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Sorghum Pathology and Biotechnology - A Fungal Disease Perspective: Part I. Grain Mold, Head Smut, and Ergot

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ABSTRACT

Three common sorghum diseases, grain mold, head smut and ergot, each of which is directly related to seed production and quality are covered in this review. Each is described with respect to the causal organism or organisms, infection process, global distribution, pathogen variability and effects on grain production. In addition, screening methods for identifying resistant cultivars and the genetic basis for host resistance including molecular tags for resistance genes are described where possible.

Keywords: *Claviceps africana*, *Claviceps sorghi, Curvularia lunata*, *Fusarium thapsinum*, *Sporisorium reilianum*

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GRAIN MOLD

Introduction

A diverse community of microorganisms is associated with the caryopses of all weed and crop plants in the *Poaceae* (formerly "*Gramineae*") that have been investigated (Pitty *et al.* 1987; Ikeda *et al.* 2006). Deleterious fungal communities, in particular, differ depending upon grass host species, environment, geographical location, and level of resistance to grain mold, weathering, post-harvest seed deterioration and associated disease complexes, such as head blight.

Grain mold of sorghum (**Fig. 1**) is caused by a complex of fungal pathogens, which infect the developing caryopsis

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Invited Review

Fig. 1 Example of grain mold resistant (*top*) and susceptible (*bottom*) sorghum varieties. Note obvious weathering and surface colonization of the susceptible panicle.

as early as anthesis, proceed through grain development, and also result in post-harvest deterioration including the production of potentially harmful mycotoxins (Leslie *et al.* 2005; Sharma *et al.* 2010c). Infection is promoted by prolonged periods of humid (> 85 to 90% relative humidity) and/or rainy weather and temperatures of 25 to 35°C throughout grain development, both before and after physiological maturity (Garud *et al.* 2000; Navi *et al.* 2005; Thakur *et al.* 2006).

In sorghum, the "grain mold complex" is composed of over 40 genera of fungi that are capable of infecting and colonizing sorghum grain at all levels of maturity and in storage (Williams and Rao 1981; Singh and Bandyopadhyay 2000; Thakur *et al.* 2003; Erpelding and Prom 2006; dos Reis *et al.* 2010). Although not all species are of equal importance, common saprophytic and facultatively pathogenic genera include *Alternaria*, *Aspergillus*, *Bipolaris*, *Cladosporium*, *Colletotrichum*, *Curvularia*, *Drechslera*, *Epicoccum*, *Exserohilum*, *Fusarium*, *Nigrospora*, *Olpitrichum*, *Penicillium*, *Phoma*, *Rhizopus*, and *Trichoderma* (**Table 1**; **Fig. 2**). For the purposes of this review, the "grain mold complex" includes those fungi introduced to the developing sorghum caryopsis as early as anthesis ("grain mold"), or after physiological maturity as defined by black layer deposition ("grain weathering"), and those that cause degradation and mycotoxin elaboration during storage.

