins and flavan-4-ols may be involved in grain mould resistance.

Jambunathan *et al.* (1992) demonstrated that grain mould resistant sorghum varieties are harder than susceptible ones. In their study, ergostrol concentration was negatively and significantly related with hardness values in resistant white sorghum without a testa. Other workers have also reported a strong relationship between seed hardness and mould resistance (Mukuru, 1992; Ghorade and Shekar, 1997; Audilakshmi *et al.*, 1999). Ghorade and Shekar (1997) found a significant and positive relationship between grain hardness, grain density, 100 seed mass, grain yield and percentage germination. The negative correlation of grain hardness with water absorption rate and fungal load of *Fusarium, Curvularia* and other mould species was significant. It was thus concluded that selection for increased grain hardness would result in an increase in mould resistance (Ghorade and Shekar, 1997). Somani and Indira (2000) demonstrated that genotypes with a greater than 5 kg strength for breakage of kernels possessed good resistance to grain mould. However, despite their importance in grain mould resistance, harder and shiny grains were reported to be less digestible compared to those with softer endosperms (Mukuru, 1992).

Recently, other mechanisms of resistance such as anti-fungal proteins (AFPs) have been reported. Kumari and Chandrashekar (1994) identified three proteins of 18, 26 and 30 KDa from endosperm of sorghum that inhibited growth of *F. thapsinum*. Seetharaman *et al.* (1997) found that sorghum seed AFPs including sormatin, chitinase, glucanase and RNA inactivating proteins (RIP) inhibited the *in vitro* spore germination of *F. thapsinum*, *C. lunata* and *A. flavus* at a concentration of 360 ppm. They proposed the possibility that these proteins might play a role in resistance under field conditions.

Rodriguez *et al.* (1999) found that the concentrations of AFPs (including sormatin, chitinase and glucanase) were correlated with each other and with grain mould resistance indicating the involvement of these AFPs in grain mould resistance. Darnetty *et al.* (1993) reported these proteins to be constitutive unlike others that are expressed in response to

infection. However, Rodriguez *et al.* (1999), from their observation under different conditions, found that infection pressure caused resistant varieties to induce and/or retain more AFPs compared to susceptible ones. Likewise, certain enzymes in sorghum including phenyl ammonia-lyase (PAL) and chalcone synthase (CHS), known to be involved in defence against grain mould, appeared to increase rapidly in response to infection (Little and Magill, 2000). Moreover, resistant sorghum varieties were found to respond to grain mould infection with localized necrotic reactions associated with dark pigmentation within or closer to the lodicules (Forbes *et al.*, 1992; Singh and Agarwal, 1993).

Seetharaman *et al.* (1996) observed sormatin, glucanase and chitinase concentrations to increase with sorghum seed development, peaking at physiological maturity. Sormatin and chitinase concentrations were significantly different between cultivars. Sormatin content at physiological maturity was correlated with the mould rating ($R^2 = 0.65$). Moreover, the AFPs were observed in the shoots of germinating seeds as well, leading to the assumption that they might protect germinating seeds in fields and in malt floors. These AFPs were leached out of immature grains but were retained in the pericarp of mature seeds. As a result, Seetharaman *et al.* (1996) suggested that the AFPs may have an important function in resistance to grain mould at high-risk times (early development stage and high humidity) and their presence in the pericarp was believed important in varieties that lack condensed tannins.

Darnetty *et al.* (1993) and Rodriguez *et al.* (1999) hypothesized that the co-expression or synergistic interaction of the four AFPs (chitinase, sormatin, glucanase and RIPs) might provide acceptable resistance in sorghum with non-pigmented testa. In other crops, synergistic interactions involving such proteins have been observed (Mauch *et al.*, 1988). It is also possible that the AFPs might act in synchrony with other resistance factors to provide higher resistance levels. It is known that overall resistance to grain mould may not be explained by any single character such as phenols (apigeninidin, flavan-4-ols and tannins),

kernel hardness and pericarp colour (Menkir *et al.*, 1996a), especially, in white sorghum without pigmented testa. Cultivars with combinations of high tannin, flavan-4-ols and grain hardness have higher resistance (Mukuru, 1992).

Other characters of sorghum including panicle shape, glume colour and cover, flowering date or maturity period and mesocarp thickness might play a role in resistance although evidence supporting their involvement is not strong (Mukuru, 1992; Waniska *et al.*, 1992; Esele *et al.*, 1993; Garud *et al.*, 1994; Menkir *et al.*, 1996a; Padma and Reddy, 1996; Audilakshmi *et al.*, 1999).

Glume colour and/or phenol contents however appear to be important resistance mechanisms. Audilakshmi *et al.* (1999) found highly significant correlations between measures of grain mould and glume colour (darker). Relatively resistant varieties with pigmented testa and darker pericarp and glume colours had more free phenolic compounds (FPC) and tannins than others (Doherty *et al.*, 1987). Mansuetus (1990) also demonstrated that resistant varieties have higher levels of free phenolic compounds in their glumes at booting than susceptible varieties and further stated that glumes of resistant sorghum were less colonized by *F. thapsinum* (*G. fujikuroi*). Waniska *et al.* (1992) reported that resistant sorghum varieties respond faster to invasion by producing increased concentrations of phenolic compounds in their glumes than susceptible ones.

According to Audilakshmi *et al.* (1999), dark coloured glumes may not always give resistance. These authors also found that the relationship between grain mould damage parameters, glume phenol and flavan-4-ol (mainly associated with red coloured glumes) contents was not strong. Susceptibility of seeds with high concentrations of phenolic compounds in their glumes may be due to low phenol levels during critical times when disease pressure is high (Melake-Berhan *et al.*, 1996).

Biotechnology and breeding for resistance. Traditional methods of breeding for resistance are time consuming. Advanced molecular techniques may help in speeding up the process of breeding for grain mould resistance. In general, studies of the genetics of grain mould resistance have indicated the involvement of few major genes (Kataria *et al.*, 1990; Esele *et al.*, 1993; Shivanna *et al.*, 1994; Sharma *et al.*, 2000). Locating and isolating such genes is of paramount importance in breeding programs.

Esele (1995) pointed out that gene pyramiding could be simplified with molecular markers and further suggested the use of recombinant DNA technology to isolate resistance genes and incorporate these into an acceptable variety. Accordingly, molecular marker assisted selection is proposed as a valuable tool in breeding for resistance to grain moulds of sorghum (Duncan, 1994). It must however to be emphasized that mould resistance traits need to be carefully identified before studying and using resistance genes as markers for selection (Duncan, 1994). Recently, the development of advanced molecular techniques that determine grain mould resistant germplasm has been reported (Klein *et al.*, 2000) and genomic regions important in reaction to the disease appear to have been identified (Klein *et al.*, 2000).

Broader utilization of biotechnology in breeding for resistance to grain moulds will also require that modes and sites of actions of the pathogen are investigated. Promising results have been obtained with other cereals and seed diseases caused by *Fusarium* spp. (Trail, 2000). Studies on the modes of attack have made it possible to identify genes in *F. graminearum* (*G. zeae*) that are responsible for protecting this pathogen from the deoxynivalenol (DON) it produces upon infecting developing wheat grains. Thus, the possibility of using this gene for engineering trichothecene resistant grains has been hypothesized (Trail, 2000). It has been found that DON inhibits protein synthesis and the protein where DON acts has been modified and expressed in transgenic tobacco plants such

that protein synthesis continues in the presence of the toxin (Trail, 2000). Subsequently, this modified gene has been transferred to wheat and barley to provide resistance to the toxin.

CONCLUSIONS

It is evident that the severity of grain mould is not significantly affected by the variation of strains within fungal species. Instead, the disease is influenced by the predominant fungal species found in a given area. In terms of importance, *Fusarium* spp. appears most important followed by *C. lunata* and *P. sorghina.* However, the importance of some *Fusarium* spp. and many other fungi on sorghum requires clarification. A complex of *Fusarium* spp. are found on grain sorghum and new species are being identified currently. But in most past literature, some of these were designated as one species, *F. moniliforme*. At present the tendency is that *F. thapsinum*, *F. semitectum*, *F. vericillioides* and *F. andiyazi* may be common colonizers grain sorghum. *F. verticilloides* is the most important in terms of mycotoxin production.

Fusarium spp. are important with regard to mycotoxin production. Studies are however required regarding the ability of *A. alternata* to produce such toxins in sorghum since this fungus is known to produce potentially dangerous mycotoxins on many other cereals. *P. sorghina* and *C. lunata* are not important from a mycotoxin point of view. Further research on the degree of mycotoxin contamination as affected by environment and host development stage (epidemiology of mycotoxin contamination) is required.

Widespread cultivation of susceptible sorghum varieties results in the development of grain mould at economically significant levels. Generally, grain mould severities appear to increase rapidly at early to mid dough stages. The use of fungicides during these growth stages may provide effective and economical control. Available data however, do not allow

for separation of the importance of growth stage from that of the pathogen and weather on disease incidence and further investigation of this aspect is thus required.

Grain mould pathogens appear to infect a broad range of plants. Even though host preference has been reported, those encountered in cereals including millets, maize and wheat are closely related to sorghum grain mould pathogens. It is therefore necessary to evaluate the role of such hosts on sorghum grain mould epidemiology. In the off-season, grain mould pathogens can survive in seeds and crop debris and most studies on survival have involved *Fusarium* spp. but still the role of inoculum sources on infection of sorghum panicles in the field is vague. Dispersal, most likely by wind and rain may be very important and requires further investigation.

Moisture is important in grain mould development and its influence on infection, sporulation or dispersal requires further study. It is also important to generate quantitative information on the relation between moisture and these elements of the infection cycle. This could lead to the development of a model that enables temporal and spatial prediction of disease severities. Favourable temperature ranges for mould development appear to be between 24 and 30°C. In one investigation on millet (Padma and Reddy, 1996), high temperatures (36°C) reduced the rate of mould development. Further study under controlled conditions is essential to define the role of temperature on sorghum grain mould. Some insect pests of sorghum mainly the head bugs, enhance grain mould development. Understanding these interactions may also assist in formulating effective control.

Host resistance is the most effective method of controlling grain mould and significant progress has been made in developing appropriate techniques for accurate identification of resistant cultivars. Sensitive methods such as the measurement of pathogen biomass (ergosterol) in seeds and the quantification of phenols and proteins involved in resistance can be used for determining host reactions. Host resistance factors of varying importance have

also been identified. High tannin varieties with pigmented testa are generally resistant. Other major resistance factors include coloured pericarp, seed hardness and anti-fungal proteins. Combinations of the different mechanisms within a single variety may provide durable and acceptable levels of resistance. Grain hardness and anti-fungal proteins appear to be important resistance factors in white grains.

Encouraging developments are occurring with the application of biotechnology in breeding programs. The possibility of isolating resistance genes for transfer into susceptible sorghum varieties exists and the possibility of obtaining resistance genes from the pathogens should also be explored. In other host-pathogen interactions, genes resistant to toxins were obtained from the pathogens themselves (Trail, 2000).

Under certain circumstances, fungicides can also be considered for control of grain mould. Fungicides thiram and carbendazim followed by mancozeb, captan, benomyl and emisan have been widely tested as seed and spray treatments and provided relatively good control. The effectiveness of certain fungicides is obviously related to the specific pathogen involved and this could be one of the reasons why mixtures of fungicides were more effective than single active ingredients.

Cultural practices also provide grain mould control methods and adjustment in planting date, choice of planting area, and timely harvesting are some examples. In the long term, it may be necessary to try seed treatments with plant extracts and microbes since they could provide environmentally and economically safe control options. Varying degrees of reduced pathogen incidence and improved seed germination have been observed using these agents.

Considering the complex nature of grain mould, it is unlikely that single, durable control methods will be found. It is therefore advisable to integrate different control methods for sustained management of this disease. Combinations of resistance, avoidance and

chemical methods may provide for effective control. In the context of sorghum grain mould where insects and pathogens were found to interact, multiple pest control methods targeted at both the insect pest and the pathogen require further consideration.

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CHAPTER 2

GRAIN MOULD PATHOGENS IN ETHIOPIAN AND SOUTH AFRICAN SORGHUM CULTIVARS: DAMAGE TO SEED, TRANSMISSION TO AND INFLUENCE ON SEEDLING VIGOUR

ABSTRACT

The major fungi associated with sorghum grain mould in Ethiopia and South Africa were determined. Sorghum cultivars from the two countries were examined for embryo infection. Fungi belonging to the genera *Fusarium, Curvularia, Alternaria, Phoma, Drechslera, Epicoccum* and *Cladosporium* were common on cultivars from both countries. In South Africa, the major *Fusarium* spp. found in seed from North West/Free State was *F. subglutinans* while at Cedara, *F. graminearum* was predominant. *F. proliferatum* was the most frequently isolated species from Ethiopian cultivars. *A. alternata* had the highest incidence at all the localities. Within species of *Drechslera* and *Phoma* from both countries, *B. sorghicola* and *P. sorghina* respectively had the highest incidence while *C. clavata* was found commonly on South African cultivars. *Colletotrichum graminicola* (*Cl. graminicola*)was encountered only on cultivars from Cedara. *F. proliferatum* was isolated from the embryos of many cultivars. Moreover, *C. clavata* was the major invader of embryos of cultivars from South Africa. *F. graminearum*, *A. alternata*, *P. sorghina* and *Cl. graminicola* were occasionally found in the embryos of some cultivars. The relative importance of the common seed fungi, based on their effect on various damage parameters (seed discolouration, 1000 grain mass and seed germination), was examined by inoculating sorghum panicles under greenhouse conditions. Using infected seeds, the transmission of the pathogens to seedlings and their effect on seedling vigour was determined. *F. subglutinans, Cl. graminicola* and *B. sorghicola* were important in causing the most damage. The range of damage appeared to increase when many species occurred together rather than separately. Most of the *Fusarium* spp., as well as *B. sorghicola* and *C. clavata*, had more than 60% transmission rate to seedlings and also significantly reduced seedling vigour. Seedling vigour was very sensitive to infection by grain mould pathogens. Pathogens with embryo invading capacities generally had higher rates of transmission. These results suggest that in addition to the various losses commonly reported, the scope of grain mould damage broadens by encompassing transmission to seedlings and reduction of seedling vigour.

INTRODUCTION

Grain mould is one of the major sorghum diseases wherever sorghum is grown (Singh and Agarwal, 1993; Mansuetus *et al.*, 1995; Menkir *et al.*, 1996b; Tsedeke, 1996). It is caused by fungi that invade seeds during development or after maturity, but before harvest (Singh and Agarwal, 1993; Doyungan, 2001). Such fungi generally cause little or no damage to grain during storage as opposed to storage fungi, which result in significant seed deterioration during storage (Agarwal and Sinclair, 1987).

The major fungi associated with this disease include *Curvularia lunata* (Wakker)Boedijn (*C. lunata)*, *Fusarium thapsinum* Klitich, Leslie, Nelson & Marasas and *Phoma sorghina* (Sacc.) Boerema, Dorenbosh and Van Kesteren (Forbes *et al.*, 1992; Singh and Bandyopadhyay, 2000). Grain mould can cause both quantitative and qualitative losses ranging from total sterility of panicles to production of discoloured and/or mycotoxin-contaminated seeds. Such grains in turn get lower market values (Williams and Rao, 1981; Singh and Agarwal, 1993).

The predominant species causing sorghum grain mould, the incidence of the disease and hence the magnitude of the resulting losses tend to vary with location (Menkir *et al.,* 1996a; Marley and Ajayi, 1999; Somani and Indira, 1999). Furthermore, species other than the major grain mould fungi referred to above may become important in some areas. For example, based on observations at 40, 50 and 60 days after anthesis, the incidence of *Fusarium graminearum (G. zeae)* was significantly related to grain discolouration at all the growth stages while *F. thapsinum* was related to discolouration only at 60 days after anthesis (Menkir *et al.*, 1996b). Accordingly, it was concluded that in this specific area, *Gibberella zeae* (Schw.) Petch. was more important than *F.thapsinum.* In Ethiopia, where sorghum grain mould has been considered economically important (Tegegne *et al.*, 1995), as well as in South Africa, there is a dearth of information on the

prevalence of major sorghum grain mould pathogens. However, such information is of paramount importance in the planning of effective grain mould management strategies in the respective areas.

Depth of grain infection by seed-borne pathogens has been considered a factor influencing the effect they have on seed germination and on transmission of the pathogens to seedlings (Colhoun, 1983). Pathogens located deeper within seeds are little affected by unfavourable conditions and hence survival and transmission to seedlings by such pathogens could be relatively higher (Bassay and Gabrielson, 1983). On the other hand, risk of seedling infection is more likely to be lower when pathogens are located further from the embryo (Gabrielson, 1988). Singh and Agarwal (1993) reported that loss in grain mass and seed viability caused by grain mould of sorghum depends on the depth of infection. Nevertheless, experimental evidence of this is limited. Thus, it is necessary to investigate the degree of invasion of sorghum seeds by the common mould pathogens prevailing in a specific area. Once the site of seed infection is understood, it may be possible to use relevant control options to effectively eradicate the pathogen from seeds.

Seed germination is significantly affected by sorghum grain moulds (Williams and Rao, 1981; Singh and Agarwal, 1989). Consequently, infected seeds can result in reduced emergence of seedlings and thus poor stand establishment in the field. On the other hand, many infected sorghum seeds may germinate but emergence and subsequent growth rate of seedlings resulting from such seeds will be reduced. It is likely that grain yields from such slow growing (stunted) plants could be lower. Neither the role of pathogens on seedling growth nor the rate of their transmission from seeds to seedlings has been ascertained with respect to grain mould of sorghum.

The objectives of this study were therefore to identify the major grain mould causal fungi within the endosperm and embryos of some South African and Ethiopian sorghum

cultivars; to determine the relative importance of these pathogens on damage to seeds; and to determine their transmission to and influence on seedling growth.

MATERIALS AND METHODS

Isolation from whole seeds. The incidence of grain mould fungi on seventeen seed lots of three sorghum hybrids grown in the North West and Free State provinces of South Africa during the 1999/2000 season was determined. Two hundred seeds were randomly drawn from each of the seed lots. One hundred seeds were surface sterilized in 1% sodium hypochlorite solution for 3 min and rinsed in three changes of sterile distilled water. The seeds were dried on sterile blotting paper and plated onto 2% malt extract agar (MEA) (Difco®) (Bandyopadhyay *et al.*, 1991) containing streptomycin sulphate, 100µl/l. Petri dishes were incubated at 25°C and each seed was examined for grain mould pathogens within 7-14 days of incubation.

The remaining 100 seeds were surface sterilized and spread onto sterile filter paper in glass petri dishes moistened with sterile distilled water and incubated at room temperature. Seven to ten days after incubation, seeds were investigated for fungal growth. Where deemed necessary, pathogens were transferred to MEA to obtain pure cultures for identification. Where possible, fungi were identified *in situ* using a low power magnification compound microscope. Commonly encountered *Fusarium* spp. were identified at the South African Medical Research Council, Tygerberg, Cape Town. The incidence of each 'pathogen' was determined. Similar tests were done on seeds of 11 sorghum cultivars obtained from 2000/2001 season plantings at Alemaya University Campus Research Station, Ethiopia.

Isolation from embryos. Four sorghum hybrids grown in the field at Cedara (South Africa) during the 1999/2000 growing season were used for this study. Three to four seed lots, harvested from plantings in mid-November, early-December and early January, were

taken from each hybrid. One hundred seeds from each seed lot (300-400 seeds per hybrid) were tested. Likewise, 100 seeds from each of 11 cultivars obtained from Ethiopia were examined for embryo infection. Seeds were soaked in sterilized distilled water for 14-16 hours (Gopinath and Shetty, 1985). The embryos were then carefully removed from each seed using a sterilized needle and scalpel. Endosperms were disinfected in 1% and embryos in 0.1% sodium hypochlorite solution, for 3 min. Rinsing, blot-drying and plating on agar medium, incubation and identification of the fungi were carried out as described above.

Pathogenicity tests. NK283, the most common commercial hybrid sorghum in South Africa, was used for pathogenicity tests under greenhouse conditions. Plastic pots (5 kg capacity) were filled with steam-sterilized soil and five seeds were planted per pot at a depth of 3 cm. After emergence, seedlings were thinned to one plant per pot. Two weeks after emergence, plants were fertilized with limestone ammonium nitrate (LAN) at a rate of 1g/kg soil. Fungal species isolated from sorghum seeds were used for the artificial inoculation of panicles at anthesis (Table 4). One treatment also consisted of a mixture of *A. alternata*, *P. sorghina*, C. *clavata*, *F. proliferatum*, *F. subglutinans* and *F. graminearum*. Three panicles (replications) per isolate were used.

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 rubbed using a glass rod. Spore suspensions of each isolate were decanted through sterilized cheesecloth and their concentrations adjusted to 10^5 spores/ml (Prasada Rao *et al.*, 1995) using a haemocytometer. The spore suspension was sprayed onto panicles until run-off using a hypodermic syringe. Each inoculated panicle was covered with a moistened plastic bag for five days to enhance infection by inducing high humidity. Control plants were treated with sterile distilled water and covered similarly.

Panicle mould rating (PMR), threshed grain mould rating (TGMR) and grain mass. Severity of grain mould on panicles was recorded three weeks after inoculation according to the percentage area of panicle that was moulded or discoloured (Menkir *et al.*, 1996a; Audilakshmi *et al.*, 1999). To measure the degree of mouldiness of threshed grains (TGMR), 30 g seeds were spread over a glass petri dish (Audilakshmi *et al.*, 1999), and assessed as the percentage of grain surface area discoloured using a stereomicroscope (Marley and Malgwi, 1999). The mass of 1000 grains was determined on a sensitive balance (Type B6100, Sartorius GMBH, Germany).