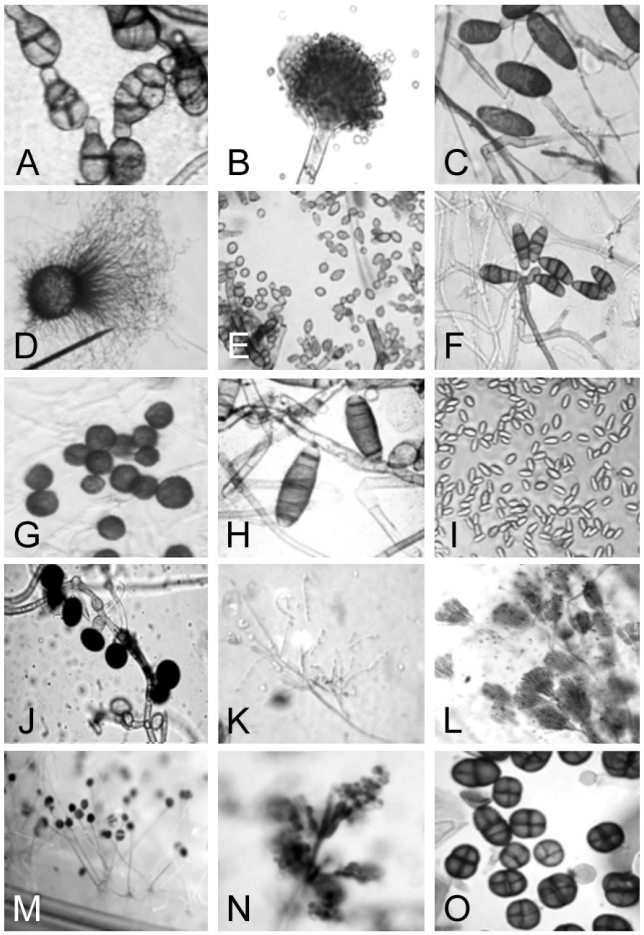

Fig. 2 Examples of fungi associated with sorghum grain mold, grain weathering, and storage molds of sorghum caryopses. (**A**) *Alternaria alternata* (conidia), (**B**) *Aspergillus flavus* (conidia, conidiophore), (**C**) *Bipolaris* sp. (conidia), (**D**) *Chaetomium globosum* (perithecium with curled setae), (**E**) *Cladosporium* sp. (conidia, hyphal fragments), (**F**) *Curvularia lunata* (conidia, conidiophore), (**G**) *Epicoccum nigrum* (conidia), (**H**) *Exserohilum* sp. (conidia, hyphae), (**I**) *Fusarium thapsinum* (microconidia), (**J**) *Nigrospora sphaerica* (aleuriconidia, hyphae), (**K**) *Paecilomyces* sp. (conidia, conidiophores), (**L**) *Penicillium* sp. (conidia, conidiophores), (**M**) *Rhizopus stolonifer* (sporangia, sporangiophores), (**N**) *Trichoderma* sp. (conidia, conidiophore), and (**O**) *Ulocladium* sp. (conidia). See **Table 1** for more information.

Pathogen biology

Over the years, numerous studies have characterized the fungal communities of sorghum caryopses. Leukel and Martin (1943) examined sorghum seed lots and found *Alternaria*, *Aspergillus*, *Helminthosporium*, *Penicillium*, *Rhizopus*, *Trichoderma*, and *Sphacelotheca*. Christensen and Kaufman (1965) showed that *Alternaria*, *Aspergillus*, *Epicoccum*, *Fusarium*, and *Penicillium* were associated with grain deterioration under storage conditions. Gray *et al.* (1971) reported that *Fusarium* spp. caused head mold in sorghum and resulted in grain with partially developed endosperm or resulted in seed abortion. Ellis (1972) found *Alternaria*, *Curvularia*, *Helminthosporium*, and *Fusarium* in sorghum grain. In 1975, Rao (1975) reported that *Curvularia*, *Fusarium*, *Helminthosporium*, *Olpitrichum*, *Penicillium*, and *Phoma* were commonly observed in grain harvested at the ICRISAT farm in Hyderabad, India. In 1982, Hepperly *et al.* (1982) isolated *F. moniliforme sensu lato*, *Curvularia lunata*, and *Alternaria* spp. from grain obtained in Mayaguez, Puerto Rico. *Alternaria*, *Aspergillus*, *Bipolaris*, *Cladosporium*, *Curvularia*, *Dreschlera*, *Exserohilum*, *Fusarium*, *Nigrospora*, *Olpitrichum*, and *Penicillium* were reported by Bandyopadhyay (1986) as being associated with grain weathering. In Texas, Prom *et al.* (2004) isolated *Alternaria*, *Fusarium*, and *Curvularia* most frequently, however, Erpelding and Prom (2006) indicated that *F. semitectum*, *Aspergillus* spp., *Rhizopus* spp., *Bipolaris*, and *Colletotrichum graminicola* were infrequently isolated in Puerto Rico. Noll *et al.* (2010) isolated *F. thapsinum*, *F. equiseti*, *F. proliferatum*, *A. alternata*, *C. lunata*, *Penicillium* spp., and *Aspergillus* spp. from naturally weathered grain produced in Kansas. Yassin *et al.* (2010) isolated *Absidia*, *Alternaria*, *Aspergillus*, *Botryodiplodia*, *Chrysosporium*, *Curvularia*, *Dreschlera*, *Epicoccum*, *Eurotium*, *Exserohilum*, *Fusarium*, *Papulaspora*, *Penicillium*, *Phoma*, *Rhizopus*, and *Stenocarpella* from sorghum grain samples in Saudi Arabia.

As a result, numerous authors have asserted that the most important grain molding fungi (in no particular order) include *Fusarium* spp. (*F. thapsinum*, *F. nygamai*, and *F. semitectum*, among others), *Curvularia lunata*, *Alternaria alternata*, *Phoma sorghina*, and *Colletotrichum graminicola* (*C. sublineolum*) (Singh and Bandyopadhyay 2000; Bandyopadhyay *et al.* 2000; Esele *et al.* 1995; Forbes *et al.* 1992; Prom *et al.* 2011). Although these species vary in importance from location to location, worldwide, *Fusarium*, *Curvularia*, and *Alternaria* spp. rank as the principle grain molding pathogens of sorghum. Thus, because of their cosmopolitan nature, these three species have been given closer attention in the following sections.

Table 1 Fungi associated with sorghum caryopses.

Genus	Species	Reference(s)
Absidia	*A. cylindrospora*	Yassin *et al.* 2010
Acremonium	*A. strictum*	Bandyopadhyay *et al.* 1987; Natural *et al.* 1982; Navi *et al.* 1999
Alternaria	*A. alternata*, *A. chlamydospora*, *A. longissima*, *A. ornatissima*, *A. oryzae*, *A. sorghicola*, *A. tenuissima*	Chilton 1940; Leukel and Martin 1943; Bain 1950; Luttrell 1954; Hsi 1956; Harris and Luttrell 1955; Swarup *et al.* 1962; Deighton *et al.* 1968; Bhale and Khare 1982; González *et al.* 1997; Simmons 2007; Thaung 2008; Husseini *et al.* 2009; Yassin *et al.* 2010
Arthrinium	*Arthrinium* sp.	Husseini *et al.* 2009
Aspergillus (and *Eurotium*)	*A. flavus*, *A. flavus* var. *colomunaris*, *A. fumigatus*, *A. glaucus* group, *A. multicolor*, *A. nidulans*, *A. niger*, *A. ochraceus*, *A. parasiticus*, *A. rubrobrunneus* (*A. ruber*), *A. sydowii*, *A. terreus*, *A. unguis*, *A. versicolor*; *E. amstelodami*, *E. chevalieri*	Swarup *et al.* 1962; Vaidehi and Ramarao 1976; Bhale and Khare 1982; González *et al.* 1997; Husseini *et al.* 2009; Yassin *et al.* 2010
Bipolaris	*B. australiensis, B. bicolor, B. dactyoctenii, B. hawaiiensis, B. kusanoi, B. maydis, B. papendorfii, B. sorghicola, B. spicifera*, *B. zeicola*	Sivanesan 1987; Rossman 2009
Chaetomium	*Chaetomium* sp.	González *et al.* 1997
Chrysosporium	*C. indicum*, *C. tropicum*	Husseini *et al.* 2009; Yassin *et al*. 2010
Cladosporium	*C. cladosporoides*, *C. herbarum*, *C. sphaerospermum*, *C. werneckii*	Swarup *et al.* 1962; Vaidehi and Ramarao 1976; Bhale and Khare 1982; González *et al.* 1997; Husseini *et al.* 2009
Claviceps	*C. africana*, *C. sorghi*, *C. sorghicola*	Bandyopadhyay *et al.* 1998; Tsukiboshi *et al.* 1999
Colletotrichum	*C. sublineolum* (*C. graminicola*)	Sutton 1980; González *et al.* 1997; Husseini *et al.* 2009
Curvularia	*C. affinis*, *C. clavata*, *C. eragrostidis*, *C. fallax*, *C. geniculata*, *C. heteropogonicola*, *C. intermedia*, *C. lunata*, *C. lunata* var. *aeria*, *C. pallescens*, *C. protuberata*, *C. sorghina*, *C. tritici*, *C. tuberculata*	Swarup *et al.* 1962; Morgan-Jones and Karr 1976; Bhale and Khare 1982; Shivas and Sivanesan 1987; Sivanesan 1987; Alcorn 1991; Navi *et al.* 1999; González *et al.* 1997; Husseini *et al.* 2009; Yassin *et al.* 2010
Dreschlera	*D. gedarefensis*, *D. longistrata*, *D. plurisepta*	Nath *et al.* 1970; El-Shaffie 1980; González *et al.* 1997; Yassin *et al.* 2010
Epicoccum	*E. nigrum*	Swarup *et al.* 1962; Melake-Berhan *et al.* 1996; Navi *et al.* 1999; González *et al.* 1997; Yassin *et al.* 2010
Exserohilum	*E. curvatum*, *E. longirostratum*, *E. rostratum*, *E. turcicum*	Sivanesan 1987; Yassin *et al.* 2010
Fusarium	*F. andiyazi*, *F. avenaceum*, *F. chlamydosporum*, *F. equiseti*, *F. graminearum*, *F. heterosporum*, *F. napiforme*, *F. oxysporum*, *F. semitectum* (*F. incarnatum*, *F. pallidoroseum*), *F. proliferatum*, *F. sacchari*, *F. sambucinum*, *F. solani*, *F. sporotrichioides*, *F. subglutinans*, *F. thapsinum*, *F. verticillioides*	Swarup *et al.* 1962; Bhale and Khare 1982; Onyike and Nelson 1992; González *et al.* 1997; Nirenberg and O'Donnell 1998; O'Donnell *et al.* 1998; Marasas *et al.* 2001; Husseini *et al.* 2009; Kvas *et al.* 2009; Sampietro *et al.* 2010; Yassin *et al.* 2010
Geotrichum	*G. candidum*	Frederiksen and Odvody 2000
Gliomastix	*Gliomastix* sp.	González *et al.* 1997
Gonatobotrys	*G. simplex*	Swarup *et al.* 1962
Gloeocercospora	*G. sorghi*	Frederiksen and Odvody 2000
Humicola	*Humicola* sp.	González *et al.* 1997
Mucor	*Mucor* sp.	Husseini *et al.* 2009
Nigrospora	*N. oryzae*, *N. sphaerica*	Swarup *et al.* 1962; González *et al.* 1997
Paecilomyces	*Paecilomyces* sp.	González *et al.* 1997
Papulaspora	*P. irregularis*	Yassin *et al.* 2010
Olpitrichum	*Olpitrichum* spp.	Frederiksen and Odvody 2000
Penicillium	*P. aculeatum*, *P. chrysogenum*, *P. citrinum*, *P. duclauxii*, *P. expansum*, *P. fellutanum*, *P. funiculosum*, *P. griseofulvum*, *P. herquei*, *P. islandicum, P. notatum*, *P. ochreaceus*, *P. oxalicum*, *P. puberulum* (*P. lanosum*), *P. purpurogenum* (*P. rubrum*), *P. restrictum*, *P. simplicissimum*, *P. variable*, and *P. verrucosum*	Bhale and Khare 1982; Swarup *et al.* 1962; Vaidehi and Ramarao 1976; Guadet and Major 1986; Ye *et al.* 1995; González *et al.* 1997; Navi *et al.* 1999; Bandyopadhyay *et al.* 2000; Yassin *et al.* 2010
Periconia	*P. circinata*	Swarup *et al.* 1962
Phoma	*P. sorghina*, *Phoma* sp.	Bhale and Khare 1982; González *et al.* 1997; Aveskamp *et al.* 2009; Husseini *et al.* 2009
Ramulispora	*R. sorghi*	Holliday, 1980
Rhizopus	*R. stolonifer*, *R. oryzae*	Leukel and Martin 1943; Christensen and Kaufman 1965; Bhale and Khare 1982; Husseini *et al.* 2009; Yassin *et al.* 2010
Rhodotorula	*R. rubra*	Husseini *et al.* 2009
Scopulariopsis	*Scopulariopsis* sp.	Husseini *et al.* 2009
Sordaria	*S. fimicola*	Swarup *et al.* 1962
Torula	*Torula* sp.	Husseini *et al.* 2009
Trichoderma	*T. harzianum*, *Trichoderma* sp.	Bhale and Khare 1982; González *et al.* 1997; Husseini *et al.* 2009