Germination and seedling height. One hundred seeds of each replicate obtained from pathogenicty tests were plated on moistened filter paper in glass petri dishes (90 mm). Five seeds were placed on each plate and incubated at room temperature. Seeds were monitored for nine days and recorded as normally germinated when roots and shoots were well-developed and showed limited lesion development (ISTA, 1999). On the tenth day, each germinated seedling was removed and their shoot and root lengths measured.

Seeds harvested from inoculated panicles were examined for recovery of inoculated pathogens. One hundred seeds per replicate were surface sterilized and plated onto sterile filter paper. After seven to ten days of incubation, the frequency of the respective pathogens was recorded.

Transmission to seedlings. Infected seeds produced from pathogenicity tests were selected. Two replicates of 50 seeds per pathogen (total of 100 seeds per fungal species) were used. The seeds were planted in plastic pots containing 5 kg mixture of sterile garden soil, manure and sand (3:2:1 ratio). Ten seeds were planted per pot to a depth of 3 cm. Fertilizer in the form of diammonium phosphate (DAP) was applied to pots at 1g/kg before planting. The average daily maximum and minimum soil temperatures were recorded during the experimental period using a digital thermometer with a sensor at 4 cm depth in the soil.

One month after planting, seedlings were removed from pots and washed in tap water to remove soil. The primary root and mesocotyl were cut into 1 cm sections and superficially sterilised in 1.5% sodium hypochlorite solutions for 3 min (Sturz and Johnston, 1983). After rinsing in sterile distilled water and blot dried, tissue pieces were transferred to MEA containing 100 µl/L streptomycin sulphate. Plates were incubated at 25°C for 7-14 days and examined daily for specific pathogens associated with the tissues. Transmission rate was calculated as the proportion of seedlings infected divided by the number of infected seeds used for planting multiplied by 100. Analyses of variance (ANOVA) and mean comparisons were carried out using the statistical package Minitab (Minitab, 1998).

RESULTS AND DISCUSSION

Incidence in whole seeds and embryos. The incidence of grain mould pathogens on sorghum seeds are indicated in Table 1. In the North West/Free State provinces of South Africa, *Alternaria alternata* (Fr.: Fr.) Keissl. was the most frequently isolated fungus followed by *Curvularia* spp.. The most common *Curvularia* spp. encountered in this area was *C. clavata* P.C. Jain (Table 2). *Fusarium*, *Phoma, Bipolaris, Cladosporium* and *Epicoccum* spp. were also isolated from many seed lots although they generally occurred at relatively low frequencies (Table 1). *Fusarium subglutinans* (Wollenw. & Reinking) Nelson, Toussoun & Marasas constituted about 60% of all *Fusarium* spp. isolated (Table 2). *Fusarium proliferatum* (Matsushima) Nirenberg, *Fusarium graminearum* Schwabe and *Fusarium chlamydosporum* Wollenw. & Reinking were also some of the main *Fusarium* spp. found in this region.

At Cedara, South Africa, *Curvularia* spp. were most prevalent followed by *A. alternata* and *Fusarium* spp. (Table 1). Here, too, the incidence of *C. clavata* exceeded that of other *Curvularia* spp.. *F. graminearum* was the major *Fusarium* spp. isolated at Cedara

(Table 2). *F. andiyazi* Marasas *et al.* constituted 6% of all the *Fusarium* spp. found at this locality while other *Fusarium* spp. made up less than 5% of the total *Fusarium* spp. isolated (Table 2). Moreover, *Cladosporium* and *Epicoccum* spp. occurred at relatively higher incidences while the incidences of *Bipolaris sorghicola* (Lefebvre & Sherwin) Alcorn. and *P. sorghina* were similar to those at the previous locality. In addition, *Colletotrichum graminicola* (Ces.) G.W. Wilson (*Cl graminicola*) was found on more than 90% of the seed lots from Cedara, even though its overall incidence was relatively low (Table 1)

A. alternata had the highest incidence among Ethiopian sorghum cultivars (Table 1). However, the frequency of *Fusarium* spp. here (over 30% incidence on some cultivars) exceeded those encountered in South Africa. The major *Fusarium* spp. isolated from Ethiopian cultivars included *F. proliferatum*, *F. graminearum* and *F. thapsinum* in decreasing order of incidence (Table 2). On the other hand, *Phoma, Curvularia, Drechslera/Bipolaris* and *Epicoccum* spp. were the least commonly isolated species (Table 1). *C. lunata* was the only *Curvularia* sp. isolated from some Ethiopian cultivars (Table 1).

F. proliferatum was found in the embryos of the seeds of many Ethiopian and South African sorghum cultivars with high incidences (> 5%) on some (Table 3). *F. graminearum* was also isolated from the embryos of some cultivars. The frequency of *Curvularia* spp. in the embryos of South African cultivars exceeded those of all other species isolated. *P. sorghina, A. alternata* and *Cl. graminicola* were isolated from some cultivars although the average incidence was $\leq$ 1% (Table 3).

Grain mould fungi recorded in the present study are similar to those reported by other investigators (Mathur *et al.*, 1975; Melake-Berhan *et al.*, 1996; Menkir *et al.*, 1996b; Frederiksen, 2000; Leslie, 2000; Singh and Bandyopadhyay *et al.*, 2000). Mathur *et al.* (1975) reported *F. thapsinum, B. sorghicola, C. lunata, Alternaria* and *Phoma* spp. as important components of sorghum seed mycoflora. Earlier studies on sorghum grown at

different locations in South Africa also indicated *Alternaria*, *Phoma* and *Fusarium* spp. as the predominant genera in grains (Bosman, 1991). Leslie (2000) reported that *F. proliferatum, F. graminearum* and *F. verticillioides* are widely distributed in sorghum growing areas of the world. Doyungan (2001) also recognizes *Alternaria, Fusarium, Cladosporium, Curvularia* and *Helminthosporium* spp. as the major genera associated with sorghum grain mould. *Cl. graminicola* has also been reported to be commonly associated with grain mould of sorghum (Williams and Rao, 1981; Singh and Bandyopadhyay, 2000). *Cladosporium* and *Epicoccum* spp. have also been isolated at relatively high frequencies even from immature sorghum seeds (Melake-Berhan *et al.*, 1996)

In this study, *Fusarium* and *Curvularia* spp. were the major invaders of sorghum seed embryos (Table 3). Mathur *et al.* (1975) and Gopinath and Shetty (1985) reported the deep-seated nature of *F. thapsinum* and other *Fusarium* spp. in sorghum seeds. *C. lunata* has also been known to invade embryos of sorghum seeds (Forbes *et al.*, 1988; Singh and Agarwal, 1993). In the present study, C. *lunata* was rarely encountered even in endosperms. Nonetheless, we found C. *clavata* to invade embryos (Table 3), which implies that this pathogen has the potential to lower sorghum seed quality.

Cl. graminicola was infrequently encountered in the embryos (Table 3). Basuchaudhany and Mathur (1979) and Prasad *et al.* (1985) reported the mycelium of *Cl. graminicola* in sorghum to be mainly located in the pericarp and endosperm with only rare occurrence in the embryos. As expected, species of *Drechslera* were not encountered in the embryos of any of the cultivars from the two countries. Mathur *et al.* (1975) observed a general reduction in infection by *B. sorghicola* from outer to internal seed parts with no embryo infection on sorghum.

Previous studies indicated that *P. sorghina* did not infect the embryos of sorghum seeds (Singh *et al.*, 1988; Singh and Agarwal, 1989). However, this pathogen was

occasionally encountered in the embryos of some cultivars (Table 3). Variations among cultivars in resistance to embryo invasion by *P. sorghina* may partly account for this difference. In other host-pathogen combinations, differences among cultivars regarding the degree of embryo colonization by seed-borne pathogens are known (Agarwal and Sinclair, 1987). Hyphae of *Phoma lingam* (black leg of crucifers) have been recorded in the radicle of sectioned cabbage (*Brassica oleraceae* L.) seeds indicating the possibility of embryo infection by *Phoma* spp. (Gabrielson, 1983).

The presence of many grain mould pathogens in the embryo suggests increased survival (Maude, 1996) and the potential for transmission to seedlings (Maude and Humpherson-Jones, 1980). Overall damage caused by fungi possessing these capacities could thus be greater. Successful eradication of such hidden pathogens necessitates the use of deep penetrating systemic fungicides.

Pathogenicity tests. In the pathogenicity study, average minimum and maximum temperature in the greenhouse from inoculation to grain harvest (± 13% moisture content) were 21 and 30°C respectively. Most of the fungi inoculated onto panicles were recovered from seeds with a range of 66.67% (*P. sorghina* and *F. andyazi*) to 90.67% (*F. subglutinans*) (Table 4). Table 5 shows damage induced by grain mould pathogens. Some *Fusarium* spp. and *P. sorghina* significantly affected germination, seed discolouration and seedling growth (Table 5). *P. sorghina* is one of the most important grain mould fungi on sorghum (Singh and Agarwal, 1993; Singh and Bandyopadhyay, 2000). *F. subglutinans* was the most important *Fusarium* spp. in causing damage to seeds and seedlings (Table 4 and 5). This pathogen decreased normal germination, seedling emergence, root length and increased TGMR and PMR compared to the control and many of the other pathogens.

The role of *F. subglutinans* as a cause of grain mould is little understood perhaps because its identity has long been confused with *F. proliferatum*/*F. verticillioides* (Leslie,

2000). However, it has been considered a complex of different biological species associated with root and stalk rot of sorghum (Leslie, 2000). This pathogen has also been known to be more commonly associated with maize seeds (Schaafsma, 1999) and in some areas, it has been reported as one of the most common causes of *Fusarium* grain mould of maize (Stack, 2000).

In this study, *F. graminearum* and *F. proliferatum* significantly increased seed discolouration (Table 5). Consistent with our results, Menkir *et al.* (1996b) also found a significant association between the incidences of *F. graminearum* and sorghum seed discolouration at different stages of grain development. Leslie (2000) stated that *F. graminearum* and *F. proliferatum* were common pathogens of sorghum seeds and associated with root and stalk rots, seedling blights as well as grain moulds. *F. proliferatum* was also reported as one of the important causes of *Fusarium* grain mould on maize (Stack, 2000).

F. chlamydosporum has not been frequently reported on sorghum, a finding which is consistent with results of the present study. It, however, caused discolouration to seeds in pathogenicity tests (Table 5) and has also been reported as a major contaminant of pearl millet seeds (Wilson *et al.*, 1995; Wilson *et al.*, 2000). Its incidence on millet (*P. glaucum or P. americanum*) was positively associated with hazardous mycotoxins (Wilson *et al.*, 1993).

F. andiyazi is a recently described species on sorghum (Marasas *et al.*, 2001). Even though a detailed pathogenicity study on this fungus has yet to be carried out, Leslie and Marasas (2001) reported *F. andiyazi* and *Fusarium thapsinum (Gibberella thapsina)* to be more aggressive on sorghum. In the present study however, *F. andiyazi* was not important in causing damage to seeds or seedlings (Table 5).

The major *Curvularia* spp. (*C. clavata)* observed in the present study was tested for pathogenicity and it significantly increased seed discolouration over the control treatment (Table 5). Its pathogenicity on sorghum has been rarely studied even though its association

with sorghum seeds has frequently been reported (Sivanesan, 1987). This species is known to cause maize leaf spot (Shurtleff *et al.*, 1993).

Mixtures of *A. alternata*, *P. sorghina*, C. *clavata*, *F. proliferatum*, *F. subglutinans* and *F. graminearum* had a significant effect on, PMR, TGMR and on seedling shoot length compared to the uninoculated control (Table 5). However, none of these species caused reductions in shoot length when inoculated separately suggesting that damage caused may depend on interactions between them. Somani and Indira (1999) found that a mixture of *C. lunata* and *F. thapsinum* inoculated on sorghum panicles caused greater seed mass loss than when each was inoculated separately.

Forbes *et al.* (1992) emphasized the need to determine the importance of the most frequently observed fungal species on grains other than the popular sorghum grain mould pathogens. *Alternaria, Drechslera, Bipolaris, Colletotrichum* and *Cladosporium* spp. are commonly isolated from sorghum seeds (Williams and Rao, 1981) and their significance as grain mould pathogens has not been established. These fungi were also frequently found in the seeds we examined in the present study. Accordingly, we tested their relative virulence after inoculation under greenhouse conditions.

Cl. graminicola significantly affected most of the loss parameters considered (Table 4 and 5). The importance of *Cl. graminicola* on sorghum seed infection is well known. Panicle infection by this pathogen can result in total discolouration of grains, which may in turn lead to poor seed germination or seedling blight (Nyvall, 1989). Thakur and Mathur (2000) reported that severe infection of sorghum panicles by *Cl. graminicola* could result in considerable yield loss (30-35%). It is believed that even late infection of the panicle by this pathogen might result in significant losses (Frederiksen, 2000).

B. sorghicola, which decreased normal and increased abnormal germinations, also significantly influenced root and shoot lengths (Table 5). Although rarely reported at species

level, the genera Drechslera/*Bioplaris* are frequently associated with sorghum grains (Williams and Rao, 1981; Menkir *et al.*, 1996b; Singh and Bandyopadhyay, 2000). However, in many countries including Ethiopia, *B. sorghicola* has been known to cause foliar disease (Odvody and Dunkle, 1975; Sivanesan, 1987; Dalmacio, 2000). In South Africa, *B. sorghicola* has been isolated from grains of different sorghum cultivars (Bosman *et al.*, 1991).

Although it was the most common isolate from the endosperm with occasional embryo invasion, *A. alternata* did not affect any of the grain damage parameters measured (Table 4 and 2.5). In other studies, the presence on sorghum seeds of *Alternaria* spp. failed to correlate with the incidence of non-germinated seeds, seed discolouration or with reduction in root and shoot lengths (Gaudet and Kokko, 1986). Menkir *et al.* (1996b) also reported very little contribution of *Alternaria* spp. to grain discolouration even though they commonly isolated it from sorghum seeds at different development stages. Our results demonstrated that *Cladosporium* spp., as a sorghum grain mould pathogen, was insignificant as also reported by other studies (Gaudet and Kokko, 1986; Forbes *et al.*, 1992; Singh and Agarwal, 1993).

Many pathogens, mainly *Fusarium* spp. and *Cl. graminicola* as well as *B. sorghicola* significantly reduced seedling length (Table 5). The significance of grain mould pathogens on the vigour of sorghum seedlings has rarely been studied. Mathur *et al.* (1975) reported that *F. thapsinum* reduced sorghum seedling growth and seed germination. Similarly, *F. graminearum* (head blight of wheat), which also causes stalk and ear rots of sorghum and maize (Watkins and Prentice, 1999), is known to reduce seedling emergence and vigour (Bohm *et al.*, 2001).

It appeared from our results that seedling vigour is affected by invasion of seed by grain mould pathogens. Many of the isolates we studied in pathogenicity tests caused significant reductions in root length and seed germination while only a few influenced seed mass (Table 5) implying the sensitivity of the former parameters as a measure of fungal

invasion compared to seed mass. This implies that even relatively low incidences of grain mould fungi may influence seedling vigour and seed germination. The relatively strong relationship between percentage germination of sorghum seeds and grain mould severity has also been found in other areas (Lukade, 1986; Forbes *et al.*, 1989).

Transmission to seedlings. The average minimum and maximum soil temperatures during the transmission study were 16 and 30°C respectively. Over 55% of the pathogens tested gave a greater than 60% transmission rate from seed to seedling (Fig. 1). These were primarily *Fusarium* spp. followed by species of *Drechslera* and *Curvularia*. The transmission of *A. alternata* to seedlings was relatively low. There is little definitive evidence of transmission of seed-borne sorghum grain mould fungi. Williams and Rao (1981) reported seedling blight resulting from transmission of grain mould pathogens as an important aspect of damage by these pathogens. In wheat, Duthie and Hall (1987) found 55 to 95% transmission efficiency of *F. graminearum* from seeds to the stem base.

Hering *et al.* (1987) suggested that infection of seedlings from wheat seed could be the possible reason for stunting of adult plants, lack of tillering and hence lower yield. In the present study, many pathogens with a relatively high rate of transmission also significantly reduced seedling length (Fig. 1 and Table 5) suggesting that grain mould pathogens can also give rise to seedling abnormalities possibly via transmission to seedlings. However, not all pathogens with high rates of transmission significantly reduced seedling vigour.

Although they have significant effect on seed discolouration, the influence of fungal species such as *F. proliferatum* on seedling vigour was negligible (Table 5 and Fig. 1). However, their rate of transmission to seedlings was high. In the field, such pathogens may continue multiplying on the growing plant. Under some circumstances, these pathogens may be able to cause damage on the plant. It has been shown that symptom-less infections of maize (Headrick and Pataky, 1989) and sorghum (Reed *et al.*, 1983) plants by *Fusarium* spp.

may sometimes lead to disease when the host is stressed or when conditions favour the pathogen.

We observed that grain mould pathogens not only result in loss of germination and/or seedling emergence, but also cause reduced seedling growth both of which can lead to yield reduction (Singh and Makne, 1985; Sturz and Johnston, 1985). Reduction of grain yield by grain mould pathogens through their effect on seedling vigour requires further investigation. On spring wheat, Sturz and Johnston (1985) found seedling vigour to correlate with yield. Infected, less vigorous roots absorb less water and nutrients (Almaras *et al.*, 1988) resulting in decreased yield.

As expected, pathogens with embryo invading capacity (e.g., *F. proliferatum and C. clavata*) had relatively higher rates of transmission (Fig. 1 and Table 3). Nevertheless, embryo infection capacity was not always necessary for higher transmission. For instance, *Drechslera* spp. were not encountered in the embryo of any of the cultivars tested (Table 3) even though they had a high transmission to seedlings (Fig. 1). Maude (1996) stated that pathogens not located in the embryo could infect the latter from surrounding tissues during germination.

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Table 1. Frequency (%) of grain mould fungi in South African and Ethiopian Sorghum Cultivars

No.	Cultivars[a]	*Alternaria*	*Fusarim spp*[b]	*Curvularia spp.*	*Phoma sorghina*	*Drechslera/ Bipolaris*	*Epicoccum spp.*	*Cladosporium spp.*	*Colletotrichum graminicola*
1.	NK283	34.6	4.2	3.3	4.0	1.0	3.3	0.7	0.0
2.	NK286	64.0	1.5	3.0	1.5	1.0	3.5	0.0	0.0
3.	PAN8446	56.0	0.0	7.0	1.3	1.7	1.7	1.3	0.0
	Mean	58.2	2.8	4.4	2.3	1.2	2.8	0.7	0.0
4.	NK283	11.0	9.8	12.0	2.2	1.4	9.4	4.2	4.2
5.	Buster	11.2	3.0	15.7	3.8	2.2	11.5	3.5	0.5
6.	AN8564	8.3	4.0	8.0	1.5	1.7	4.2	9.5	0.8
7.	SNK3939	11.2	7.4	12.4	1.8	2.0	6.2	9.0	0.0
8.	PAN8446	8.8	1.2	10.4	2.0	2.8	3.8	1.8	0.6
	Mean	10.1	5.1	11.7	2.3	2.0	7.0	5.6	1.2
9.	ETS2111	17.0	31.0	3.0	2.0	0.0	3.0	2.0	0.0
10.	ETS2752	15.0	0.0	0.0	0.0	2.0	0.0	2.0	0.0
11.	ETS3235	22.0	13.0	0.0	1.0	2.0	1.0	1.0	0.0
12.	IS9302	6.0	5.0	0.0	1.0	0.0	0.0	2.0	0.0
13.	IS8686	5.0	0.0	0.0	1.0	0.0	0.0	6.0	0.0
14.	Gambella1107	7.0	0.0	1.0	0.0	0.0	0.0	3.0	0.0
15.	Red Fendisha	21.0	8.0	1.0	0.0	1.0	3.0	3.0	0.0
16.	White Fendisha	6.0	10.0	0.0	1.0	1.0	0.0	8.0	0.0
17.	ETS1005	11.0	6.0	0.0	0.0	2.0	0.0	10.0	0.0
18.	ETS576	20.0	15.0	0.0	0.0	0.0	1.0	5.0	0.0
19.	Awash1050	6.0	10.0	0.0	0.0	0.0	0.0	6.0	0.0
	Mean	12.4	8.9	0.5	0.6	0.7	0.7	4.3	0.4

[a] At N. West and Free State provinces (No. 1-3), 12 seed lots from NK283, 2 from NK286, and 3 from PAN8446 were examined; 500-600 seeds were tested for each cultivar (4-8) grown at Cedara; 100 seeds were evaluated for each cultivar (9-19) from Ethiopia. [b] The *Curvularia* spp. reported under Ethiopian cultivars were *C. lunata.*

Table 2. Major grain mould pathogens isolated from South African and Ethiopian sorghum cultivars

	Composition of each species (%)		
Pathogen	South Africa		Ethiopia
	N. West/Free State	Cedara	
Fusarium subglutinans	61.0	4.4	2.1
Fusarium proliferatum	11.0	9.4	83.0
Fusarium graminearum	19.0	70.3	7.9
Fusarium chlamydosporum	6.0	4.4	0.0
Fusarium andyazi	0.0	5.8	0.0
Fusarium thapsinum	0.0	0.0	7.0
Fusarium spp.	3.0	5.8	0.0
Curvularia clavata	59.0	83.5	0.0
Bipolaris sorghicola	42.1	38.9	57.5

Table 3. Incidence of grain mould fungi in the embryos of grain sorghum cultivars

Cultivars[1]	Incidence in embryo (%)					
	Curvularia clavata	*Alternaria alternata*	*Fusarium graminearum*	*Fusarium proliferatum*	*Phoma sorghina*	*Colletotrichum graminicola*
Ethiopia:						
1. ETS2111	0.0	0.0	0.0	20.0	0.0	0.0
2. ETS2752	0.0	0.0	0.0	0.0	0.0	0.0
3. ETS3235	0.0	2.0	0.0	5.0	0.0	0.0
4. IS9302	0.0	1.0	0.0	0.0	0.0	0.0
5. IS8686	0.0	0.0	0.0	1.0	0.0	0.0
6. Gambella1107	0.0	0.0	0.0	0.0	0.0	0.0
7. Red Fendisha	0.0	1.0	0.0	5.0	0.0	0.0
8. White Fendisha	0.0	1.0	0.0	2.0	0.0	0.0
9. ETS1005	0.0	0.0	0.0	3.0	0.0	0.0
10. ETS576	0.0	0.0	0.0	15.0	0.0	0.0
11. Awash1050	0.0	1.0	0.0	5.0	0.0	0.0
Mean	**0.0**	**0.5**	**0.0**	**5.1**	**0.0**	**0.0**
South Africa:						
12. NK283	4.7	1.0	2.0	0.3	0.0	0.7
13. Buster	6.5	0.5	0.5	0.5	2.5	0.0
14. PAN8564	1.5	0.5	0.0	0.0	0.0	0.0
15. PAN8446	2.3	0.0	0.0	0.3	0.5	0.0
Mean	**3.7**	**0.5**	**0.63**	**0.3**	**0.8**	0.9

[1]One hundred and 300-400 seeds respectively were evaluated for each cultivar.