Alternaria spp. are associated with numerous seed diseases and foliar leaf spots on grass hosts. *Alternaria* spp. appear as dark dematiaceous hyphal growth where conidia in chains are observed growing from the surface of sorghum kernels (Singh and Bandyopadhyay 2000). Several species of *Alternaria* have been isolated from sorghum tissues, including caryopses, stalks and leaves including *A. alternata*, *A. chlamydospora*, *A. longissima*, *A. ornatissima*, *A. oryzae*, *A. sorghicola*, and *A. tenuissima* (**Table 1**). However, *A. alternata* (Fr.:Fr) Keissl. (syn. *A. tenuis* Nees) is the most commonly isolated *Alternaria* species from sorghum grain (Navi *et al.* 1999; Husseini *et al.* 2009; Yassin *et al.* 2010). This fungus is characterized by dematiaceous hyphae, conidia, and conidiophores. Castor (1981) indicated that the presence of *Alternaria* spp. in a seed sample was normal and not necessarily associated with poor seed quality.

Numerous authors (Esele *et al.* 1993; Esele *et al.* 1995; Forbes *et al.* 1992; Singh and Bandyopadhyay 2000; Bandyopadhyay and Chandrashekar 2002; Prom *et al.* 2011) have identified *Curvularia lunata* (Wakker) Boedijn as a

major pathogen responsible for grain mold throughout sorghum producing regions. However, *C. lunata* is not the only species that has been isolated from sorghum caryopses. At least twelve other species have been isolated from sorghum caryopses, and many of these have also been found in association with close relatives of sorghum and common weedy grasses in the *Andropogoneae* (Sivanesan 1987; Navi *et al.* 1999) (**Table 1**).

C. lunata can infect developing caryopses prior to physiological maturity. Infection at this point in grain development leads to pre-mature black layer deposition of at least two weeks in susceptible sorghum lines (Castor 1981). Somani *et al.* (1994) showed that maltose and starch were the optimum carbon sources for all isolates of *C. lunata* collected in India, whereas xylose could be used by some isolates. Additionally, ammonium salts were a preferred nitrogen source.

Fusarium spp. are the dominant fungi in the sorghum caryopses and compose the majority of the important pathogens in the grain mold complex (Sharma *et al.* 2010c). Many species within this genus are pathogenic and cause grain mold, head blight, and stalk rot (Leslie *et al.* 2005). However, most are facultative saprophytes or may live as endophytes within plant tissues (Prom and Erpelding 2009). *Fusarium* spp. function as saprophytes, endophytes and pathogens within the agroecosystems of other *Poaceae*, including maize, pearl millet, rice and sugarcane, as well as many weedy grass relatives such as johnsongrass (Marasas *et al.* 2001; Leslie *et al.* 2005; Leslie and Summerell 2006. Funnell-Harris and Pederson (2011) showed the presence of numerous sorghum-associated *Fusarium* spp., including *F. thapsinum*, *F. verticillioides*, *F. proliferatum*, and *F. andiyazi* among others, in soil and air samples obtained from sorghum fields in Nebraska providing some evidence for the source and dispersal of *Fusarium* conidia associated with grain mold infections.

Fusarium spp. are characterized by chains of microconidia produced from mono- or polyphialides which appear to predominate in sorghum tissues and especially caryopses. Thus, this character was used for many years as a quick diagnostic tool and as a result many species were "lumped" into a single morphological species. Historically, *F. moniliforme* Sheldon (*Gibberella fujikuroi* 'species complex') has represented the most important *Fusarium* pathogen isolated from grain. However, over the last two decades, "*F. moniliforme*" (*sensu lato*) has been replaced through more accurate subdivision into multiple *G. fujikuroi* mating populations, which in many cases represent species with unique sexual stages. Of the more than fifteen *Fusarium* spp. in the "*G. fujikuroi* species complex" (or at least thought to be, based upon morphology or genetic relatedness data) that produce chains of microcondia, seven have been isolated from sorghum. These include *F. andiyazi* Marasas, Rheeder, Lamprecht, Zeller & Leslie (sexual stage unknown), *F. napiforme* Marasas, Nelson & Rabie (sexual stage unknown), *F. nygamai* Burgess & Trimboli (mating population G; *G. nygamai* Klaasen & Nelson sexual stage), *F. proliferatum* (Matsushima) Nirenberg (mating population D; *G. intermedia* (Kuhlman) Samuels, Nirenberg & Seifert sexual stage), *F. pseudonygamai* O'Donnell & Nirenberg (sexual stage unknown; more common on millets), *F. thapsinum* Klittich, Leslie, Nelson & Marasas (mating population F; *G. thapsina* Klittich, Leslie, Nelson & Marasas sexual stage), and *F. verticillioides* (Saccardo) Nirenberg (mating population A; *G. moniliformis* Wineland sexual stage). Other members of the *G. fujikuroi* species complex that are found but do not produce microconidia in chains include *F. konzum* Zeller, Summerell & Leslie (mating population I; *G. konzum* Zeller, Summerell & Leslie sexual stage), *F. sacchari* (E.J. Butler) W. Gams (mating population B; *G. sacchari* Summerell & Leslie sexual stage), and *F. subglutinans* Wollenweber, Reinking, Nelson, Tousson & Marasas (mating population E; *G. subglutinans* Nelson, Tousson & Marasas sexual stage).

Other *Fusarium* spp. have also been recovered from sorghum caryopses, but may be less important as saprophytes and facultative pathogens. These include *F. culmorum* (W.G. Smith) Saccardo, *F. equiseti* (Corda) Saccardo (*G. intricans* Wollenweber sexual stage), *F. graminearum* Schwabe (*G. zeae* Schwein (Petch) sexual stage), *F. polyphialidicum* Marasas, Nelson, Tousson & van Wyk (sexual stage unknown), and *F. semitectum* Berkeley & Ravenel (sexual stage unknown) (González *et al.* 1997; Klittich *et al.* 1997; Marasas *et al.* 2001; Leslie and Summerell 2006; Sharma *et al.* 2010c). Funnell-Harris and Pedersen (2011) desribe *F. equiseti* in the *F. incarnatum-F. equiseti* complex, or "FIESC". Although the sexual stage is not always important for the pathogen life cycle or disease, the distinction of these *Fusarium* species is important because it suggests that different sources of resistance may be required to combat different species or *Fusarium* species complexes.