Table 4. Recovery of grain mould pathogens from seeds of artificially inoculated panicles of grain sorghumand their influence on seedling emergence

Pathogen	Recovery from inoculated seeds (%)	Seedling emergence[1]
Alternaria alternata	83.3ab[2]	44.5a
Curvularia clavata	83.7ab	45.0a
Phoma sorghina	66.7c	45.5a
Fusarium proliferatum	83.7ab	41.0ab
Fusarium graminearum	76.3b	44.5a
Fusarium chlamydosporum	85.3a	44.0a
Fusarium subglutinans	90.7a	39.0b
Fusarium andyazi	66.7c	43.5ab
Colletotrichum graminicola	82.7ab	28.5c
Bipolaris sorghicola	85.7a	41.5ab
Mixture	-	44.0a
Control	-	45.0a
SE (±)	2.6	1.4

[1]For each pathogen, 50 seeds of 2 replications were used in emergence study.

[2]Means followed by the same letters within a column, are not significantly different at 5% level based on Duncan's Multiple Range Test.

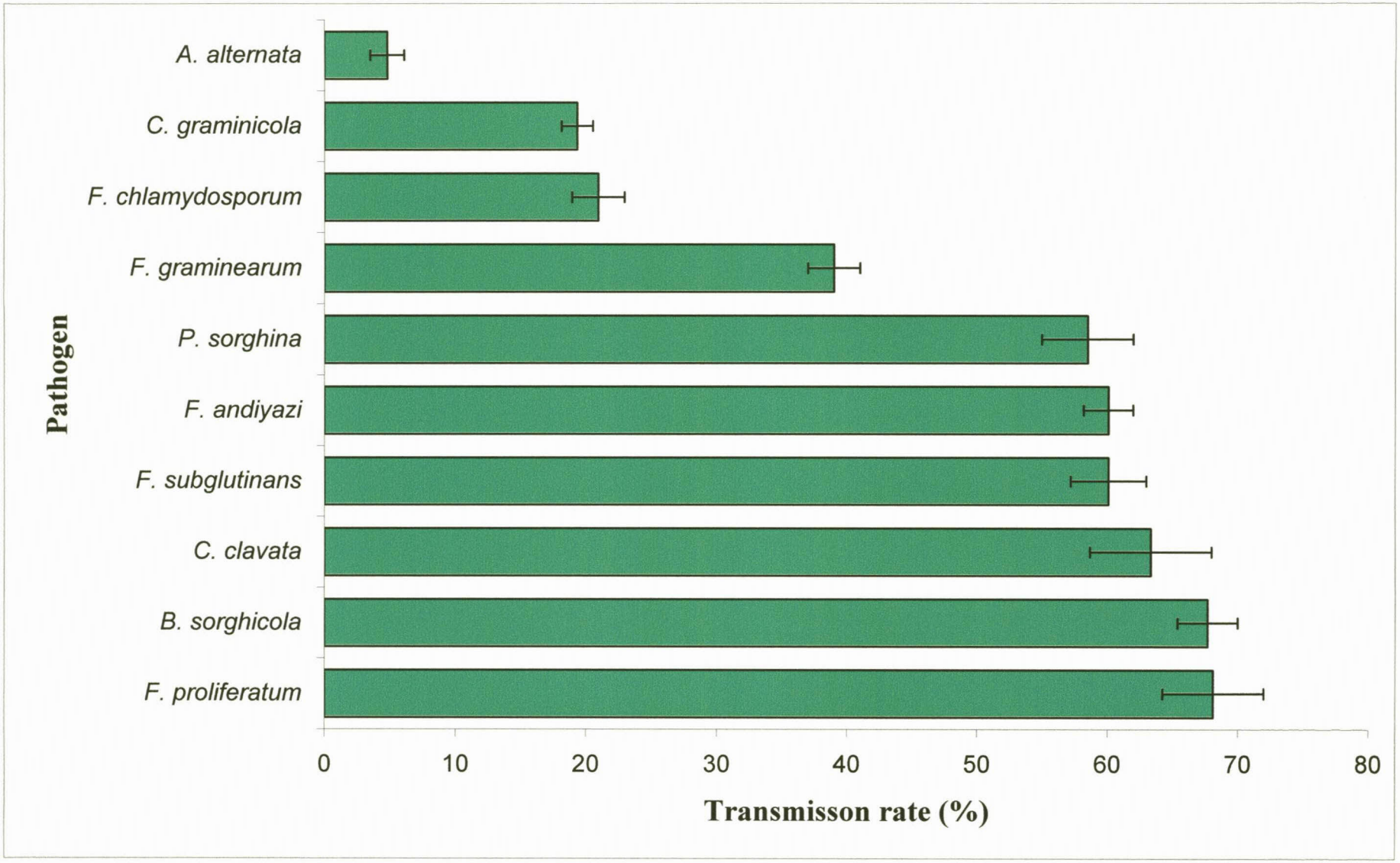
Table 5. Damage caused by grain mould pathogens on sorghum after panicle inoculation in the greenhouse

Pathogen	PMR[1]	TGMR	1000 seed wt	Normal germination	Abnormal germination	Root length	Shoot length
Alternaria alternata	3.0g[2]	5.3fgh	26.0ab	91.0a	7.0bcd	10.6a	3.9a
Curvularia clavata	32.0b	17.3cd	24.7ab	88.7ab	5.3bcde	9.8abc	2.9bcde
Phoma sorghina	4.0g	8.3ef	29.0a	81.3bc	8.3bc	9.8abc	3.1abcde
Fusarium proliferatum	14.0de	8.3ef	26.3ab	89.3ab	7.3bcd	10.84a	3.9a
Fusarium graminearum	18.3cd	16.7cd	31.7a	88.0ab	5.0cde	7.9bcde	3.4abc
Fusarium chlamydosporum	9.3f	12.7de	25.3ab	87.7ab	8.3bc	8.8abcd	3.6ab
Fusarium subglutinans	22.0c	17.7c	27.0ab	76.3c	9.3b	6.7de	2.9bcde
Cladosporium	10.3ef	8.0efg	32.3a	92.7a	1.7e	10.9a	3.4abcd
Epicoccum	3.3g	2.7h	27.3ab	88.7ab	3.7de	10.2ab	3.6ab
Fusarium andyazi	2.0g	8.3ef	29.7a	89.3ab	5.7bcde	7.6cde	2.4e
Colletotrichum graminicola	40.7a	32.7a	18.0b	59.0d	8.3bc	7.9bcd	2.4e
Bipolaris sorghicola	17.7cd	16.7cd	26.3ab	73.3c	20.0a	6.0e	2.4de
Mixture	33.3b	27.7b	25.0ab	84.7ab	5.3bcde	8.1bcde	2.5cde
Control	1.3g	3.3gh	30.7a	92.0a	4.0de	9.3abc	3.5ab
SE (±)	0.9	0.9	1.8	1.6	0.8	0.4	0.2

[1]PMR = Panicle mould rating; TGMR = Threshed grain mould rating.

[2]Within a column, means followed by the same letter are not significantly different at 5% level using Tukey's Honestly Significant Difference Test.

Figure 1. Transmission of grain mould pathogens from seeds to seedlings


Pathogen
A. alternata
C. graminicola
F. chlamydosporum
F. graminearum
P. sorghina
F. andiyazi
F. subglutinans
C. clavata
B. sorghicola
F. proliferatum
0
10
20
30
40
50
60
70
80
Transmisson rate (%)

CHAPTER 3

THE EFFECT OF WEATHER ON GRAIN MOULD OF SORGHUM AT CEDARA, SOUTH AFRICA

ABSTRACT

Field trials were carried out at Cedara, South Africa over a three-year period (1999/2000 to 2001/2002 planting seasons) to study the effects of weather variables on grain mould development. Five sorghum hybrids were planted at different dates to ensure flowering that would expose developing seeds to different weather conditions. At harvest, incidence of grain mould fungi was determined by plating out seeds on 2% malt extract agar (MEA) (Difco®). Averages of weather data were determined for all permutations of weekly time intervals for a two-month post-flowering period in order to identify the time when weather variables and pathogen incidence significantly correlate. The weather variables used were maximum and minimum temperature, maximum relative humidity, and total and frequency of precipitation. Weather variables that showed a significant correlation with the incidence of mould fungi were used for model development to quantify the relationships between variables. The models were developed based on two years' data. Data from the third season were used to evaluate the regression model. Strong and significant positive correlations were observed between the incidence of mould fungi and weather during 4-6 weeks after flowering for the shorter season hybrid Buster and 5-8 weeks after flowering in the remaining hybrids. In most hybrids, correlations between the incidence of grain mould pathogens including *Alternaria alternata*, *Curvularia* spp. (*C. lunata* and *C. clavata*), *Fusarium* spp. (*F. proliferatum* and *F. graminearum)*, *Bipolaris sorghicola* and average minimum temperature, total and frequency of rainfall over the indicated period were significant ($P \leq 0.05$). In four hybrids, models showing a linear relationship between pathogen incidence and minimum temperature and in one hybrid, between pathogen incidence and rainfall frequency, were developed. Depending on the hybrid, models that used minimum temperature as predictor described 60 to 82% of variation in the incidence of pathogens. Frequency of rainfall explained 93% of the variation in pathogen incidence in one hybrid. Evaluation of the models using an independent data set yielded average prediction errors near zero indicating that the models were acceptable.

INTRODUCTION

Sorghum (*Sorghum bicolor* (L) Moench) is an important food crop for many people of Asia and Africa (FAO, 1995). However, yield and quality of sorghum grains in these areas are by far lower than those attained in developed countries. Grain mould constitutes one of the main biotic constraints that limit sorghum production (Singh and Agarwal, 1993; Mansuetus *et al.*, 1995; Menkir *et al.*, 1996; Tsedeke, 1996). This disease affects quality and yield of sorghum grains worldwide (Williams and Rao, 1981; Jardine and Leslie, 1992; Singh and Agrawal, 1993; Somani *et al.*, 1993). The major causal fungi of grain mould are *Fusarium thapsinum* Klitich, Leslie, Nelson & Marasas, *Curvularia lunata* (Wakk.) Boedijin, *Fusarium semitectum* Berk. and Ravenel and *Phoma sorghina* (Sacc.) Boerema, Dorenbosch and van Kesteren (Forbes *et al.*, 1992; Singh and Agrawal, 1993; Singh and Bandyopadhyay, 2000). Other species such as *Fusarium graminearum* (*Gibberella zeae)* are also considered important grain mould pathogens in other areas (Menkir *et al.*, 1996). Moreover, although their role as grain mould pathogens has not been well understood, fungi within the genera *Alternaria, Helminthosporium, Bipolaris, Colletotrichum* and *Cladosporium* have commonly been isolated from sorghum grain (Williams and Rao, 1981; Menkir *et al.*, 1996).

Many sorghum farmers are unable to afford fungicides with which to control grain mould fungi (Williams and Rao, 1981). It is therefore necessary to explore alternatives for the economic management of grain mould. The use of host resistance combined with minimal fungicide application is a possible option. A better understanding of the epidemiology of grain mould will assist in establishing efficient control measures. Weather variables are among the important abiotic factors that affect plant disease epidemics. Climatic variables may influence infection, sporulation and dispersal of grain mould pathogens (Ooka and Kommedahl, 1977; Fakir *et al.*, 1989; Bandyopadhyay *et al.*, 1991). A good understanding of the effect of weather conditions on grain mould will therefore be essential in developing

appropriate control strategies (Maloy, 1993; McGee, 1995). Such knowledge may help in timely application of fungicides only when favourable conditions for disease development are met. This in turn reduces the number of fungicide sprays required and maximizes their effectiveness thereby ensuring the employment of economically feasible and environmentally safe control practices.

In South Africa as well as in other sorghum growing regions of the world, definitive information about grain mould-weather relationships is not available. Singh and Agarwal (1993) reported that post-flowering temperature and rain might influence grain mould development in the field. Tarr (1962) also stated that the occurrence of moist conditions any time after flowering was conducive to infection. Similarly, Mansuetus *et al.* (1997) observed increased grain mould severity in areas with high rainfall and relative humidity. Many of these reports indicate only that moist and warm weather conditions generally favour grain mould development. Evidence of quantitative relationships between weather variables and sorghum grain mould are in particular are very limited. Such information however, is believed to play an important role in the production and improvement of grain sorghum (Hall *et al.*, 2000).

The main objectives of this study were therefore, firstly, to investigate the possible correlation between weather variables and incidence of grain mould under field conditions in South Africa and secondly, to develop a model describing the relationship between the two variables using different hybrid sorghums.

MATERIALS AND METHODS

Field trials. Sorghums hybrids NK283, PAN8446, SNK3939, PAN8564 and Buster were planted in the field during mid-November, mid-December and early-January over a period of three planting seasons (1999/2000 to 2001/2002) to ensure flowering at different

times and expose developing grains to varying weather conditions. Plots consisted of five rows 10 m in length with 25 cm inter-row and 4 cm intra-row spacing. The experimental site was situated at Cedara (South Africa). Standard agronomic practices for the area were applied as required through out the season. The incidence of grain mould fungi was determined on 200 grains collected for each planting date per hybrid. Grains were randomly drawn from each of seed lots, surface sterilized in 1% sodium hypochlorite for 3 min and rinsed in three changes of sterile distilled water, dried on blotting paper at room temperature and plated out on 2% malt extract agar (MEA) (Difco®) containing streptomycin sulphate, 100 µl/L (Bandyopadhyay *et al.*, 1991). Petri dishes were incubated at 25°C and each seed was examined for grain mould fungi after 7-14 days after incubation.

The degree of mouldiness (discolouration) of threshed grains was examined on 30 g of seeds spread over a glass dish using a stereo microscope (30x magnification) (Audilakshmi *et al.*, 1999). Discolouration was assessed as a percentage of the discoloured grain surface per total area of the grain (Marley and Malgwi, 1999). The mass of 1000 grains was determined on a sensitive balance (Type B6100, Sartorius GMBH, Germany). Germination tests were also conducted on 200 grains collected for each planting date by placing five grains on moistened filter paper in a glass Petri dish (90 mm). Germination was recorded after seven days of incubation at room temperature (ISTA, 1999). The various damage parameters (grain discolouration, germination and 1000-grain masses) were examined for possible correlation with the incidence of different grain mould pathogens.

Weather data for duration of two months post-flowering were obtained from a nearby weather station. Weather variables monitored included maximum and minimum temperature, maximum relative humidity, total and frequency of precipitation. The time when weather variables were highly significantly correlated with pathogen incidence was identified by calculating mean daily weather scores for each week from flowering to two months after

flowering. Furthermore, all permutations of weekly weather scores for the two months post-flowering were calculated.

Statistical analysis. Correlation analysis between weather variables and incidence of pathogens was performed using Minitab (Minitab, 1998). Variables that indicated a significant correlation ($P \leq 0.05$) with incidence of mould fungi were used in regression analyses. Before proceeding to regression analysis, scatter plots of pathogen incidence against scores of each independent variable were examined to estimate the type of relationship between variables. The independent variables used in regression analysis were minimum temperature, total and frequency of rainfall (averages of daily scores for 4-6 weeks post-flowering in the hybrid Buster, and average of daily scores for 5 to 8 weeks post-flowering in the remaining varieties). Dependent variables were the incidence of grain mould fungi that showed a statistically significant correlation with weather variables (Table 1).

Scatter plots suggested linear relationships between pathogen incidence and weather variables. However, a few points in some hybrids tended to be outliers. Natural logarithm transformation of the data was therefore employed to improve the estimation of the parameters in the regression model (Montgomery and Peck, 1982). Procedures including best subsets, stepwise and multiple regression analyses were used in quantifying relationships between variables (Minitab, 1998). Models were developed based on two years' data. Independent data from the third cropping season were used to evaluate the regression model. In the evaluation process, the observed pathogen incidence was regressed on the predicted incidence using the respective models developed in each hybrid.

RESULTS

Correlation between incidence of grain mould and weather variables. Strong and significant correlations were observed between the incidence of many mould fungi and

averages of weather variables during the period 4-6 weeks post-flowering in Buster and 5-8 weeks post-flowering in the remaining varieties (Table 1). In all hybrids, coefficients of correlation between the incidence of grain mould pathogens isolated from seeds and average minimum temperature, total rainfall and frequency of rainfall were highly significant ($P \leq 0.05$). In four hybrids, the incidence of *Alternaria alternata* (Fr.:Fr) Keissl was positively associated with minimum temperature in the range of $r = 0.73$ to 0.87 ($P < 0.01$). Positive correlations were also observed in all hybrids between the incidence of *Curvularia* spp. (*C. lunata* and *C. clavata*) and minimum temperature ($r = 0.68$ to 0.84, $P \leq 0.05$). Furthermore, correlations between *Fusarium* spp. (*F. proliferatum* and *F. graminearum)* or *B. sorghicola* and minimum temperature ranged from $r = 0.59$ to 0.72 and 0.65 to 0.88 ($P \leq 0.05$) respectively. Similarly, total rainfall and the incidence of different fungi were significantly correlated ($r = 0.56$ for *Fusarium* spp in the hybrid PAN8564 and $r = 0.89$ for *A. alternata* in SNK3939). The frequency of rainfall was also significantly correlated to different fungi in four hybrids ($r = 0.61$ to 0.89). The correlations between the remaining variables and pathogen incidence were either non-significant or inconsistent (Table 1). In most hybrids, the correlations between seed damage parameters and the incidence of grain mould fungi were not significant (Table 2).

Model development. Based on the maximum coefficient of determination (R^2), the best subsets regression procedure (Minitab, 1998) generated a number of regression models (Table 3). The program selected a one-predictor model with the largest R^2. Subsequently, a two-predictor model was examined and those with significantly larger R^2 were listed. This process continued until all the specified predictors had been selected. Each row in Table 3 represents a specific model with the respective R^2 values and standard deviations. In the hybrid PAN8564, the best one-variable model used minimum temperature ($R^2 = 81\%$) and the second best one-predictor model used total rainfall ($R^2 = 44.3\%$). The best two-variable

model used minimum temperature and frequency of rainfall (R^2 = 83.9%). The different models listed for the remaining hybrids can be visualized in the same way (Table 3). In most hybrids, minimum temperature was selected as the best one-predictor model while in the hybrid SNK3939 minimum temperature was the second-best alternative. In the latter, frequency of rainfall was considered the best predictor (R^2 = 93.41%). The best two-variable models usually included minimum temperature and total or frequency of rainfall (Table 3).

The different models fitted by the best subset regression procedure were further analysed using stepwise regression procedures. The stepwise regression procedure of Minitab evaluates all possible combinations of independent variables by removing and adding variables each time to identify the best predictor based on criteria such as R^2, standard deviation and F-statistics of model parameters (Minitab, 1998). Except for SNK3939 where rainfall frequency was selected, the stepwise regression procedure consistently yielded a simple linear model with minimum temperatures as the best predictor. The statistical information on the models generated by stepwise regression for different hybrids is given in Table 4. The significance of the selected predictors was determined by simple regression analyses and the resulting P-values are shown in Table 4.

The model with minimum temperature as predictor yielded R^2 values ranging from 59.5% in the hybrid NK283 to 82. 23% in PAN8446 (Table 4). In SNK3939, frequency of rainfall as regressor gave the highest R^2 value, i.e., 93.41%. Estimates of regression slopes (parameter b) for the effects of minimum temperature on incidence of grain mould pathogens were significant and ranged from 2.32 ($P < 0.01$) in the hybrid NK283 to 4.57 ($P < 0.01$) on Buster (Table 4). Fig. 1 shows the fitted models for different hybrids after selection using different regression procedures. The observed data randomly scattered around the prediction line suggesting that the fitted model is reasonable (Campbell and Madden, 1990). In the model evaluation procedure, comparison of observed incidence with expected grain mould

incidence using the prediction model in the different hybrids gave an average prediction error between 0.27 in SNK3939 and 0.41 in NK283. Furthermore, regression analysis of observed incidence on predicted incidence gave intercepts not significantly different from zero and the slopes of the regression equations for different varieties fell between 0.83 and 0.93 (Table 5).