DNA-based diversity measures

Little work has been done to characterize diversity within the many groups of fungi associated with grain mold and grain weathering *per se*. However, several studies have noted diversity among grain mold species as measured using morphological, cultural characteristics, toxin elaboration, or pathogenicity (El Shafie and Webster 1981; Girish *et al.* 2004; Horn 2006; Thakur *et al.* 2006; Campos *et al.* 2008). To date, this type of data has only rarely been tied to molecular data from a comprehensive genetic study within genera or species described as part of the "grain mold complex". In one example, de Hoog and Horré (2002) showed that *Alternaria tenuissima*, including an isolate from sorghum, and *A. alternata* isolates from a wide range of plant sources were grouped according to ITS 1-2 rDNA (internal transcribed spacers of ribosomal DNA) sequences as saprotrophs and thus separated from clusters including plant pathogenic species, such as *A. porri* and *A. solani*. Also, Shimizu *et al.* (1998) constructed a *Bipolaris* spp. phylogeny using sequences of the melanin biosynthesis gene, *Brn1*. Within the framework of this phylogeny (via neighbor-joining and maximum likelihood tree methods) isolates from sorghum, including *B. australiensis*, appear to belong to a cluster associated with the teleomorph *Pseudocochliobolus*, whereas *B. bicolor*, *B. cookei*, and *B. panici-milliacei* corresponded to *Cochliobolus* teleomorph(s). Further, several unidentified *Bipolaris* spp. from sorghum may be new, yet unidentified species (Shimizu *et al.* 1998). *B. sorghicola* (syn. *Dreschlera sorghicola*) may be obtained from grain, but is generally known for causing target leaf spot and is conditioned by the resistance gene, *ds1*. Borges (1983) noted differential pathogenicity of *B. sorghicola* isolates on sorghum in Venezuela, and more recently, Katewa *et al.* (2005) noted variation in isolate growth and morphology, lesion development and overall aggressiveness, indicating potential heterogeneity among *B. sorghicola* isolates. However, no studies to our knowledge have examined the DNA-based genetic diversity of these and other related "*Helminthosporium sensu lato*" fungi from sorghum caryopses. To our knowledge, little or no work has been done to characterize the genetic diversity or interspecies relationships of other important dematiaceous grain mold fungi such as *Cladosporium* and *Curvularia* spp.

Pažoutová (2009) examined 41 isolates of *Phoma sorghina* from sorghum and millet caryopses originating from Africa and Texas and found that rep-PCR marker haplotypes showed UPGMA clustering according to geographic location and host origin, e.g., Corpus Christi and Beeville (Texas, USA) versus Dalmas-Paltrand (South Africa) and Namibia, and sorghum versus millet hosts, respectively. Although no isolate from sorghum was tested, isolates of *Epicoccum nigrum*, a caryopsis-associated fungus closely related to *Phoma*, were genetically heterogeneous as shown by arbitrary primed (AP)-PCR, tDNA-PCR, microsatellite-primed PCR, ARDRA (amplified rDNA restriction analysis) and AFLP (amplified fragment length polymorphisms); however, genetic differences did not correlate with geogra-

phical or ecological origins of the isolates (Arenal *et al.* 1999).

In *Fusarium*, genetic diversity studies from the past are confounded by the use of the name "*Fusarium moniliforme*" in earlier literature (see detailed discussion above). Marasas *et al.* (2001) compared members of the *Gibberella fujikurori* species complex: *F. andiyazi*, *F. verticilloides*, *F. thapsinum*, and *F. nygamai*, all previously classified as "*F. moniliforme*", among themselves and other *Fusarium* spp., and found an average AFLP similarity of 24% (5.3% minimum, 72% maximum). *F. thapsinum* and *F. andiyazi*, both grain pathogens of sorghum, possessed the highest similarity to one another (40%), but *F. andiyazi* did not produce perithecia when crossed with *F. thapsinum* tester strains (*MATF1*, *MATF2*) or the testers for the other mating populations of *Fusarium* spp. once categorized as *F. monilforme sensu lato*. Leslie *et al.* (2005) used both isozyme and AFLP analyses to compare isolates of *F. andiyazi*, *F. nygamai*, *F. pseudonygamai*, *F. thapsinum*, and *F. verticillioides* from sorghum, millet, and maize. Both techniques were useful in reliably differentiating species. While few species were compared within genera, AFLP analysis showed differing levels of similarities between six isolates of the same species, e.g. 92% to 100% similarity between *F. andiyazi* isolates, 23% to 100% similarity between *F. nygamai* isolates (the authors suggest that one isolate likely represented a different species), and 74 to 100% similarity between *F. thapsinum* isolates.