DISCUSSION

Results of the present study indicated that averages of minimum temperature, total and frequency of rain during post-flowering periods significantly affected development of sorghum grain mould caused by *Curvularia* spp. (*C. lunata* and *C. clavata), Fusarium* spp. (*F. proliferatum* and *F. graminearum*), *A. alternata* and *B. sorghicola* in the field. The positive correlation between pathogen incidence and weather variables implies that disease severity increases as minimum temperature and precipitation increase thus becoming warmer. Based on the hybrid used, the linear models indicated that increasing the ln (minimum temperature) by a single unit results in an increase in ln (pathogen incidence) between 2.32 in hybrid NK283 and 4.57 in Buster. The models ascribed 60, 70, 81 and 82% of the variation in observed values of ln (pathogen incidence) to minimum temperature in NK283, Buster, PAN8564 and PAN8446 respectively.

It should however be noted that the selection of minimum temperature as a predictor in most hybrids does not mean that other weather variables did not have a significant effect on grain mould development. For example, total and frequency of precipitation were clearly shown to influence grain mould incidence. Both these variables were significantly correlated ($P < 0.05$) with the incidence of the various grain mould pathogens (Table 1). As a second choice in three hybrids, total rainfall accounted for 44.3 to 91% of variation in pathogen incidence (Table 3). Furthermore, frequency of rainfall explained 93% of the variation in incidence of pathogens recorded in the hybrid SNK3939 and was selected as the best

predictor by stepwise regression procedure (Table 4). Results of this study therefore clearly showed that in addition to minimum temperature, moisture related factors (total and frequency of precipitation) are also important in affecting grain mould incidence.

The question may arise why multiple regression models combining minimum temperature with total and/ or frequency of rain were not the best choices. Table 3 indicates that in most cases, the addition of total or frequency of rainfall to the model using minimum temperature, did not improve the quality of fit (no obvious improvements in R^2 and standard deviations) and hence multiple regression models combining variables could not be justified. Furthermore, where slight increases in R^2 were observed, the results of subsequent multiple regression analyses using minimum temperature and rainfall parameters revealed an absence of a significant contribution by the latter. Since similar results were found in other hybrids when rain-related predictors were considered in addition to minimum temperature, the hybrid PAN8446 is presented for illustration. Multiple regression output from fitting minimum temperature and precipitation frequency on pathogen incidence in PAN8446 is shown in Table 6. The best fit in this hybrid was minimum temperature ($R^2 = 82.29$) and frequency of rain was second choice with $R^2 = 76.9\%$ (Table 3). The inclusion of rainfall frequency in the model not only reduced the significance of minimum temperature from P-value < 0.01 when used alone to 0.17, but also rainfall frequency itself did not have significant effect on the regression (Table 6). However, when considered alone in simple linear regression analysis, rainfall frequency resulted in a significant model ($P < 0.01$). The results therefore illustrated the irrelevance of using both variables in a single model although they yielded a significant model when treated separately.

An absence of significant effects of independent variables when combined in a model may arise from significant correlation between predictors, i.e., multicollinearity (Weisberg, 1985). Accordingly, correlation analyses performed between weather variables used in this

study and those considered in model development were significantly correlated (Table 7), which also proved the assumption of multicollinearity. Furthermore, R^2 obtained by the inclusion of minimum temperature and total or frequency of rainfall in a single model was lower than the sum of R-squares from fitting these variables separately (Table 3). This is also an indication that multicollinearity of independent variables exists (Weisberg, 1985). The problem of multicollinearity is minimised usually by removing one of the regressors and a model fit using the regressor with high R^2. This could be the main reason why the stepwise regression preferred a model containing a single best independent variable and ignoring the other variables although they showed high correlations with incidence of pathogens. The one-variable models selected in all hybrids showed small prediction errors in model evaluation procedures. Furthermore, closeness of the slopes to 1 as well as the insignificant difference of the intercepts from 0 (Table 5), suggest that the models provide unbiased predictions (Farnum and Stanton, 1989).

The increase in grain mould incidence observed in the present study as temperature and moisture increased are consistent with current understanding that moist and warm conditions during post-flowering favour mould development (Tarr, 1962; Williams and Rao, 1981; Forbes *et al.*, 1992; Singh and Agrawal, 1993; Esele, 1995). Average air temperatures > 25°C and relative humidity > 75% were found to favour sorghum grain mould development (Gray et *al.*, 1971; Nyvall, 1989; Melake-Berhan *et al.*, 1996). Moreover, average precipitation of 100 mm/month was reported as ideal for the development of *Fusarium* spp. on sorghum heads (Gray *et al.*, 1971). In a related study, Padma and Reddy (1996) reported that continuous rain for 20 days was conducive to grain mould infection and development in pearl millet (*Pennisetum glaucum* (L). R. Br. In the present study, even if maximum temperatures were not significantly related to incidence of pathogens (Table 1), incidence of grain mould pathogens increased linearly with increasing average minimum temperatures

from 6.32 to 13.92, rainfall from 1.1 to 148.2 mm and frequency of rainfall from 3 to 19 days. During these periods, the range in mean values for maximum temperatures was not so high (18 to 23°C). In other crops also low temperatures (< 9.5°C) are known to retard production and release of spores of some fungi (Couture and Sutton, 1978).

Although high correlations between weather variables and pathogen incidence were observed in this study, there were no meaningful correlations between incidence of grain mould pathogens and loss parameters such as seed germination and 1000-grain masses (Table 2). Several factors could have contributed to this. While we attempted to ensure the exposure of flowering to different weather conditions by employing different planting dates, we could not avoid the influence of environmental factors on yield and quality of seeds. For instance, late sowing enables plants to escape pathogens (Padma and Reddy, 1996). Accordingly, we isolated fewer pathogens from late sown crops. However, such grains were found to be poorly filled and were generally shrivelled (had light masses). This has resulted probably due to the effects of cooler temperature conditions on seed development. Another possible reason for lack of disease effect on loss parameters could be that the severity of infection may not have reached the damage threshold. Differences in the incidences of sorghum grain mould pathogens have been found without significant differences in grain loss parameters such as grain yield, 100 seed mass and seed germinations (Lukade, 1986; Somani *et al.*, 1995). It may, therefore be important in the future to determine the level of grain mould required to induce economic loss.

Reports on how temperature and rain influence grain mould development are limited. These factors may help in infection, sporulation and dissemination of grain mould pathogens. Bandyopadhyay (1986) suggested that rain splash may disseminate sorghum grain mould pathogens. Furthermore, it has been demonstrated that wet conditions and high humidity encourage spore production and dispersal and that concentrations of spores of grain mould

pathogens in the air over sorghum field increase under wet and decrease under dry seasons (Bandyopadhyay *et al.*, 1991). The latter authors also found that rain contributed to the dispersal of *Fusarium* spp. Hence, higher pathogen incidences encountered in this study as rainfall increased could be due to the effect on spore production and dispersal to the panicle. On maize, rain has been considered important in the spread of *F. verticillioides* from different parts of the plant and from crop residues to maize ears (Ooka and Kommedahl (1977). Likewise, moist and warm conditions have been considered important in the production and dispersal of spores of many fungi including *B. sorghicola* on maize (Leach *et al.*, 1977; Lyon *et al.,* 1984). Furthermore, an increase in pathogen incidence observed in the present study as the number of rainy days increased, may suggest that precipitation frequency may play a role in the development of grain mould by favouring repeated infections.

The production of grain mould pathogens may commence early in the season. However, the build-up and dispersal to the panicle may take longer. In the present study, the significant correlations between the incidence of grain mould pathogens and weather variables during late growth stages (commencing four weeks post-flowering) could partly be attributed to greater inoculum pressure than during the early development stage. Spores of wheat and barley pathogens including *Alternaria* and *Bipolaris* spp. initially appear at or near ground level, gradually increasing towards late crop development stages (Eversmeyer and Kramer, 1975; Couture and Sutton, 1978; Martin and Clough, 1984). Alternatively, strong correlations during growth stages observed in this study could also suggest that secondary inoculum may have contributed to rapid increase and spread of spores during these latter periods. Sporulation of grain mould pathogens on sorghum panicles is usually observed during the dough growth stages although infection may occur earlier after flowering (Audilakshmi *et al.*, 1991). The role of secondary inoculum on sorghum grain mould incidence has not been studied.

Padma and Reddy (1996) found that high temperatures (36.4°C) during pearl millet heading stages, reduced the incidence of grain mould compared to the maximum incidence recorded when maturity of plants coincided with a temperature of 32.1°C. Increased temperatures, beyond those observed in the present experiment, may therefore result in a different model for example, an optimum type of relationship in which incidence increases and then declines after a critical point). It may be essential to conduct controlled experiments including temperature ranges not encountered in this study as this may lead to a better understanding of optimum as well as limiting temperature conditions to grain mould development.

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Table 1. Pearson's correlation coefficients between incidence[1] of grain mould pathogens (%) on seeds at harvest and post-flowering weather conditions on different sorghum hybrids

	Hybrid							
	NK283				SNK3939			
Weather	*Alternata alternata*	*Curvularia* spp.	*Bipolaris sorghicola*	*Fusarium* spp.	*Alternaria alternata*	*Curvularia* spp.	*Bipolaris sorghicola*	*Fusarium* spp.
Min. Temp.	0.74**	0.77**	0.87**	0.63*	0.87**	0.81**	0.88**	0.52
Max. Temp.	0.19	0.36	0.32	0.19	0.31	0.33	0.49	0.09
Max. RH	0.81**	0.47	0.45	0.22	0.46	0.43	0.56	-0.01
Total RF	0.65*	0.73**	0.79**	0.57	0.89**	0.77**	0.85**	0.63 *
No. of rain days	0.66*	0.78**	0.79**	0.66*	0.89**	0.82**	0.80**	0.72**
	PAN8446				PAN8564			
Min. Temp.	0.76**	0.68*	0.65*	0.63*	0.73**	0.84**	0.12	0.59*
Max. Temp.	0.08	0.24	0.22	0.36	0.44	0.44	0.40	0.31
Max. RH	0.50	0.16	0.32	-0.04	0.39	0.28	-0.11	-0.08
Total RF	0.81**	0.60	0.71*	0.61	0.74**	0.80**	0.17	0.56*
No. of rain days	0.78**	0.61*	0.69*	0.57	0.61*	0.79**	-0.04	0.48
	Buster							
Min. Temp.	-0.14	0.81**	0.51a	0.59*				
Max. Temp.	-0.23	0.78 **	0.38	0.39				
Max. RH	0.42	-0.75**	-0.08	-0.50				
Total RF	0.34	0.28	0.35	0.49				
No. of rain days	0.35	0.15	0.34	0.37				

[1] *Fusarium* spp. include *F. proliferatum* and *F. graminearum* and *Curvularia* spp. include *C. lunata* and *C. clavata.*

** Significant at P < 0.01; * Significant at P <0.05.

Table 2. Pearson's correlation coefficients between grain damage parameters and the incidence of grain mould pathogens in different sorghum hybrids

	Hybrids				
	NK283	Buster	PAN8564	SNK3939	PAN8446
Grain discolouration	- 0.44	- 0.36	0.13	-0.40	0.46
Seed germination	0.58	0.30	0.67	0.69*	0.24
1000 grain weight	0.65*	0.64*	0.50	0.51	0.08

Table 3. Models using different predictors with respective standard deviation and R-square values as generated by best subsets regression procedures for five sorghum hybrids

PAN8564			PAN8446		
R^2	SD	Predictors[1]	R^2	SD	Predictors
81.0	0.3845	Min. temp.	82.2	0.3368	Min. temp
44.3	0.6590	Total rainfall	76.9	0.3837	Rainfall frequency
83.9	0.3695	Min. temp. + Rainfall frequency	82.7	0.3553	Min. temp. + Rainfall frequency
81.5	0.3971	Min. temp. + Total rainfall	82.3	0.3598	Min. temp. + Total rainfall
88.0	0.3348	Min. temp. + Total rainfall+ Rainfall frequency	83.4	0.3758	Min. temp. + Total rainfall + Rainfall frequency
NK283			Buster		
59.5	0.5196	Min. temp.	69.6	0.6942	Min. temp.
55.5	0.5449	Total rainfall	55.1	0.8447	Maximum temp.
65.1	0.5119	Min. temp. + Total rainfall	69.8	0.7301	Min. temp + Maximum temp.
62.9	0.5273	Min. temp. + Rainfall frequency	69.7	0.7314	Min. temp. + Max. RH
65.1	0.5465	Min. temp. + Total rainfall+ Rainfall frequency	69.8	0.7743	Min. temp. + Maximum temp +. Max. RH
SNK3939					
93.4	0.2922	Rainfall frequency			
91.2	0.3386	Total rainfall			
94.0	0.2963	Min. temp. + Rainfall frequency			
93.4	0.3098	Total rainfall+ Rainfall frequency			
94.1	0.3141	Min. temp. + Total rainfall+ Rainfall frequency			

[1]Predictors are weather variables during 4-6 and 5-8 weeks after flowering in Buster and the remaining hybrid respectively; response variables are pooled incidences of *A. alternata*, *Curvularia* spp., *Fusarium* spp. and *B. sorghicola* in hybrids NK283, SNK3939 and PAN8446; *A. alternata, Curvularia* spp. and *Fusarium* spp. in PAN8564 and *Curvularia* spp. and *Fusarium* spp in Buster.

Table 4. Statistical values for regressions of natural logarithm of incidence of grain mould pathogens on weather variables for different sorghum hybrids

Hybrid	Coefficients	Constant (P-value)	R^2 (%)	SD
NK283	2.32 (0.005)[1]	-1.94 (0.207)	59.5	0.52
PAN8564	3.15 (0.000)	-4.21(0.001)	81.04	0.38
SNK3939[2]	1.83 (0.000)	-1.02 (0.024)	93.41	0.292
PAN8446	3.61 (0.000)	-5.35 (0.005)	82.23	0.337
Buster	4.57 (0.001)	-8.73 (0.004)	69.64	0.694

[1]Numbers in parenthesis are P-values.
[2]in SNK3939 and the remaining hybrids, frequency of rainfall and minimum temperature respectively were used as regressors.

Table 5. The relationship between natural logarithm of predicted and observed incidences of grain mould pathogens in model evaluation process on different sorghum hybrids

Hybrid	Prediction error	Regression equation[1]	R^2 (%)	P-value constant	P-value slope
NK283	0.41	Ln (Y) = 0.47 + 0.88X	81	0.395	0.003
Buster	0.32	Ln (Y) = 0.51 + 0.83X	92	0.057	0.000
PAN8564	0.35	Ln (Y) = 0.43 + 0.86X	89	0.116	0.000
SNK3939	0.27	Ln (Y) = 0.23 + 0.93X	91	0.388	0.000
PAN8446	0.33	Ln (Y) = 0.49 + 0. 86X	90	0.062	0.000

[1]Y = observed value and X = predicted value.

Table 6. Analysis of variance output for simple and multiple linear regressions of natural logarithm of grain mould incidence on minimum temperature and rainfall frequency separately or together using the hybrid PAN8446

Simple linear regression

Source	Degree Freedom	Sum of square	Mean square	F statistic	P-value	Predictor	Coefficient	Standard deviation	T statistic	P-value
Regression	1	3.9274	3.9274	26.68	0.001	Constant	0.49	0.50	0.97	0.361
Residual Error	8	1.1777	0.1472			Ln (rain frequency)	1.20	0.21	5.17	0.001
Total	9	5.1050								
							Standard deviation = 0.38		R^2 = 76.9%	

Multiple linear regression

Regression	2	4.2212	2.1106	16.72	0.002	Constant	-4.17	3.09	-1.35	0.219
Residual Error	7	0.8838	0.1263			Ln (Minimum temp.)	2.85	1.87	1.53	0.171
Total	9	5.1050				Ln (Rain frequency)	0.25	0.59	0.43	0.679
							Standard deviation = 0.36		R^2 = 82.7%	

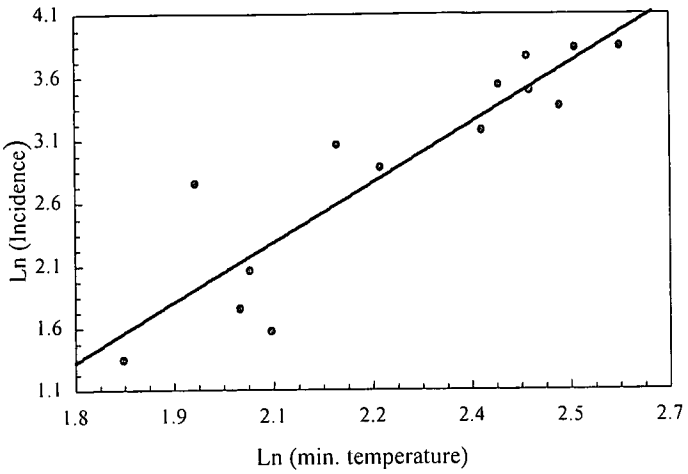
Table 7. Pearson's correlation coefficients between means of post-flowering weather variables that associated with incidence of garin mould pathogens in sorghum hybrids

	PAN8564				NK283			
	Maximum temp.	Minimum temp.	Relative humidity	Total rainfall	Maximum temp.	Minimum temp.	Relative humidity	Total rainfall
Minimum temp.	0.44				0.53			
Relative humidity	-0.48	0.33			-0.08	0.52		
Total rainfall	0.35	0.95***	0.34		0.42	0.91***	0.42	
Rainfall frequency	0.12	0.89***	0.48	0.94***	0.17	0.85***	0.47	0.94***
	PAN8446				Buster			
Minimum temp	0.17				0.92***			
Relative humidity	-0.52	0.48			-0.83***	-0.74***		
Total rainfall	0.25	0.99***	0.40		0.29	0.48	-0.07	
Rainfall frequency	0.03	0.96***	0.51	0.94***	0.34	0.57	-0.08	0.86***
	SNK3939							
Minimum temp.	0.53							
Relative humidity	-0.09	0.51						
Total rainfall	0.33	0.90***	0.42					
Rainfall frequency	0.14	0.84***	0.47	0.95***				

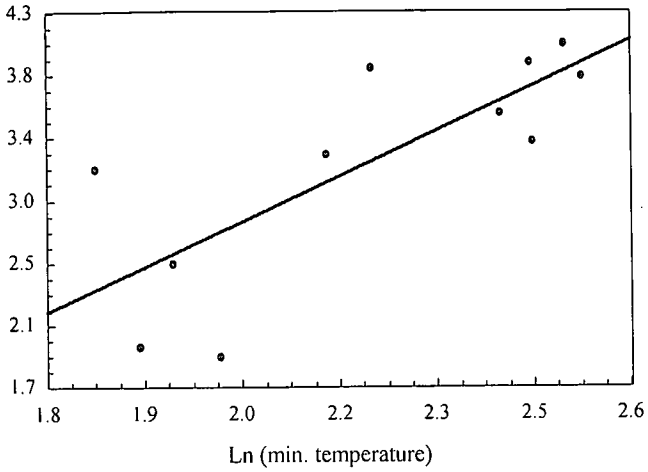
** Significant at P < 0.01; * Significant at P <0.05.

Fig. 1. Linear regression models fitted to the incidence of grain mould pathogens on weather variables in five sorghum hybrids

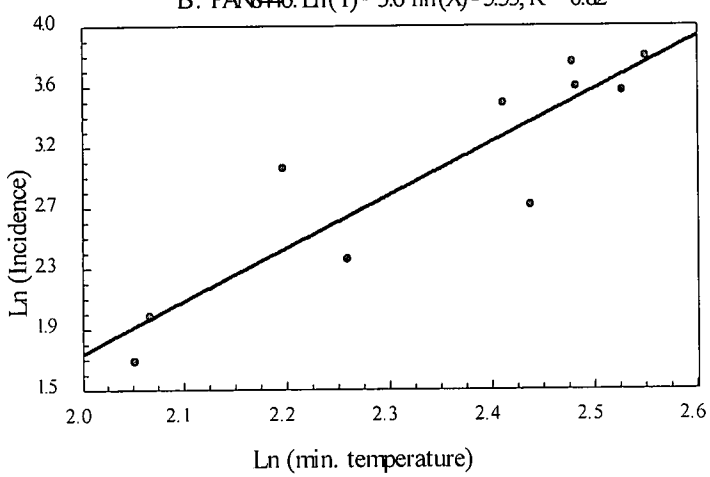
A. PAN8564: Ln (Y) = 3.15ln (X) - 4.21; R^2 = 0.93



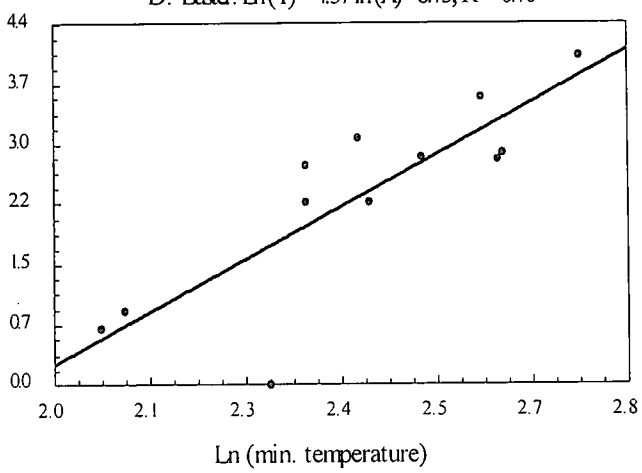
C. NK283: Ln (Y) = 2.32ln (X) - 1.94; R^2 = 0.60



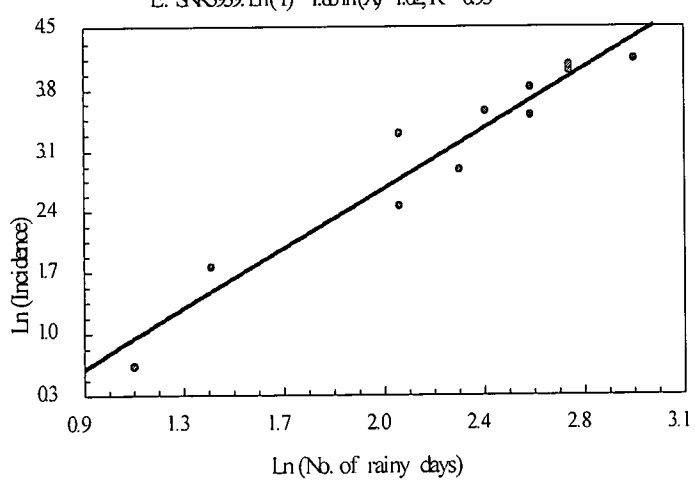
B. PAN8446: Ln(Y) = 3.6 1ln(X) - 5.35; R^2 = 0.82



D. Buster: Ln(Y) = 4.57 ln(X) - 8.73; R^2 = 0.70



E. SNK999: Ln(Y) = 1.83 ln(X) - 1.02; R^2 = 0.93



CHAPTER 4

CULTIVAR SUSCEPTIBILITY TO GRAIN MOULD: RELATIONSHIPS WITH GRAIN DEVELOPMENT STAGES IN SORGHUM (*SORGHUM BICOLOR* (L.) MOENCH)

ABSTRACT

The susceptibility of five sorghum cultivars was evaluated in field trials with the primary objective of determining grain development stage (s) susceptible to infection by mould pathogens. Four growth stages (anthesis, milk, dough and physiological maturity) were compared. Infection during different growth stages was enhanced by daily application of moisture to panicles. Damage to grains and the incidence of most fungi depended significantly ($P \leq 0.05$) on cultivar by growth stage interactions. Significant interaction effects resulted mainly from change in susceptibility between milk and dough stage in some cultivars or from lack of growth stage effects in others. Accordingly, higher infection and damage to grains occurred when moist conditions prevailed at dough or milk stages. In general, high incidences of the major mould fungi (*Fusarium proliferatum, F. thapsinum, F. graminearum* and *Bipolaris sorghicola*) consistently occurred during milk to dough stages. Incidences of *C. lunata* and *P. sorghina* were generally lower at all stages. However, infection by *P. sorghina* increased at anthesis. Frequency of *Alternaria* spp. and *Epicoccum* spp. increased significantly starting from dough growth stage. Grain colonisation by *Cladosporium* spp. was not affected significantly by growth stage. As with incidences of fungi, significantly higher grain damages were associated with application of moisture to panicles during dough and/or milk stages. Differences in incidence of fungi over growth stages were more evident in cultivars Awash1050, Seredo and ETS2111, on which significantly greater grain discolouration and lower percentage germination were recorded as well. The incidence of *Fusarium* spp. and *B. sorghicola* correlated negatively with percentage germination and positively with grain discolouration ($P \leq 0.05$). Moreover, a negative correlation ($P < 0.05$) was found between the incidence of *B. sorghicola* and 1000-grain masses. In the greenhouse, artificial inoculation of potted plants at soft dough growth stage by *C. lunata, F.verticillioides* and *P. sorghina* reduced seed germination by 52, 46 and 48% respectively compared with inoculation at anthesis. Results suggested that susceptible sorghum cultivars could respond better to control practices during late milk to soft dough growth stages than at other stages of development. Chemical control of sorghum grain mould, which has often been discouraged mainly for economic reasons, may thus be applied efficiently and economically at such susceptible growth stages.

INTRODUCTION

Grain mould is a major sorghum disease that limits sorghum production worldwide (Mansuetus *et al.*, 1995; Menkir *et al.*, 1996; Tsedeke, 1996). The commonly reported grain mould causal fungi include *Fusarium* spp. (formally referred to as *Fusarium moniliforme* Sheld.), *Curvularia lunata* (Wakk.) Boedijin, *Fusarium semitectum* Berk. and Ravenel and *Phoma sorghina* (Sacc.) Boerema, Dorenbosch and van Kesteren (Forbes *et al.*, 1992; Singh and Agarwal, 1993; Singh and Bandyopadhyay, 2000). Moreover, fungi within the genera *Alternaria, Helminthosporium, Drechslera, Bipolaris, Colletotrichum* and *Cladosporium* have commonly been isolated from sorghum seeds (Williams and Rao, 1981; Menkir, *et al.*, 1996). However, the importance of these fungi as grain mould pathogens has not been ascertained (Forbes *et al.*, 1992). Common symptoms of grain mould on the surface of seeds may be pink, white, orange, grey or black discolouration (Esele *et al.*, 1993). Infected panicles usually produce smaller sized and/or discoloured kernels of lower viability, which may also be contaminated by mycotoxins that are harmful to animals (Williams and Rao, 1981; Jardine and Leslie, 1992; Singh and Agarwal, 1993; Somani *et al.*, 1993). Grain mould can also be important in the malting and brewing industry because moulded grain is not suitable for malting. Mould pathogens can also survive the malting processes and may consequently contaminate beer with their toxic metabolic products which are a risk to human health (Ilori *et al.*, 1991).

Identification of the susceptible host stage is considered one of the main prerequisites in the development of plant disease forecasting systems (Maloy, 1993). By understanding the relationship between plant growth stage and disease development, the time at which control measures need to be implemented can be predicted more precisely. Consequently, unnecessary use of crop protection chemicals such as fungicides will be avoided, thereby

reducing management costs and contamination of the environment. Tarr (1962) stated that infection of panicles could occur at any stage from young inflorescence to maturity, providing free moisture was present. However, whether the degree of infection at different development stages varies or not, has yet to be ascertained.

Reports suggest that susceptible stages may vary for different sorghum varieties. For instance, studies on resistance mechanisms have indicated that the incidence of grain mould pathogens increases significantly during dough stages and decreases at maturity (Singh and Agarwal, 1993; Melake-Berhan *et al.*, 1996; Menkir *et al.*, 1996). In another investigation, susceptibility appeared to extend beyond physiological maturity (Bandyopadhyay *et al.*, 1988). But Singh and Agarwal (1993) believe that infection at later seed development stages is generally limited to the outer surface of seed coats. Therefore, an increase in disease severity during or after seed maturity may not significantly affect grains since the host is able tolerate disease at these late growth stages. It is however essential to confirm this hypothesis with experimental evidence.

Somani and Indira (1999) found that the reduction in grain mass due to mould pathogens under greenhouse conditions was less as grains developed from anthesis to maturity. Such findings need to be confirmed under greenhouse and field conditions. Since grain damage associated with moulds is complex and diverse (Williams and Rao, 1981; Jardine and Leslie, 1992; Singh and Agarwal, 1993; Somani *et al.*, 1993), it is also essential that additional grain damage parameters, in addition to grain mass be considered in the study of relationships between mould and grain development stage. In many Ethiopian sorghum cultivars, knowledge is lacking, not only about mould progress in relation to grain development stages, but also with regard to their overall susceptibility to grain moulds.

The present study was conducted to assess the susceptibility of five sorghum cultivars to grain mould pathogens. The major objective was to determine the role of grain development stages on incidence of mould pathogens and on damage resulting to grains under field conditions. One hybrid sorghum from South Africa was also evaluated for its reaction to mould pathogens following artificial inoculation at different growth stages in the greenhouse.

MATERIALS AND METHODS

Field experiment. Ethiopian sorghum cultivars Awash1050, Seredo, ETS2111, ETS2752 and IS9302 were planted in field plots during the 2000/2001 planting season at Alemaya University Campus Research Station, Raaree, Ethiopia. Plots consisted of four rows, 5 m in length with a 75 cm inter-row spacing. Fertilizer in the form of diammonium phosphate (DAP) was applied at planting by broadcasting (150 kg/ha). Plots were over-seeded and two weeks after emergence, seedlings were thinned to 20 cm intra-row spacing. When ± 50 cm high, plants were fertilized with urea (100 kg/ha) as side-dressing (Temam, 1992). Throughout the experimental period, cultivation and weeding were carried out manually as required.

Four growth stages were selected for comparison. Growth stages were determined based on the descriptions in Shapiro and Peterson (1997) and the Zadoks decimal growth stages for cereals (Zadoks, *et al.*, 1974). The following grain development stages were used

1. Head emergence to complete anthesis (Zadoks code 59-69).
2. Blister (partially formed grains with uncoloured liquid) to milk (grains have thick milky liquid throughout) (Zadoks code 71-75).
3. Late milk (kernels possess semi-solid white liquid) to dough (almost white solid substances fill the grain) (Zadoks code 77-85).

4. Hard dough (firm grain, difficult to crush between fingers) to physiological maturity (formation of black spot on the kernel tip) (Zadoks code 87 to 91).

For the sake of simplicity, in the subsequent text, the above growth stages will, respectively be referred to as anthesis, milk, dough and physiological maturity.

Moisture was applied to panicles at different growth stages with some modification of the method described by Bandyopadhyay *et al.* (1988). When the respective growth stages were reached, four random panicles per replication were sprayed with tap water to run-off each morning and afternoon. After wetting, panicles were covered with plastic bags for 3 hours (9:00 to 12:00 am and 3:00 to 6:00 pm local times (Ethiopia). To minimize the occurrence of abnormally high temperature in the plastic bags, the latter were placed on the panicles in such a way that air circulation was not hindered. Treatments were continued during each major seed development stage until the onset of the subsequent growth stage. Control treatments were not watered nor covered with plastic bags throughout all growth stages. Plots were arranged in a split plot design of three blocks, sorghum cultivars and growth stages being main and subplot treatments respectively.

Incidence of fungi and seed damage. Grain was harvested at physiological maturity (± 13% moisture content). Panicles from all treatments were threshed manually and seeds from four panicles were pooled representing one replication. One hundred grains were randomly selected from each of the three replications of a particular growth stage, surface disinfected in 1% sodium hypochlorite solution for three minutes and rinsed three times in sterile distilled water. After drying on sterile blotting paper, five seeds were plated onto each of 20 plastic petri dishes (9 cm) containing 2% malt extract agar (MEA) (Difco®) and 100 µl/L streptomycin sulphate (Bandyopadhyay *et al.*, 1991). Plates were incubated at 25°C and each grain was examined for grain mould pathogens within 7-14 days of incubation. Fungi

were identified *in situ* using a light microscope where possible. When required, pure cultures were obtained for identification by transferring isolates to MEA. Subsequently, percentage incidence of each specific grain mould pathogen was determined.

Grains (30 g) were spread over a glass petri dish to measure the degree of mouldiness (discolouration) of threshed grains (Audilakshmi *et al.*, 1999). Using a stereo microscope (30X magnification), grain mould severity was assessed as a visual estimate of the percentage of grain surface discoloured (Marley and Malgwi, 1999). The mass of 1000 grains was determined using a sensitive balance (Type B6100, Sartorius GMBH, Germany). A further 100 grains from each replication were used to evaluate seed germination on moistened, sterile filter paper in glass petri dishes. Five grains per plate were incubated at room temperature for nine days during which time they were monitored for germination. Grains were recorded as having germinated normally when they had well developed roots and shoots and where limited lesion development occurred on roots and shoots (ISTA, 1999).

All data were subjected to an analysis of variance (ANOVA) using the statistical package Minitab (Minitab, 1998) and mean comparisons were performed according to Duncan's multiple range tests.

Greenhouse experiment. Anthesis (Zadoks code 69) and soft dough (Zadoks code 85) stages were compared using hybrid sorghum NK283. Seeds were sown in plastic pots filled with soil (5 kg capacity). Two weeks after emergence, one plant per pot was retained. Panicles were inoculated with different grain mould pathogens. A spore suspension made from cultures of *C. lunata, F.verticillioides* and *P. sorghina,* grown for 10 days on MEA at 25°C, was prepared in sterile distilled water. Concentrations were adjusted to 10^5 spores/ml (Prasada Rao *et al.*, 1995) using a haemocytometer. At the respective growth stages, the suspension was sprayed onto the panicles until run-off using a hypodermic syringe.

Inoculated panicles as well as uninoculated control treatments were covered with plastic bags (with out blocking air movement so that unnaturally high temperature may not occur) for five days. Three panicles were used per isolate. The grain mould parameter used was seed viability (percentage germination). Seed germination was determined as above. When roots and/or shoots were underdeveloped relative to the control or when systemic lesions developed on these structures, grains were considered abnormally germinated.

RESULTS

Field experiment: *Effect of cultivar x growth stage interaction on grain mould incidence.* Analysis of variance indicated that, with the exception of *C. lunata*, whose incidence was significantly ($P < 0.01$) affected by cultivar only, cultivar x growth stage interaction significantly affected the incidence of remaining fungi from sorghum grain. This suggests that the incidence of grain mould pathogens at a specific growth stage differs over cultivars. Accordingly, significantly more *F. proliferatum* was isolated from moisture treated grains at dough growth stages in cultivars Seredo, ETS2111 and Awash1050 (Table 1). However, while isolation frequencies at milk and maturity growth stages were not significantly different from isolations at dough stage in Awash1050, a significantly lower incidence of *F. proliferatum* was associated with anthesis than remaining growth stages in ETS2111. In IS9302 and ETS2752, growth stages did not significantly affect isolation frequency of *F. proliferatum* from grains. Cultivar x growth stage interaction affected the incidence of *F. thapsinum* in the same way as *F. proliferatum* except that in Awash1050, the milk stage had a significantly lower incidence than the dough stage and higher incidence at other growth stages. The frequency of *F. graminearum*, in moisture treated grains during dough stage was significantly greater than milk stage although a high incidence occurred in

the latter than in other growth stages in ETS2111 (Table 1). However, there was no significant difference in incidence of *F. graminearum* from grains treated with moisture during anthesis, milk and maturity growth stages in Seredo. *P. sorghina* occurred at low incidence in all cultivars. However, a significantly higher isolation frequency was associated with anthesis, followed by dough and milk stages in Seredo. Similarly, most cultivars were resistant to infection by *B. sorghicola.* The exception was Seredo, which had the highest incidence of this species at dough stage although incidences significantly greater than the control were recorded at other growth stages (Table 1).

Alternaria spp. were frequently isolated at all growth stages from all cultivars and isolation frequencies in Seredo, IS9302 and Awash1050 in moisture treated grains did not differ significantly with growth stage or from the control (Table 2). In ETS2111, isolation frequencies significantly greater than the control were associated with milk, dough and maturity growth stages while in ETS2752, a significant increase in frequency of *Alternaria* spp. occurred at maturity. The incidence of *Cladosporium* spp. was not consistently associated with growth stage (Table 2). No significant difference in *Cladosporium* spp. incidence was recorded in Seredo, while in ETS2111 incidence prior to maturity was significantly less than the control. Similarly, in Awash1050, the incidence of *Cladosporium* spp. was significantly less at anthesis than at dough and maturity growth stages. Conversely, in IS9302, incidence of *Cladosporium* spp. at anthesis and milk stages was significantly greater than the control. The frequency of *Epicoccum* spp. was lower in ETS2111, IS9302 and ETS2752 although in the latter, there was an increased incidence at dough stage relative to the control. In Seredo and Awash1050, an increased incidence of *Epicoccum* spp. was recorded at post-anthesis stages.

Cultivar and growth stage main effects on incidence. In Table 1, incidence values under column six (mean) for each pathogen indicate the main effects of growth stages while mean values in rows show main effects of cultivars. Cultivars Seredo, ETS2111 and Awash1050 were more susceptible to many grain mould pathogens. A significantly higher incidence of *Fusarium* spp. was recorded in these cultivars. Moreover, isolation frequencies of *P. sorghina* and *B. sorghicola* were significantly higher in Seredo than in other cultivars. The incidence of *C. lunata* in all cultivars was very low although Seredo, ETS2111 and IS9302 tended to be more prone to infection by this pathogen. Almost all cultivars had a high incidence of *Alternaria* spp. with a significantly greater frequency of this genus in Awash1050. Similarly, *Cladosporium* spp. was common in all cultivars although the highest incidence was recorded in IS9302 and the lowest in Seredo (Table 2). Awash1050 and Seredo were colonised more by *Epicoccum* spp. while the incidence of this genus was significantly lower in IS9302 and ETS2752 compared with its incidence in Awash1050 and Seredo. Within growth stage main effects, the dough followed by the milk stage, was more susceptible to *F. proliferatum* and *F. thapsinum* (Table 2). Although significantly more infection by *F. graminearum* occurred at the dough stage, there was no significant difference ($P = 0.05$) between remaining growth stages in infection by this species. Similarly, other growth stages were less susceptible to *B. sorghicola* compared to the dough stage. While grain colonisation by *Cladosporium* spp. was not significantly affected by growth stage, *Alternaria* spp. and *Epicoccum* spp. increased significantly after the dough stage.

Effect of cultivar x *growth stage interaction on grain damage.* Damage to grains was significantly affected by cultivar x growth stage interaction ($P \leq 0.05$). Although there was significantly more grain discolouration associated with moisture application to panicles during dough growth stages in cultivars Seredo, ETS2111 and Awash1050, moisture

application during milk stage resulted in a significantly higher level of discolouration than other stages in IS9302 (Table. 3). Moreover, there was no significant difference in grain discolouration associated with growth stages in ETS2752 and discolouration levels were very low. While grains from panicles treated with moisture during milk stage showed significantly more discolouration in Seredo, they did not differ significantly (P = 0.05) from other stages or from the control in ETS2111 and IS9302. The effect of dough growth stage on grain discolouration was most pronounced in ETS2111 (Table. 3).

Significantly lower seed germination in ETS2111 was associated with panicles treated with moisture at dough stage. However, there were no significant differences in percentage germination among treatments at anthesis, milk and dough stages in Seredo and Awash1050 (Table. 3). Growth stage had no significant effect on germination in IS9302 and ETS2752. In ETS2752 however, there was significantly lower percentage germination at anthesis compared with the control. Significantly lower 1000-grain masses were recorded at milk than dough stage in ETS2111 and Awash1050 (Table 3). No significant difference in grain mass was found between these growth stages in Seredo. Moisture applications at different growth stages did not result in significantly different grain masses in IS9302. However, significantly lower 1000-grain masses were recorded at anthesis to dough stages than at maturity in ETS2752.

Cultivar and growth stage main effects on grain damage. Significantly greater discolouration of grains was recorded in cultivar Seredo and Awash1050 and the least in ETS2752 (Table 3). Percentage germination was significantly lower in Seredo and ETS2111 compared with other cultivars. There was no significant difference among most cultivars with regard to 1000-grain mass. A significantly lower grain mass was however recorded in cultivars other than Awash1050. Averaged over cultivars, moisture application during dough

stage alone or at dough and milk stages caused the highest seed damage (Table 3). Seed germination was significantly lower at dough than at other growth stages. In the same way, moistening of panicles at dough stage resulted in significantly greater grain discolouration and lower 1000-grain mass. The level of grain discolouration at milk stage was significantly higher but grain masses at this stage were not significantly different from those observed at other stages (Table 3).

Correlation analyses revealed significant relationships between the incidence of some mould fungi and grain damage parameters (Table 4). Based on average values from susceptible cultivars Awash1050, Seredo and ETS2111, incidences of *Fusarium* spp. and *B. sorghicola* correlated negatively with percentage germination and positively with grain discolouration ($P \leq 0.05$). A strong, negative correlation ($P < 0.05$) was found between isolation frequency of *B. sorghicola* and 1000-grain masses. Correlations between incidences of the remaining fungi and grain damage parameters were not significant (Table 4).

Greenhouse experiment. Inoculation of plants in the greenhouse with three pathogens at soft dough growth stage resulted in consistently lower germination and greater abnormal germination than inoculation during anthesis (Fig. 1). Inoculation at soft dough stage with *C. lunata*, F.verticillioides and *Phoma sorghina* reduced seed germination by 52, 46 and 48% respectively, while increasing abnormal germination by 57, 51 and 60%.

DISCUSSION

Results of the present study indicated that in the field, infection of sorghum grains by grain mould pathogens was greater when moisture was applied to panicles during the milk to dough development stages compared with physiological maturity and/or anthesis stages. The greater susceptibility of the dough stage was also confirmed by artificial inoculation with

different grain mould pathogens under greenhouse conditions. Examination of results indicated that the cultivar x growth stage interaction effects were due to a change in susceptibility between milk and dough stages in some cultivars and/or absence of growth stage effects in others (Table 1, 2 and 3). Comparison of growth stage effects averaged over cultivars also showed that high levels of grain damage and infection by many mould pathogens occurred when moist conditions were applied during dough stage alone or during milk and dough stages. Panicle moistening during dough stages increased seed discoloration by 56 and 51% over anthesis and maturity stages while treatments at milk stage increased discolouration by 28 and 18% compared with anthesis and maturity stages. Similarly, watering of panicles during dough and milk stages decreased seed mass by about 12 and 10% respectively, compared with treatment during maturity stages (Table 1 and 3).

Generally, differences in seed damage and the incidence of grain mould pathogens over growth stages were more evident on cultivars Seredo, ETS2111 and Awash1050. Overall, seed damage and the incidence of grain mould pathogens were significantly greater in these cultivars than in others. The reduction in germination at dough growth stage was about 26% more than at maturity or anthesis growth stages in ETS2111. Moisture application during milk stage in ETS2111 reduced grain mass by 23 and 17% compared with maturity and anthesis stages respectively. Similarly, the presence of moist conditions at dough growth stage in Seredo reduced 1000-grain mass by 17 and 14% compared with milk and anthesis stages.