Kvas *et al.* (2009) indicated that *Fusarium* spp. in the *G. fujikuroi* complex can be separated into African, American, and Asian clades using maximum likelihood phylogeny based upon translation elongation factor 1-alpha and beta-tubulin gene sequences. Of these, isolates from sorghum, including *F. nygamai* and *F. thapsinum* fall into the African clade. However, other grain associated species, including *F. subglutinans*, and *F. proliferatum* and *F. fujikuroi*, fall into the American and Asian clades, respectively. da Silva *et al.* (2006) examined 43 strains of *F. verticillioides* from corn and sorghum and compared them to *F. thapsinum*, *F. verticillioides*, and *F. subglutinans* reference strains using SPAR (single primer amplification reaction) markers. The resulting polymorphisms resulted in the *F. verticillioides* isolates being divided into two groups in a strongly host-dependent manner, with the *F. thapsinum* reference strains grouping with *F. verticillioides* strains from sorghum. These sorts of results suggest that crop host and geography has had a strong selection pressure for evolution and diversity in the *G. fujikuroi* complex.

DNA-based detection methods for mycotoxigenic grain molding fungi, such as *Aspergillus flavus* and *A. parasiticus*, including PCR-based detection methods (standard PCR, reverse transcriptase PCR, real time PCR), DNA fingerprinting (RAPDs, RFLPs, AFLPs), and DNA microarray-based methods are reviewed by Abdin *et al.* (2010). Thus, it is logical that these techniques could also be applied to better understand population biology and genetic diversity within diverse grain molding species. Other important aspergilli that constitute the grain weathering and storage mold aspects of the grain mold complex include those in the *Aspergillus* section *Nigri*, e.g. *A. niger* and *A. carbonarius*. Perrone *et al.* (2007) used AFLP, calmodulin, and beta-tubulin sequences to compare *Aspergillus* section *Niger* isolates from grape. Since these techniques were effective in differentiating aspergilli into uniseriate and biseriate clusters as well as individual species, an equivalent approach should help to characterize the black aspergilli commonly found in stored sorghum caryopses, especially since some strains are producers of ochratoxin A. Additionally, AFLP data showed considerable intraspecies variability within *A. niger*, *A. carbonarius*, and *A. tubingensis*. Varga *et al.* (2004) examined *Aspergillus* section *Nigri* isolates from numerous crop species using ITS sequence analysis, and found that *A. niger* and *A. aculeatus* from sorghum grain were clearly separated in the *Nigri* section along with closely related species such as *A. carbonarius* and *A. tubingensis*.

An additional fungus causing weathering and storage mold in sorghum, *Rhizopus*, has been poorly studied in sorghum in terms of genetic diversity. A study of the genus by Abe *et al.* (2006) showed that rDNA (18S, ITS, and 28S) sequences could be used to differentiate *Rhizopus* species and could group numerous species into three consistent clusters. Representatives of the most common caryopsis-associated species, *R. stolonifer* and *R. oryzae*, clustered separately. However, isolate diversity within these species was apparently low.

For further information concerning genetic diversity in sorghum-associated *Claviceps* spp., *Sporisorium reilianum*, and *Colletotrichum sublineolum*, please see this review (*below*) and this paper's accompanying review (Tesso *et al.* 2012), respectively.

Disease symptoms and losses

Grain mold of sorghum was ranked 35th in importance among multiple crops for all abiotic and biotic constraints within Asian, African, and Latin American production systems by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), and specifically among the top three constraints for sorghum (ICRISAT 1992; Bantilan *et al.* 2004). Annual losses to this disease have been estimated to be $130 million USD per year, however in highly susceptible cultivars losses may reach 100% (Ibrahim *et al.* 1985; ICRISAT 1992; Bandyopadhyay *et al.* 2002). These yield losses are due to a reduction in yield (caryopsis abortion, reduced seed filling, and lower grain density), reduced seed quality (cosmetic deterioration, endosperm and embryo degradation, and mycotoxin contamination), reduced nutritional value, and poor storability (Ibrahim *et al.* 1985; Thakur *et al.* 2006; Little and Magill 2009). These factors combine to result in grain with lower marketability and reduced utilization characteristics for end-use processors (Rooney and Serna-Saldivar 2000).

As alluded to earlier, the sorghum seed is a “caryopsis”, thus the endosperm is fused directly with the pericarp. Near the base of the seed are the embryo tissues (**Fig. 3**). The typical sorghum caryopsis is composed of 12-14% protein (as protein bodies, protein matrix, and enzymes) and 65-70% starch (as starch granules). The size of the sorghum caryopses is small (~35 mg) compared to that of corn (~375 mg). Translocation efficiency affects seed size. Abiotic (environmental) and biotic (disease, insects) stressors also affect seed size (Waniska *et al.* 2001).