The present study demonstrated that the milk and dough grain development stages are more susceptible to infection by grain mould fungi, dough stage being the most susceptible. Moreover, infections at these development stages were shown to cause a significant loss in grain mass and viability of seeds. Elsewhere, field experiments involving observations at

different growth stages indicated the highest incidences of grain mould on sorghum between 25 and 35 days after anthesis with an increase in infection starting at soft dough stage (Melake-Berhan *et al.*, 1996). Forbes *et al.* (1988) also observed that colonization of sorghum seeds by *F. tahpsinum* increased towards the dough stage. Similarly, Narendrappa *et al.* (1988) found the highest percentage of seed infection (95.6%) when sorghum heads were inoculated at soft dough stages with the grain mould fungus, *Gonatobotrys ramose*. The incidence of seed infection was 81.6, 89.9 and 42% with inoculations at anthesis, grain-fill and fully formed grains, respectively. Similar trends have been observed in susceptibility of hosts other than sorghum with regard to seed infection in the field. *A. alternata, C. lunata, F. moniliforme, F. semitectum* and *Phoma* spp. were isolated from seeds of pearl millet (*Pennisetum glaucum* (L) R. Br.) at all seed development stages; however increased incidence was more evident during grain-fill to physiological maturity stages (Ingle and Raut, 1993).

Susceptibility of growth stages observed in different cultivars in the present study could be due to reduced expression of resistance mechanisms during the respective stages. Studies on temporal expression of sorghum resistance factors against grain moulds showed significant reductions in concentrations of flavan-4-ols in susceptible cultivars beginning at the soft dough stage while in resistant varieties concentrations remained higher throughout seed development stages (Melake-Berhan *et al.*, 1996). Similarly, Jambunathan *et al.* (1991) found a detectable difference in the concentrations of flavan-4-ols in susceptible and resistant varieties at or after 30 days post-flowering. It is therefore important that the expression of resistance factors in relation to grain developmental stages be investigated in the cultivars used in this study.

Somani and Indira (1999) found that sorghum inoculated with *C. lunata* and *F. tahpsinum* at anthesis did not set seed. They further reported that inoculation of panicles as seeds developed to maturity caused only smaller reduction grain mass. This finding is consistent with the present study where 1000-grain masses generally increased with increasing growth stage. In contrast to their finding however, in majority of the cultivars moist conditions applied at flowering did not have significant effects on seed mass in this study. Such inconsistency of results might partially arise from differences in the susceptibility of the sorghum varieties used. Our finding however partly agrees with the results of Narendrappa *et al.* (1988) who indicated that artificial inoculation of freshly emerging sorghum panicles with *G. ramosa* failed to give infection. Furthermore, inoculation at anthesis gave a lower incidence of the fungus than inoculation at the soft dough and milk stages. The present results are also consistent with those of Mills (1983) who stated that most biotic interactions related to seed deterioration occur during seed enlargement but that at anthesis and ripening, food and moisture respectively, become limiting.

Fungi within the genera *Alternaria, Cladosporium,* and *Epicoccum* have been considered seed surface colonizers at later development stages with little capacity to penetrate deep into grains (Forbes *et al.*, 1992; Singh and Agarwal, 1993). Accordingly, these fungi have often been regarded as not being true pathogens with no significant influence on grain loss parameters (Gaudet and Kokko, 1986; Menkir *et al.*, 1996). In the present study, despite relatively higher isolation frequencies of these fungi at most growth stages, their incidence was not significantly associated with grain discolouration, seed mass or percentage germination (Table 4). Nevertheless, significant correlations of grain damage parameters with *Fusarium* spp. and *B. sorghicola* (Table 4) indicated the importance of the latter as grain mould pathogens at the specific locality where the cultivars were grown.

Results of this study suggest that sorghum is most likely to respond to control practices during at least late milk to soft dough growth stages. Consequently, if favourable conditions for infection prevail, application of fungicides at these stages may result in greater returns. Chemical control of sorghum grain mould has not often been encouraged mainly from an economical point of view (Williams and Rao, 1981; Mukuru, 1992; Singh and Bandyopadhyay, 2000). However, under occasional conditions of severe mould pressure, fungicides could be profitably used through well-timed application at susceptible growth stages.

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Table 1. Incidence of grain mould pathogens on sorghum cultivars after moisture application to panicles at different growth stages in the field

Cultivars	Seredo	ETS2111	IS9302	ETS2752	Awash1050		Seredo	ETS2111	IS9302	ETS2752	Awash1050	
Growth Stage	*Fusarium proliferatum* (%)					Mean	*Fusrium thapsinum* (%)					Mean
Anthesis	4.0b[1]	6.3c	0.0a	0.0a	7.3c	3.5c	1.7b	3.0c	0.0a	0.0a	4.3c	1.8c
Milk	3.0b	12.0b	0.7a	0.0a	10.3ab	5.2b	1.7b	6.0b	0.3a	0.0a	5.7b	2.7b
Dough	14.3a	26.3a	2.3a	0.3a	11.3a	10.9a	5.7a	11.0a	0.0a	0.3a	6.7a	4.7a
Maturity	3.7b	6.7c	0.7a	0.0a	10.0ab	4.2c	1.7b	3.3c	0.0a	0.0a	4.3c	1.9c
Control	2.3b	6.3c	0.0a	0.3a	8.7bc	3.5c	0.7c	3.7c	0.0a	0.0a	4.7c	1.8c
Mean[2]	5.5c	11.5a	0.7d	0.1d	9.5b		2.3b	5.4a	0.1c	0.1c	5.1a	
	Fusrium graminearum (%)						*Curvularia lunata* (%)					
Anthesis	2.3b	3.3d	0.00a	0.0a	5.0c	2.1b	1.3a	2.3a	0.7a	0.3a	0.7a	1.0a
Milk	1.7b	6.0b	0.33a	0.0a	6.7ab	2.9b	0.7a	0.7b	1.3a	0.3a	0.0a	0.6b
Dough	6.7a	15.7a	0.67a	0.0a	7.7a	6.1a	0.7a	2.3a	1.0a	1.0a	0.3a	1.1a
Maturity	1.7b	4.7c	0.00a	0.0a	5.7bc	2.4b	1.0a	1.7a	0.3a	0.3a	0.3a	0.7ab
Control	1.7b	3.3d	0.33a	0.0a	4.7c	2.0b	1.3a	1.3ab	1.3a	0.7a	0.3a	1.0ab
Mean	2.8c	6.6a	0.27d	0.0d	5.9b		1.0ab	1.7a	0.9ab	0.5b	0.3d	
	Phoma sorghina (%)						*Bipolaris sorghicola (%)*					
Anthesis	5.0a	0.7ab	1.0a	0.0a	0.0b	1.3a	10.0b	1.0a	0.3a	1.3a	0.3a	2.6bc
Milk	3.0c	0.7ab	0.7ab	0.0a	0.0b	0.8b	10.7b	1.0a	0.7a	0.7a	1.3a	2.8b
Dough	4.0b	0.0b	0.7ab	0.0a	0.7b	1.1ab	15.3a	1.0a	0.7a	1.3a	0.7a	3.8a
Maturity	1.7d	0.7ab	0.0b	0.0a	2.0a	0.8b	8.3c	1.3a	0.7a	0.7a	0.0a	2.2cd
Control	1.7d	1.3a	1.0a	0.0a	0.0b	0.8b	6.7d	1.0a	0.7a	1.0a	0.0a	1.9d
Mean	3.07a	0.7b	0.7b	0.0b	0.5b		10.2a	1.1b	0.6b	1.0b	0.5b	

[1]Within a column, means followed by the same letter are not significantly different (P = 0.05) based on Duncan's multiple range test.

[2]Means of cultivars within a row followed by different letters are significantly different (P = 0.05) according to Duncan's multiple range test.

Table 2. Incidence of 'saprophytic' fungi on sorghum cultivars after moisture application to panicles at different growth stages in the field

Cultivars	Seredo	ETS2111	IS9302	ETS2752	Awash1050		Seredo	ETS2111	IS9302	ETS2752	Awash1050	
Growth Stage	*Alternaria* spp.					Mean	*Cladosporium* spp. (%)					Mean
Anthesis	26.7a	24.0bc	17.0a	24.3c	30.7a	24.5b	8.0a	13.7bc	23.3a	16.7bc	10.0b	14.3a
Milk	28.0a	30.3a	15.3a	22.3c	30.7a	25.3b	10.7a	12.3bc	23.7a	19.3ab	13.7ab	15.9a
Dough	27.0a	27.0ab	19.3a	29.7b	33.7a	27.3a	10.3a	11.3c	21.3ab	14.3c	17.7a	14.9a
Maturity	29.0a	30.7a	17.0a	38.3a	30.0a	29.0a	10.7a	19.0a	22.3ab	19.7ab	18.0a	17.9a
Control	24.7a	21.7c	15.0a	20.7c	30.0a	22.4c	9.3a	16.7ab	18.0b	22.7a	15.0a	16.3a
Mean[2]	27.1b	26.7b	16.7c	27.1b	31.0a		9.8d	14.6c	21.7a	18.5b	14.8c	

	Epicoccum spp. (%)					
Anthesis	11.0c	6.3ab	5.0bc	2.0c	6.7b	6.2c
Milk	17.0a	4.3b	3.0c	6.0b	10.7a	8.3b
Dough	16.7a	6.7ab	6.0ab	9.3a	10.0a	9.9a
Maturity	16.7a	8.3a	8.0a	6.0b	11.3a	10.1a
Control	13.7b	8.0a	6.0ab	4.0bc	5.0b	7.3b
Mean	15.0a	6.7c	5.8cd	5.5d	8.7b	

[1]Within a column, means followed by the same letter are not significantly different (P = 0.05) based on Duncan's multiple range test.
[2]Means of cultivars within a row followed by different letters are significantly different (P = 0.05) according to Duncan's multiple range test.

Table 3. Grain damage on sorghum cultivars after moisture application to panicles during different growth stages in the field

Grain discolouration (%)						
	Cultivars					
Growth stage	Seredo	ETS2111	IS9302	ETS2752	Awash1050	Mean
Anthesis	16.7c[1]	10.0b	5.7b	3.7a	10.7c	9.3c
Milk	20.7b	10.7b	13.7a	3.3a	16.0b	12.9b
Dough	25.7a	36.7a	7.3b	5.3a	31.7a	21.3a
Maturity	16.7c	9.7b	5.3b	4.7a	16.7b	10.6c
Control	13.3c	7.68b	4.7b	3.7a	9.0c	7.7d
Mean[2]	18.6a	14.9b	7.3c	4.3d	16.8a	
	Seed germination (%)					
Anthesis	75.7abc	82.0a	91.0a	82.7b	79.7ab	82.2bc
Milk	73.3bc	72.0b	86.3a	86.0ab	81.3ab	79.8c
Dough	69.7c	60.7c	88.0a	85.7ab	74.0b	75.6d
Maturity	81.7a	82.0a	91.0a	86.7ab	85.0a	85.3ab
Control	80.7ab	82.7a	91.3a	93.0a	84.0a	86.3a
Mean	76.2c	75.9c	89.5a	86.8a	80.8b	
	1000 seed mass (g)					
Anthesis	26.7ab	29.0ab	23.7b	26.0bc	31.0ab	27.7b
Milk	25.3bc	24.3c	25.3ab	23.7c	32.7a	26.3b
Dough	23.0c	27.7b	22.7b	27.0b	29.3b	25.9b
Maturity	27.7ab	31.0a	25.0ab	30.0a	33.0a	29.3a
Control	28.7a	29.7a	27.0a	31.0a	33.7a	30.0 a
Mean	26.3bc	28.3bc	24.7b	27.5b	31.9a	

[1]Means followed by the same letter within a column are not significantly different at 5% level of significance using Duncan's multiple range test.

[2]Means of cultivars in a row followed by different letters are significantly different according to Duncan's multiple range test.

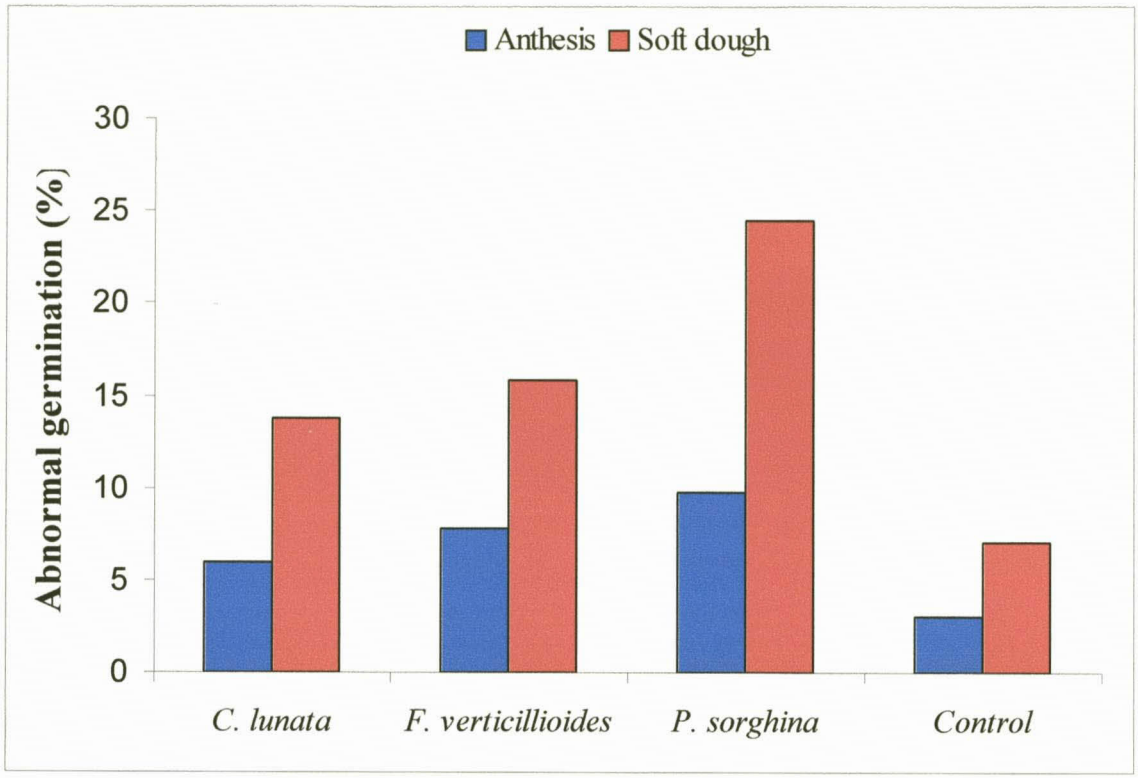
Table 4. Pearson's correlation coefficients between incidences of fungi isolated from sorghum grains and grain damage parameters

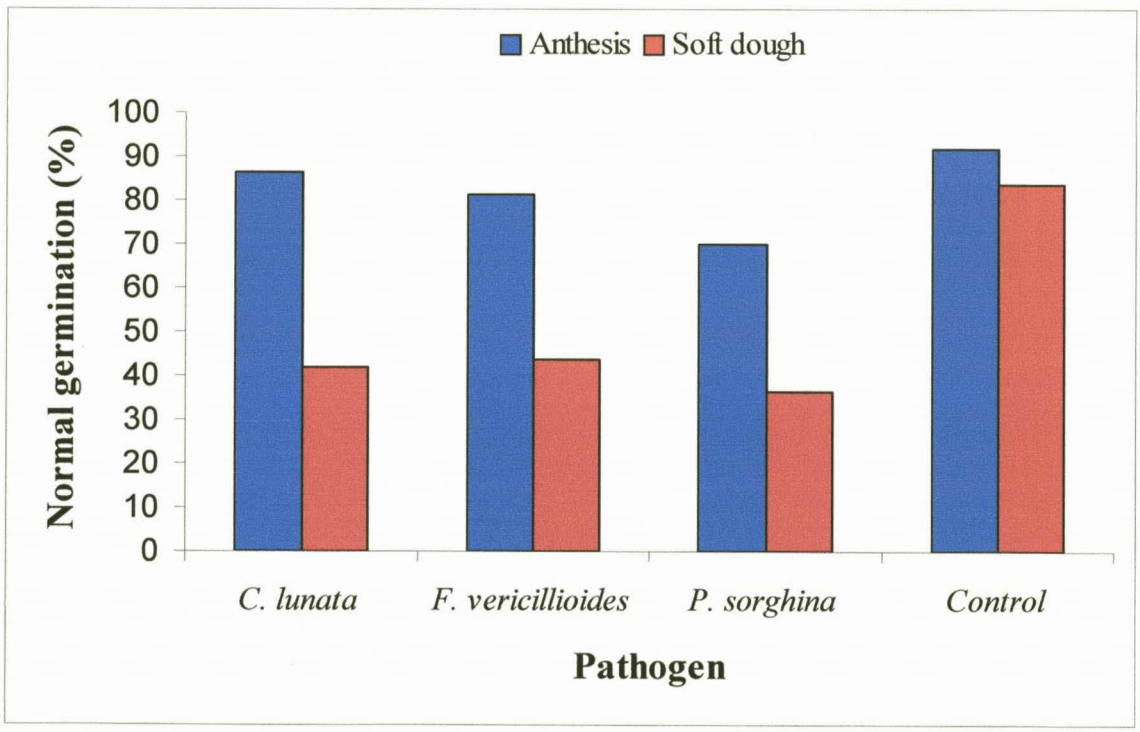
	Grain discolouration	Seed germination	1000-grain mass
Fusarium spp.[1]	0.99**	- 0.94*	- 0.79
Curvularia lunata	0.02	0.08	0.21
Phoma sorghina	0.26	- 0.25	- 0.29
Bipolaris sorghicola	0.92*	- 0.97**	- 0.95*
Alternaria spp.	0.52	- 0.42	- 0.47
Cladosporium spp.	0.02	0.32	0.50
Epicoccum spp.	0.47	- 0.23	- 0.15

**Significant at $P \leq 0.01$; *Significant at $P \leq 0.05$; the remaining coefficients are insignificant at $P \leq 0.05$.

[1] *Fusarium* spp. is the average of *F. proliferatum*, *F. thapsinum* and *F. graminearum* isolates.

Fig. 1. Effect of inoculation of sorghum panicles with grain mould pathogens at different growth stages on normal and abnormal germination of seed in the greenhouse


Anthesis
Soft dough
Abnormal germination (%)
30
25
20
15
10
5
0
C. lunata
F. verticillioides
P. sorghina
Control


Anthesis
Soft dough
Normal germination (%)
100
90
80
70
60
50
40
30
20
10
0
C. lunata
F. vericillioides
P. sorghina
Control
Pathogen

CHAPTER 5

HOST CHARACTERISTICS RELATED TO GRAIN MOULD RESISTANCE IN SORGHUM CULTIVARS FROM ETHIOPIA AND SOUTH AFRICA

ABSTRACT

Seeds of different sorghum cultivars from Ethiopia and South Africa were evaluated for resistance to grain mould, phenol content and morphological traits. The reaction of these cultivars to grain mould was determined in the greenhouse and field. The susceptibility of plants was rated by determining ergosterol content of grains as a measure of invasion by mould pathogens. The varieties were characterized for seed and glume proanthocyanidins and flavan-4-ols, for seed apigeninidins and luteolinidins. Ergosterol contents of grains were compared with their chemical and physical characters to establish possible resistance mechanisms. Resistant varieties contained significantly greater amounts of glume proanthocyanidins, seed flavan-4-ols, apigeninidins and/or luteolinidins than susceptible varieties suggesting the involvement of these compounds in determining resistance to grain mould. There was a highly significant negative correlation between seed ergosterol concentration and level of glume proanthocyanidins both in the greenhouse and field experiments indicating the role of glume proanthocyanidins in mould resistance. Generally, cultivars with diverse and increased concentrations of phenolic compounds had moderate to high levels of resistance. Most of the resistant cultivars have coloured seed pericarps and glumes. However, brown or red pericarps or darker glume colours were not always associated with resistance and resistant cultivars with white seeds and straw-coloured glumes were also encountered. Similarly, resistance was found in early, medium as well as late maturing cultivars.

INTRODUCTION

Grain mould is a major disease of sorghum worldwide (Singh and Agarwal, 1993; Mansuetus *et al.*, 1995; Menkir *et al.*, 1996b; Tsedeke, 1996). It is caused by a complex of fungi, which include primarily *Fusarium thapsinum* Klitich, Leslie, Nelson & Marasas, *Curvularia lunata* (Wakk.) Boedijin, *Fusarium semitectum* Berk. & Ravenel and *Phoma sorghina* (Sacc.) Boerema, Dorenbosch and van Kesteren (Forbes *et al.*, 1992; Singh and Agarwal, 1993; Singh and Bandyopadhyay, 2000). Other fungi such as species of *Bipolaris* and *Colletotrichum* may also infect sorghum grain (Williams and Rao, 1981; Menkir, *et al.*, 1996b). Losses due to grain mould vary from yield reduction (lower seed set and/or smaller seeds) to grain discolouration with poor viability and low market value (Chandrashekar *et al.*, 2000). Furthermore, grain mould pathogens can contaminate grain with mycotoxins, with severe implications to animals and human health (Williams and Rao, 1981; Jardine and Leslie, 1992; Singh and Agarwal, 1993; Somani *et al.*, 1993).

A number of methods have been utilized to control grain mould. These include avoidance, treatment of seeds with hot water and fungicidal plant extracts (Utikar and Shinde, 1989; Forbes *et al.*, 1992), biological control (Bjornberg and Schnurer, 1993; Gupta and Raut, 1997), fungicides and the genetic improvement of cultivars. Avoidance is commonly practised through timely harvesting (Seitz *et al.*, 1983; Bandyopadhyay *et al.*, 1988; Garud *et al.*; 1998), adjusting planting times so that flowering and maturity do not coincide with favourable conditions for infection and growing sorghum in relatively dry areas (Forbes *et al.*, 1992; Singh and Bandyopadhyay, 2000). Many researchers consider avoidance to be impractical in grain mould control (Williams and Rao, 1981; Bandyopadhyay and Mughogho, 1988; Hall *et al.*, 2000). Fungicides have been used as spray treatments in the field (Lukade, 1986; Somani *et al.*, 1995) and as seed treatments to eradicate seed borne grain mould

pathogens (Singh and Agarwal, 1988; Singh *et al.*, 1989; Gopinath and Shetty, 1992). Their high costs however limit many small-scale farmers from using them (Williams and Rao, 1981; Mukuru, 1992; Singh and Bandyopadhyay, 2000)

Currently, the most economical and effective control method of sorghum grain mould appears to be the use of genetic resistance (Bandyopadhyay and Mughogho, 1988; Mukuru, 1992; Menkir *et al.*, 1996a; Chandrashekar *et al.*, 2000; Reddy *et al.*, 2000; Waniska, 2000). An understanding of host plant resistance mechanisms is however essential for effective breeding of mould resistant varieties. Once different resistance factors are identified, it may be possible to combine them into a single host thereby developing a variety with a range of resistance factors (Audilakshmi, 1999; Chandrashekar *et al.*, 2000; Waniska, 2000). Understanding host resistance mechanisms is also a fundamental requirement in breeding grain mould resistant sorghum using molecular techniques such as marker-assisted selection (Duncan, 1994; Chandrashekar *et al.*, 2000)

Mechanisms involving both morphological and biochemical characteristics of grains have been associated with grain mould resistance (Williams and Rao, 1981; Jambunathan *et al.*, 1992; Melake-Berhan *et al.*, 1996; Menkir *et al.*, 1996a). Phenolic compounds are among the most important chemical factors offering resistance to grain mould. Phenolic compounds are secondary plant metabolites found in seeds of a wide variety of plant species (Shirley, 1998). Phenols in sorghum that could be involved in resistance to grain mould include proanthocyanidins (condensed tannins)(Harris and Burns, 1973; Waniska *et al.*, 1989; Melake-Berhan, *et al.*, 1996; Menkir *et al.*, 1996a), leucoanthocyanidins (flavan-4-ols) (Jambunathan *et al.*, 1990; Esele *et al.*, 1993; Martinez, *et al.*, 1994; Melake-Berhan *et al.*, 1996) and 3-deoxyanthocyanidins such as apigeninidins and luteolinidins (Menkir, *et al.*, 1996a).

The concentration and type of phenolic compounds in seeds and hence the degree of their contribution to grain mould resistance tends to vary according to variety and environmental conditions (Jambunathan *et al.*, 1991; Menkir *et al.*, 1996a; Murty, 2000). In breeding for resistance, it is therefore necessary to determine the kinds and quantities of phenolic compounds present in varieties growing in specific areas. Despite the importance of grain mould in Ethiopia and South Africa (Bosman *et al.*, 1991; Tegegne *et al.*, 1995; Tsedeke, 1996), many common sorghum cultivars adapted to these two countries have not been evaluated for their reactions to grain mould nor have they been assessed for traits that may offer resistance to grain mould.

The aim of the present study was therefore to determine the reaction of selected Ethiopian and South African improved sorghum cultivars to grain mould pathogens and to examine relationships of resistance with grain phenol content and physical characteristics in order to identify possible mechanisms of resistance.

MATERIALS AND METHODS

Agronomy and physical characters. Seven improved sorghum cultivars commonly grown in eastern Hararghe, Ethiopia were planted in the field during the 2000/2001 cropping season at Alemaya University Campus Research Station, Raaree, Ethiopia. Seeds were sown in 5 m long plots of four rows with a 75 cm inter-row spacing. After emergence, seedlings were thinned to a 20 cm spacing between plants. Diammonium phosphate (DAP) was applied at planting (150 kg/ha) and urea (100 kg/ha) when plants were 50 cm in length (Temam, 1992). Weeding, cultivation and other agronomic practices were carried out as required for the duration of the experimental period. Plots were arranged in a randomized complete block design with three replications.

At anthesis, six panicles per replication were randomly selected. At the soft dough growth stage (Zadoks decimal code 85), panicles were inoculated with suspensions prepared from mixtures of four known grain mould pathogens (*Fusarium proliferatum, F. subglutinans, Curvularia lunata* and *Phoma sorghina*). Each fungus was grown on malt extract agar (MEA) at 25°C for 10 days. Spore suspensions prepared by homogenizing cultures in sterile distilled water decanting the inoculum suspension through sterile cheesecloth. After adjusting concentrations to 10^5 spores/ml using a haemocytometer (Prasada Rao *et al.*, 1995), the inoculum suspension was sprayed onto panicles until run-off using a hypodermic syringe. Panicles were then covered with moistened plastic sheets for seven days to enhance humidity and thereby facilitate infection. To reduce growth of saprophytic fungi that may be favoured by high temperature, the bags were placed on panicles with out hindering air circulation. Another eight sorghum cultivars were evaluated for their reaction to grain mould after artificial inoculation under greenhouse conditions. After sowing seeds of each cultivar in plastic pots with a 5 kg soil capacity, two plants per pot were retained. Nitrogen, in the form of limestone ammonium nitrate (LAN) was applied at a rate of 1g/kg soil two weeks after emergence. Artificial inoculation of the panicles was performed as described above. Three replications were used for each cultivar.

Morphological data including grain and glume colour were recorded at maturity based on the descriptions in IBPGR and ICRISAT (1993). Number of days to flowering were recorded starting from planting to when 50% of plants commenced flowering (Menkir *et al.* (1996a). Panicle compactness was also determined visually as described in IBPGR and ICRISAT (1993).

Phenol analysis. *Proanthocyanidins and leucoanthocyanidins.* Tannin and flavan-4-ol contents of seeds and glumes were analysed using the methods of Watterson and Butler

(1983) as modified by Menkir *et al.* (1996a) and Melake-Berhan *et al.* (1996). After harvest, seeds and the glumes were threshed manually from the panicle. After grinding sufficient amounts of both the glumes and the seeds, 250 mg flour from each cultivar were extracted in 15 ml of 0.5% HCl in methanol for 20 minutes. The suspension was centrifuged for 5 minutes at 5000 rpm and the supernatant was retained. A 1 ml aliquot was taken from the supernatant and mixed with 14 ml of 30% HCl in 1-butanol. Blanks for both flavan-4-ol and tannin analyses were prepared by adding 0.5 ml of the extract to 7 ml of a mixture of methanol, 0.1N acetic acid and butanol (15:15:70 v/v). The sample and the blank were vortexed and left for 1 hour. The absorbance of the supernatant was measured using a spectrophotometer set to 550 nm for flavan-4-ols after correcting for blanks. Test tubes containing the supernatant were then maintained in boiling water for 2 hours. After cooling at room temperature, the concentration of tannins (proanthocyanidins) was determined by reading the absorbance at 550 nm after correcting for blanks.

3-deoxyanthocyanidin pigments. Two hundred and fifty milligrams of flour was extracted in 15 ml of 100% ethyl acetate for 30 minutes. The supernatant was retained and the residue was re-extracted with 15 ml of 0.5% HCl in methanol for 20 minutes. Acid treated poly-vinylpyrrolidone (PVP) was prepared as described in Watterson and Butler (1983) and Loomis and Battaile (1966). The PVP was boiled for 10 minutes in 10% HCl and repeatedly washed with distilled water. It was then washed with acetone and the residue was filtered and allowed to dry. The acid treated PVP (0.4 g) was then added to 8 ml of the extract and mixed thoroughly using a vortex mixer. The mixture was incubated at room temperature for 10 minutes and centrifuged at 5000 rpm for 5 minutes. Absorbance of the supernatant was determined at 475 nm for apigeninidin and at 495 nm for luteolinidin.

Results of phenol concentrations are expressed as A550/g dry sample for seed and glume proanthocyanidins and flavan-4-ols. Apigeninidin and luteolinidin concentrations are presented as A475 and A495/g dry sample (Melake-Berhan *et al.*, 1996; Menkir *et al.*, 1996a).

Ergosterol analysis. Ergosterol was analysed based on the method of Seitz *et al.* (1977) as modified by Jambunathan *et al.* (1991). Two panicles from each replication of a specific cultivar were harvested and threshed manually. Grain samples from the six panicles were uniformly mixed and a sufficient amount ground using an Udy Cyclone mill. Twenty grams of flour was extracted in 50 ml methanol by vigorous mixing in a 100 ml beaker for 30 minutes using a magnetic stirrer. When the suspension settled, 25 ml clear extract was added to test tubes containing 3 g KOH. These were agitated vigorously on a vortex mixer to dissolve the KOH. Ten millilitres of n-hexane was added to the mixture after which it was incubated in a water bath at 75°C for 30 minutes. After adding 5 ml of distilled water and mixing thoroughly, the mixture was cooled to room temperature. The upper hexane layer was transferred to clean beakers while 10 ml hexane was added to the remaining suspension and then thoroughly mixed. The hexane layer was again removed and added to the previous aliquot and this procedure was repeated once more. Finally the pooled hexane extracts were evaporated to dryness on a hot water bath. The residue was then re-dissolved in 5 ml high performance liquid chromatograph (HPLC) grade methanol and filtered through 0.45µm filters (Millex, Millipore Corporation, Bedford, USA).

From the filtrate, ergosterol content was determined using HPLC (Waters 600E). 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. To calibrate the equipment, standard ergosterol

(Sigma) was injected several times at a concentration of 5μg and the HPLC peak area of the standard was recorded. Subsequently, each sample was injected by mixing with the standard ergosterol (5:5μg ratio). The standard ergosterol had a retention time of ±7 minutes. The peak area of the sample and standard mixture was then recorded. The differences between peak area of the sample plus standard and the peak area of the standard alone gave the peak area of the sample. Based on these data, the concentration of ergosterol in μg/g of seed was calculated.

Statistical analyses, including mean comparison, analysis of variance (ANOVA) and Pearson correlations, were performed using the statistical package Minitab for windows (Minitab, 1998).

RESULTS

Physical characters. Most of the cultivars tested in the present study flowered approximately 80 days after planting with the lowest and highest flowering dates being 65 and 130 respectively (Table 1). The majority of cultivars have coloured seed pericarps (yellow to brown) and glumes (greyed orange to purple) and panicle shapes varied between semi-compact and compact (Table 1). The minimum and maximum air temperature means from inoculation of panicles to physiological maturity of grains were 12.67 and 29.03°C in the greenhouse and 9.5 and 23.5°C in the field experiments.

Phenol and ergosterol analysis. In the greenhouse, cultivars including Gambella1107, NK286, Buster and SNK3939 had significantly lower concentrations of ergosterol than remaining cultivars (3.56-23.58 μg/g seed) (Table 2). Within these cultivars, NK286 and SNK3939 had relatively higher concentration of seed proanthocyanidins while Gambella1107 showed lower seed proanthocyanidin content. Gambella1107 and NK286 did

not have seed flavan-4-ols while SNK3939 had the highest concentration followed by PAN8446 and Buster. Significantly higher levels of apigeninidin and luteolinidin were observed in NK286, SNK39339 and Buster than in the remaining cultivars.

SNK3939 had significantly higher concentration of glume flavan-4-ols than the remaining cultivars. Nonetheless, NK286 and Buster generally exhibited lower levels of glume flavan-4-ols with Gambella1107 having the lowest amount of this compound. Gambella1107 was also among cultivars with the highest levels of glume proanthocyanidins. High concentration of the latter was found also in NK286, Buster and PAN8446 (Table 2).

NK283 and Seredo respectively contained 135.6 and 117.8 μg/g ergosterol (Table 2). Conversely, these cultivars had significantly lower seed flavan-4-ol than other cultivars. Furthermore, NK283 and Seredo had the lowest glume proanthocyanidin concentrations. PAN8446 and IS9302 appeared to have intermediate levels of ergosterol concentration (44.24 and 63.20 μg/g sample respectively) (Table 2).

Among cultivars tested in field trials, significantly lower concentrations of ergosterol were observed in AL-70, IS8686 and ETS2752 (Table 3). These cultivars however possessed significantly higher levels of glume proanthocyanidin than other cultivars. Furthermore, IS8686 had higher levels of seed apigeninidin, luteolinidin, flavan-4-ol and proanthocyanidin. Similarly, higher levels of seed flavan-4-ol and luteolinidin were noted in ETS2752. However, AL-70 had the lowest level of seed apigeninidin and seed proanthocyanidin and did not contain seed flavan-4-ol, luteolinidin and glume flavan-4-ol (Table 3).

Awash1050 and ETS2111 had significantly higher ergosterol concentrations (Table 3). These cultivars also had the lowest levels of glume proanthocyanidins. Furthermore, Awash1050 contained lower concentrations of seed apigeninidin, luteolinidin as well as proanthocyanidins and did not have seed flavan-4-ols (Table 3). Similarly, negligible

amounts of luteolinidin as well as seed and glume flavan-4-ol were found in ETS2111 while the levels of seed apigeninidin and proanthocyanidin were comparatively lower in the latter cultivar. ETS1105 and ETS576 possessed intermediate levels of ergosterol (over 57μg/g seed) (Table 3). Furthermore, these cultivars had a generally higher seed proanthocyanidin concentration with ETS1005 also having the highest levels of glume flavans. In addition, ETS1005 had higher seed apigeninidin and luteolinidin compared to most of the remaining cultivars. ETS576 had a significantly higher concentration of apigeninidin than Awash1050 and AL-70 and significantly higher glume proanthocyanidin concentrations than Awash1050 and ETS2111 (Table 3).

The trend in type and level of phenols produced by cultivars is shown in Fig 1. Generally, resistant to moderately resistant cultivars correspond to longer bars in the figure hence containing more diverse and greater quantities of phenolic compounds. Susceptible cultivars had fewer and/or lower amount of the various phenol types (Fig. 1).

DISCUSSION

Results of the present study show that grain mould is only slightly affected by the maturity of the respective cultivars (Table 1). Resistant cultivars were found in the early (< 66 days), medium (67 –80 days) and late (> 80 days to 50% flowering) maturity types. In studies carried out by Garud *et al.* (1994), flowering date (maturity time) appeared to influence grain mould severity with late maturity types showing lower mould levels. On the other hand, Menkir *et al.* (1996a) found that late maturity might not always be related to resistance. Perhaps the resistance of late maturing cultivars could partly be due to flowering and maturity beginning during conditions unfavourable for mould development and thereby escaping inoculum pressure. Panicle shape is unlikely to have influenced the variation in

resistance observed in the present study since the cultivars tested were found to be closely related in degree of panicle compactness (Table 1).

Coloured pericarp grains may have higher grain mould resistance than white grains (Hahn and Rooney, 1986; Hiremath *et al.*, 1993; Waniska, 2000). Even though most of the resistant cultivars in the present study were brown or red, darker pericarp colour was not always associated with resistance (Tables 1, 2, and 3). Furthermore, cultivars such as Gambella1107, which have a white pericarp, were highly resistant. Similarly, in other areas, the susceptibility of grain with a coloured pericarp has been reported (Williams and Rao, 1981). Waniska *et al.* (1989) and Audilakshmi, *et al.* (1999) also reported that grain colour has no a major role in grain mould resistance. The majority of moderately resistant to resistant cultivars have pigmented glumes (Table 1 and Fig. 1). Nonetheless, cultivars with straw-coloured glumes were also found to have resistance and there are those also with pigmented glumes that are susceptible. Audilakshmi *et al.* (1999) found a strong relationship between darker glume colours and grain mould resistance. However, the same authors reported that dark-coloured glumes may not always offer resistance to grain mould.

The quantification of fungal biomass in seeds using biochemical characters specific to fungi (ergosterol method) is preferred to other criteria (Seitz *et al.*, 1983b; Forbes *et al.*, 1992; Magan, 1993; Singh and Agarwal, 1993). This method has the advantage of high sensitivity in detecting the degree of internal seed colonization by fungi. According to Audilaksmi *et al.* (1999), the ergosterol content of the majority of sorghum cultivars resistant to grain mould was not more than 20 µg/g seed. In contrast, cultivars that are susceptible had more than 100µg ergosterol per gram of seeds (Audilakshmi *et al.*, 1999). In the present study, after artificial inoculation with grain mould pathogens, cultivars including Gambella1107, NK286, SNK3939 and Buster had lower concentrations of ergosterol (< 24 µg/g) suggesting their

resistance to grain mould. In a field trial, AL-70, IS8686 and ETS2752 had lower ergosterol concentrations (< 20 μg/g), which also suggests their resistance to grain mould. On the other hand, the high ergosterol concentration (> 100 μg/g) in cultivars including Seredo, NK283, Awash1050 and ETS2111 is an indication of their susceptibility to grain mould. The remaining cultivars had intermediate levels of ergosterol that may indicate their having moderate resistance (Table 2 and 3).

NK286 and IS 8686 had higher seed and glume proanthocyanidins as well as greater amounts of seed apigeninidin and luteolinidins suggesting the role of these compounds to resistance (Table 2 and 3). Higher levels of proanthocyanidins are known to associate with increased resistance to grain mould (Harris and Burns, 1973; Waniska *et al.*, 1989; Melake-Berhan, *et al.*, 1996; Menkir *et al.*, 1996a). Likewise, SNK3939 seems to obtain resistance from seed and glume proanthocyanidin although this cultivar also had higher levels of seed flavan-4-ol, which may enhance its resistance. Flavan-4-ols have been reported to impart resistance to grain moulds in some sorghum varieties (Jambunathan *et al.*, 1990; Esele *et al.*, 1993; Martinez, *et al.*, 1994; Melake-Berhan *et al.*, 1996).

In addition to having increased amounts of seed flavan-4-ols, the resistant cultivar Buster also had greater amounts of seed apigeninidin and luteolinidin, as did the other resistant cultivar NK286. Furthermore, most of the moderately resistant cultivars also had relatively higher levels of one or both apigeninidin and luteolinidin compared to susceptible cultivars (Table 2 and 3). Most likely, apigeninidins and luteolinidins could have contributed to the resistance of these cultivars. Although flavanoids such as apigeninidin and luteolinidin are known to occur specifically in sorghum (Shirley, 1998), they have not been recognized to offer resistance to grain mould. However, Menkir *et al.* (1996a) recently reported apigeninidin (highly associated with luteolinidin) as one of the principal phenols contributing

to grain mould resistance in sorghum. Apigeninidin has also been reported to inhibit the growth of fungi *in vitro* (Schutt and Netzly, 1991).

Many of the cultivars used in the present study have not previously been characterized for phenol content. Some, eg. NK283 were previously found to contain no proanthcyanidin (Prof. J. Taylor, University of Pretoria, South Africa and Dr. L. Rooney, Texas A&M University, College Station, Texas, personal communication). In the present study, significant levels of proanthocyanidin (condensed tannin) were however recorded in NK283. These differences may partly be attributed to variation in environmental conditions under which the cultivar was grown. Environment has been known to significantly influence phenol synthesis in plants. Wong (1976) has summarized many works where factors such as light, stresses from wounding and infection by pathogens influence flavonoid synthesis in different plants including peas and buckwheat. Nutrient imbalances such as excess nitrogen are also known to inhibit phenol accumulation (Margna, 1977). In aspen tree (*Populus tremuloides* Michx.), the same clone exposed to varying levels of CO_2 yielded significantly different concentrations of condensed tannin in leaves (Teeri *et al.*, 1995).

Gambella1107 and AL-70, both resistant cultivars, had either lower or none of the phenols mentioned above. Nonetheless, these two cultivars stand out among resistant cultivars with the highest levels of glume proanthocyanidins (Table 2 and 5.3) thus indicating that this compound may offer resistance in these cultivars. ETS2752 also appears to derive its resistance largely from glume proanthocyanidins (Table 3). Furthermore, a strong negative correlation was found between the concentration of ergosterol and glume proanthocyanidin in both the greenhouse ($r = - 0.83$, $P = 0.01$) and field ($r = - 0.87$, $P = 0.01$) experiments (Table 4) suggesting the greater influence of this trait on grain mould resistance. Little information is available from literature on the role of glume phenols in grain mould resistance. Mansuetus

(1990) found that compared to susceptible varieties, resistant ones have higher levels of phenolic compounds in their glumes and that such glumes were less colonized by *F. thapsinum*. Similarly, Waniska *et al.* (1992) reported that resistant sorghums respond to invasion by grain mould pathogens faster than susceptible varieties by producing increased concentrations of phenolic compounds in their glumes. In contrast, Audilakshmi *et al.* (1999) found a weak relationship between measures of invasion by grain mould and glume phenol contents. Further studies are required on the influences of glume tannin on grain mould using a large number of cultivars. If glume tannins are not always linked to seed tannins, white seeded, tannin-free resistant varieties that are preferred for food in many sorghum-growing areas may be developed (Waniska, 2000).

There is evidence to suggest that the contribution of some phenols to resistance may be greater than others (Table 2, 3 and Fig. 1). Glume proanthocyanidins appear important in offering resistance to Gambella1107, AL-70 and ETS2752. In these cultivars, the level of seed proanthocyanidin was not significantly higher than in the susceptible cultivars Seredo, NK283, Awash1050 and ETS2111. Moreover, they also contain none or negligible amounts of the other compounds evaluated with the exception of ETS2752, which contains moderate levels of seed apigeninidin and luteolinidin. In addition to glume proanthocyanidins, factors other than those considered in the present study may possibly have contributed to the resistance of cultivars such as Gambella1107 and ETS2752. Anti-fungal proteins and grain hardness have been implicated in grain mould resistance (Mukuru, 1992; Kumari and Chandrashekar, 1994; Menkir *et al.*, 1996a; Seetharaman *et al.*, 1997; Rodriguez *et al.*, 1999). Preliminary studies on some cultivars tested in the present study however, indicated the absence of a significant correlation between grain hardness and mould resistance (Table 1).

In general, cultivars possessing wide range and increased levels of phenolic compounds appear to have improved resistances to grain mould (Fig. 1). Conversely, susceptible cultivars had few and/or relatively smaller quantities of phenols (Fig. 1). Corresponding to our results, Menkir *et al.* (1996a) found that the overall resistance of sorghum to grain mould may not be explained by a single chemical or physical characteristic of the grain. Accordingly, they developed a selection index consisting of a mixture of five chemical and physical traits including apigeninidin, flavan-4-ols, and tannin concentrations in grains as well as endosperm texture and grain colours. Mukuru (1992) also reported that cultivars with combinations of high tannin, flavan-4-ols and grain hardness had relatively higher resistance. Many other authors also hold the view that better grain mould resistance results mainly from a combination of resistance factors than a single mechanism alone (Esele, *et al.*, 1993; Seetharaman *et al.*, 1996; Audilakshmi *et al.*, 1999; Chandrashekar *et al.*, 2000; Reddy *et al.*, 2000).

Results of the present study provide important information on chemical and physical characteristics of grains and resistance of major cultivars adapted to Ethiopia and South Africa. This knowledge may help sorghum breeders of the two countries involved in the development of resistant cultivars. The presence of cultivars with white grain and light coloured glume (with high tannin that imparted resistance to grain mould) is significant. Probably, it will be possible in the future to develop grain mould resistant cultivars with a better nutritional value having low seed- but high glume-tannin. Such cultivars also give products that are less pigmented and are more preferred for food in many countries including Ethiopia. The coloured resistant South African cultivars can be more suitable for purposes other than food, for example, the brewing of opaque beers as is presently being practiced.

The reaction of cultivars based on ergosterol content needs to be confirmed by evaluating them for mould severity or incidence and through correlating the latter with the ergosterol content.

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Table 1. Days to 50% flowering, panicle shape, seed and glume colour of sorghum varieties and hybrids evaluated for grain mould resistance

Cultivar	Days to 50% flowering	Panicle shape	Seed colour	Glume colour	Grain hardness[1]
NK286	67	Semi-compact, elliptic	Brown	Greyed orange	-
PAN8446	67	Semi-compact, elliptic	Light brown	purple	7.76
SNK3939	78	Semi-compact, elliptic	Light brown	Red	8.02
Buster	65	Semi-compact, elliptic	Brown	Purple	10.54
NK283	78	Semi-compact elliptic	Light brown	Greyed orange	11.09
Gambella1107	78	Compact, elliptic	White	Orange	-
Seredo	86	Compact elliptic	Light brown	Greyed orange	-
IS9302	78	Compact, elliptic	Red	Greyed orange	11.15
ETS1005	130	Compact, oval	Red	Greyed orange	10.87
ETS2752	118	Compact, elliptic	Straw	Straw	-
IS8686	80	Compact, elliptic	Red	Straw	-
Awash1050	97	Compact, elliptic	Yellow	Red	-
ETS2111	97	Compact, oval	Straw	Greyed orange	-
ETS576	130	Compact, oval	White	Purple	-
Al-70	127	Semi-compact, elliptic	White	Greyed orange	9.95

[1]Figures are times in seconds required to abrade 1 % of sorghum kernel; Harder grains require longer abrasion times; Pearson correlation analysis between ergosterol concentration and grain hardness gave $r = 0.43$, $P = 0.34$.

Table 2. Concentrations of ergosterol and phenolic compounds in seeds and glumes of sorghum evaluated under greenhouse conditions

Cultivar	Ergosterol (μg/g)	Seed proanthocyanidins (A550/g)[1]	Glume proanthocyanidins (A550/g)	Seed Flavan-4-ols (A550/g)	Seed Apigeninidin (A475/g)	Seed luteolinidin (A495/g)	Glume flavan-4-ols (A550/g)
NK286	12.37f[1]	1.85a	2.07a	0.00d	1.85a	1.86b	0.02d
PAN8446	48.24d	1.61b	2.02ab	0.06b	0.68c	0.66c	0.02d
SNK3939	23.58e	1.83ab	2.01ab	0.09a	1.85a	2.03a	0.08a
Buster	12.77f	1.76ab	1.79c	0.06b	1.04b	0.73c	0.03c
NK283	135.60a	1.72ab	1.58d	0.01c	0.28e	0.03e	0.06b
Gambella1107	3.56g	1.69ab	2.00ab	0.00d	0.03f	0.00e	0.01e
Seredo	117.80b	1.67ab	1.70cd	0.01cd	0.48d	0.44d	0.03e
IS9302	63.20c	1.79ab	1.84bc	0.01cd	0.19e	0.09e	0.06b
SE	2.27	0.07	0.06	0.01	0.04	0.05	0.002

[1]Means within a column followed by the same letter are not significantly different at 5 % level based on Duncan's Multiple Range Test.

Table 3. Concentrations of ergosterol and phenolic compounds in seeds and glumes of sorghum evaluated under field conditions

Cultivar	Ergosterol (µg/g)	Seed proanthocyanidins (A550/g)	Glume proanthocyanidins (A550/g)	Seed Flavan-4-ols (A550/g)	Seed Apigeninidin (A475/g)	Seed luteolinidin (A495/g)	Glume flavan-4-ols (A550/g)
ETS1005	57.38c[1]	1.83a	1.77c	0.03b	0.71b	0.81b	0.06a
ETS2752	19.65d	1.52c	2.25a	0.03b	0.49cd	0.41c	0.01c
IS8686	18.37de	1.91a	1.96b	0.04a	1.17a	0.93a	0.00d
Awash1050	171.80b	1.67abc	1.68cd	0.00c	0.30d	0.28d	0.02b
ETS2111	183.40a	1.51c	1.52d	0.00c	0.47cd	0.00e	0.01c
ETS576	57.38c	1.81ab	1.95b	0.00c	0.60bc	0.21d	0.00d
Al-70	12.34e	1.58bc	2.25a	0.00c	0.05e	0.00e	0.00d
SE	2.25	0.07	0.05	0.002	0.07	0.04	0.002

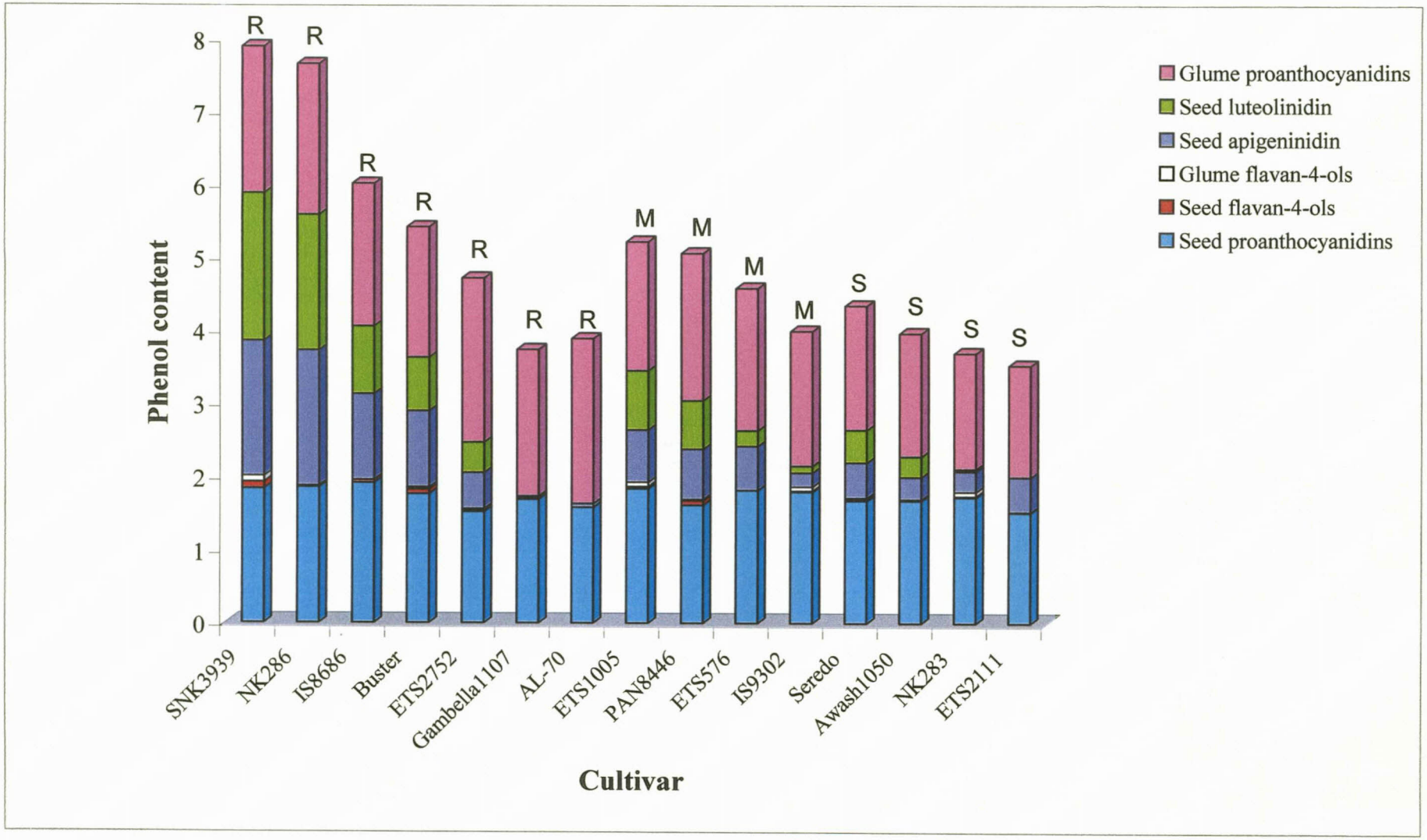
[1]Means within a column followed by the same letter are not significantly different at 5 % level based on Duncan's Multiple Range Test.

Table 4. Coefficients of correlation between ergosterol concentrations and phenolic contents of sorghum seed and glumes

Character	Correlation coefficient (r)	
	Greenhouse	Field
Seed proanthocyanidin	-0.11	-0.62
Seed flavan-4-ols	-0.27	-0.52
Seed apigeninidin	-0.42	-0.24
Seed luteolinidin	-0.46	-0.39
Glume flavan-4-ols	0.35	0.10
Glume proanthocyanidin	-0.83**	-0.87**

** Highly significant (P = 0.01).

Fig. 1. Phenol contents of sorghum cultivars. Each division of the bar shows concentrations of specific compound present in the respective varieties. R = resistant; M = moderately resistant; S = Susceptible.


Phenol content
0
1
2
3
4
5
6
7
8
R
R
R
R
R
R
R
M
M
M
M
S
S
S
S
SNK3939
NK286
IS8686
Buster
ETS2752
Gambella1107
AL-70
ETS1005
PAN8446
ETS576
IS9302
Seredo
Awash1050
NK283
ETS2111
Cultivar
Glume proanthocyanidins
Seed luteolinidin
Seed apigeninidin
Glume flavan-4-ols
Seed flavan-4-ols
Seed proanthocyanidins

SUMMARY

A study aimed to determine grain mould fungi associated with sorghum cultivars grown in two localities (Cedara and North West/Free State) in South Africa indicated *Fusarium subglutinans* and *F. graminearum* to be the predominant species. *Fusarium proliferatum* followed by *F. thapsinum* was however more commonly isolated from Ethiopian cultivars. *Bipolaris sorghicola* and *Phoma sorghina* were found in some cultivars from both countries. *C.urvularia clavata* was frequently isolated from South African sorghum cultivars. *C. lunata* was encountered occasionally although its prevalence was relatively high in Ethiopian cultivars. *Alternaria alternata* had the highest incidence in cultivars from both countries. *Colletotrichum graminicola (Cl. Graminicola)* occurred only in grains from Cedara. *F. proliferatum* and *C. clavata* were isolated from the embryos of many cultivars. *F. graminearum*, *A. alternata*, *P. sorghina* and *Cl. graminicola* were occasionally found in embryos of sorghum grains. Pathogenicity studies showed that *F. subglutinans, Cl. graminicola* and *B. sorghicola* were important in causing various kinds of grain damage (seed discolouration, 1000 grain mass and seed germination). Grain damage tends to increase when many species occurred together rather than separately. Most *Fusarium* spp., *B. sorghicola* and *C. clavata* had relatively higher transmission rates to seedlings and significantly reduced seedling vigour. Pathogens with embryo invading capacity had generally higher rates of transmission.

During a three-year period (1999/2000 to 2001/2002), the relationship between weather variables and grain mould development was assessed in field trials at Cedara, South Africa. Significant positive correlations were observed between the incidence of *A. alternata*, *Curvularia* spp. (*C. lunata* and *C. clavata*), *Fusarium* spp. (*F. proliferatum* and *F. graminearum)*, *B. sorghicola* and minimum temperature, total and frequency of rainfall (average during 4-6 or 5-8 weeks after flowering based on hybrid sorghum used). However,

significant correlations could not be established between seed damage parameters such as seed germination and 1000-grain mass and incidence of different grain mould fungi. Linear models that described relationships between disease incidence and minimum temperature and between disease incidence and rainfall frequency were developed. Models that used minimum temperature as predictor explained 60 to 82% of variation in disease incidence. Frequency of rainfall explained 93% of the variation in disease incidence in one cultivar.

The most susceptible grain development stage of major Ethiopian sorghum cultivars was determined in field trials. Higher infection incidences and damage to grains were associated mainly with dough stage of grain development. Significantly higher incidence of major mould fungi (*Fusarium proliferatum*, *F. thapsinum* and *F. graminearum* and *B. sorghicola*) consistently occurred during milk to dough stages. Incidences of *C. lunata* and *P. sorghina* were generally lower at all stages. Infection by *P. sorghina* increased at anthesis. Frequency of *Alternaria* spp. and *Epicoccum* spp. increased significantly after the dough growth stage. Grain colonisation by *Cladosporium* spp. was not affected significantly by growth stage. Differences in the incidence of fungi over growth stages were more evident in susceptible than resistant cultivars. The incidence of *Fusarium* spp. and *B. sorghicola* was negatively correlated with percentage germination but positively correlated with grain discolouration. A negative correlation was found between the incidence of *B. sorghicola* and 1000-grain mass. In the greenhouse, artificial inoculation at the soft dough growth stage with *C. lunata*, *Fusarium moniliforme* and *P. sorghina*, resulted in a greater reduction in seed germination than inoculation at anthesis.

Sorghum cultivars from South Africa and Ethiopia were evaluated for resistance to grain mould and characterized for physical and chemical characteristics of grains. The objective was to determine resistance factors associated with grains. Resistant cultivars contained significantly greater amounts of phenols including glume proanthocyanidins, seed

flavan-4-ols, apigeninidins and/or luteolinidins compared with susceptible cultivars. A highly significant negative correlation was observed between ergosterol content of grains and level of glume proanthocyanidins suggesting the role of glume proanthocyanidins in mould resistance. Cultivars containing diverse and increased concentrations of phenolic compounds had moderate to high resistance levels. Most of the resistant cultivars have coloured seed pericarps and glumes. Resistance was found in early, medium as well as late maturing cultivars.

The present study revealed the widespread occurrence of grain mould pathogens among cultivars commonly grown in Ethiopia and South Africa. Hence, susceptible cultivars maturing during moist and warm conditions may suffer significant grain yield and quality losses. This dissertation provides important information mainly about the influence of meteorological factors and plant host growth stage on grain mould dynamics. It is hoped that these findings may motivate future studies particularly on the epidemiology of grain mould, to which little attention has been paid.

OPSOMMING

'n Ondersoek is gedoen om swamspesies te identifiseer wat graanskimmel by sorghumkultivars veroorsaak. *Fusarium subglutinans* en *F. graminearum* was oorheersende vanuit die twee lokaliteite in Suid-Afrika (Cedara en Bethlehem) geisoleer. *Fusarium proliferatum* gevolg deur *F. thapsinum* was egter meer algemeen vanaf Ethiopiëse kultivars geïsoleer. *Curvularia clavata* was gereeld vanaf Suid-Afrikaanse kultivars geïsoleer terwyl *C. lunata* net soms opgemerk is, maar laasgenoemde was baie meer opvallend by Ethiopiëse kultivars. *Alternaria alternata* het die hoogste voorkoms op kultivars uit beide lande getoon. *Colletotrichum graminicola* (*C. graminicola*) het net op graan vanaf Cedara voorgekom. *F. proliferatum* en *C. clavata* was vanuit die embrios van meeste kultivars geïsoleer. *F. graminearum*, *A. alternata*, *P. sorghina* en *C. graminicola* het egter net sporadies in embrios voorgekom. Patogenisiteitstoetse het getoon dat *F. subglutinans*, *C. graminicola*, en *B. sorghicola* verskeie tipes graanskade (saadverkleuring, 1000-graan massa en saadontkieming) veroorsaak. Graanskade neig om toe te neem wanneer spesies gesamentlik optree eerder as afsonderlik. Meeste *Fusarium* spp., *B. sorghicola* en *C. clavata* kan teen hoë frekwensie na saailinge oorgedra word en ook kiemkragtigheid van saailinge beduidend verlaag.

Die verwantskap tussen weerveranderlikes en graanskimmel was oor 3- seisoene (1999/200 tot 2001/2002) by Cedara gekwantifiseer. Beduidende positiewe korrelasies is waargeneem tussen die voorkoms van *A. alternata*, *Curvularia* spp. (*C. lunata* en *C. clavata*), *Fusarium* spp. (*F. proliferatum* en *F. graminearum*) *B. sorghicola* en minimum temperatuur, totale reënval asook die frekwensie van reën (gemiddeld tussen 4-6 of 5-8 weke na blomtyd nagelang groeiperiode van sorghumvariteite). Beduidende korrelasies kon nie tussen saadeienskappe soos ontkieming, 1000-graan massa en voorkoms van verskillende

graanskimmelswamme vasgestel word nie. Lineêre modelle is ontwikkel wat die verwantskap tussen siektevoorkoms en minimum temperatuur asook reën frekwensie beskryf. Modelle wat minimum temperatuur as voorspeller gebruik het, kon tussen 60 en 80% van die variasie in siektevoorkoms verduidelik. Frekewensie van reën het 93% van die variasie in siektevoorkoms in een spesifieke kultivar beskryf.

Die mees vatbare graan-onwikkelingstadium van vername Ethiopiëse kultivars was in veldproewe vasgestel. n Hoër voorkoms van infeksie en skade by graan was hoofsaaklik waargeneem by deegstadium van graanontwikkeling. Beduidende hoër voorkoms van die vernaamste swamme (*F. proliferatum*, *F. thapsinum* en *F. graminearum en B. sorghicola)* het gereeld tussen melk- en deegstadiums voorgekom. Die voorkoms van *C. lunata* en *P. sorghina* was oor die algemeen laer by alle groeistadiums. Infeksie deur *P. sorghina* het by stuifmeelstort toegeneem. Die frekwensie van *Alternaria* spp. en *Epicoccum* spp. het noemenswaardig na afloop van deegstadium toegeneem. Kolonisasie van graan deur *Cladosporium* spp. was nie beduidend deur groeistadiums beïnvloed nie. Die invloed van groeistadiums was minder opvallend in weerstandbiedende kultivars. Die voorkoms van *Fusarium* spp. en *B. sorghicola* was negatief gekorreleerd met ontkieming, maar postief met verkleuring van graan. 'n Negatiewe korrelasie was tussen die voorkoms van *B. sorghicola* en 1000-graan massa waargeneem. Onder kunsmatige toestande in die glashuis, het inokulasie met *C. lunata*, *F. moniliforme* en *P. sorghina* 'n groter afname in saadontkieming by sagtedeegstadium meegebring as by stuifmeelstort

Sorghumkultivars van Suid-Afrika en Ethiopië was geëvalueer ten opsigte van fisiese- en chemise-weerstandsmeganismes teen graanskimmel. Saad van weerstandbiedende kultivars het groter hoeveelhede fenole, proantosianidiene, flavan-4-ole, apigenidiene en/of luteolinidiene

bevat in vergelyking met vatbare kultivars. 'n Hoogs beduidende negatiewe korrelasie was tussen ergosterolinhoud van sade en vlakke van proantosianidiene in saadkaffies waargeneem, wat die moontlike rol van proantosianidiene in weerstand teen graanskimmel aandui Verskeie fenoliese verbindings het ook bygedra tot weerstandsvlakke. Weerstand is in alle groeiseisonlengte kultivars waargeneem. Die huidige studie het getoon dat graanskimmelpatogene by sorghum in Suid-Afrika en Ethiopië wyd verspreid voorkom. Vatbare kultivars wat tydens vogtige en warm toestande ryp word, kan verliese in opbrengs en kwalitiet ly. Hierdie proefskrif bevat belangrike inligting met betrekking tot die invloed van meteorologiese faktore en plant groeistadiums op die dinamika van graanskimmel. Dit sal hopelik verdere studies stimuleer, veral op die epidemiologie van graanskimmel wat tot dusver min aandag geniet het .