Grain mold can be distinguished from grain weathering due to the time at which the associated fungi infect and colonize the developing grain. Grain mold infection occurs early in caryopsis development, between anthesis and black layer formation (physiological maturity), whereas colonization in the “grain weathering” sense occurs throughout grain development. Grain mold fungi colonize the internal tissues of the floret and developing grain, whereas grain weathering fungi (many species of which may also fall into the grain mold category) colonize external surfaces of the grain.

Fusarium and *Curvularia* conidia that infect at flowering, first colonize lemma tissues when glumes are open. Colonization proceeds to the lodicule, located at the base of the floret, and then to the pedicel and the structures associated with the ovule (including the placenta, nucellus, and ovarian lodicule) (Castor 1981). Once the base of the floret is colonized, the fungi may move basipetally towards the phloem parenchyma and transfer tissue acropetally towards the nucellar pad, transfer cells, ovary wall, and peripheral endosperm. In susceptible varieties, extensive colonization occurs in the germ and endosperm tissue (Castor 1981).

Only a few studies have addressed the issue of seed abortion in particular. In general, yield analyses of grain molded sorghum varieties show reduced yield in susceptible varieties, which is expected. Some of this yield loss is due to the failure of seed to form under high disease pressure at the time of flowering although the underlying genetics of

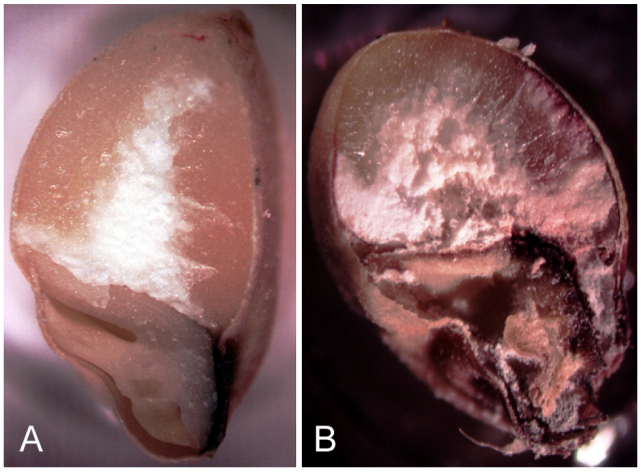

Fig. 3 Example cross-sections of healthy (A) and molded (B) sorghum caryopses. In the healthy caryopsis (**A**), the embryo (*hilar* portion of caryopsis) is intact and clearly distinquishable from the endosperm (*stylar* portion of caryopsis). Note the degradation of the embryo and endosperm as well as the presence of white, fungal mycelium in the lower portion of the molded caryopsis (**B**). In this example, the healthy caryopsis was inoculated with sterile-distilled water at anthesis, while the molded caryopsis was inoculated with a *Fusarium thapsinum* conidia suspension at anthesis, then grain was allowed to mature.

panicle architecture and abiotic stressors can also impact glume and flowering site development (Brown *et al.* 2006).

In China, glume blight is a widespread problem in the Shanxi province; here, *A. alternata* was shown to be the second most important pathogen causing withered glumes and small seed (Ye *et al.* 1995). Ye *et al.* (1995) have indicated that *Penicillium oxalicum* also causes "glume blight" and premature death/ripening of sorghum seed in Shanxi province China.

Little and Magill (2009) established a "caryopsis formation frequency" (CFF) value for sorghum panicles. In this case, it was clear that inoculation of flowering spikelets at anthesis with *F. thapsinum* resulted in significantly reduced CFF values for panicles in grain mold susceptible lines. This study showed that early infection by a grain mold pathogen can significantly reduce the number of grain when a panicle is exposed to high relative humidity and high inoculum pressure.

Menkir *et al.* (1996) found that kernel weight was the main factor that differed between susceptible and resistant sorghum accessions. It is assumed that kernel weight is not a predictor of grain mold resistance, but low weight is a result of early infections that progress throughout grain development and induce pre-mature black layer deposition, reduce the formation of, or degrade endosperm tissues. Thus, numerous authors have found strong associations between grain mold resistance levels and seed weight (Bandyopadhyay *et al.* 2000; Bandyopadhyay *et al.* 2002). For example, Prom *et al.* (2005) demonstrated a highly significant interaction between sorghum line and treatment with several fungal pathogens.

Earlier work with storage fungi, especially species in the *Aspergillus glaucus* group, demonstrated that significant damage occurs on fully developed embryos during storage (Lopez and Christensen 1963). Seedling blights and damping-off found to occur at low soil temperatures (15°C) were attributed to *A. niger* (Leukel and Martin 1943). Gaudet and Major (1986) indicated that *P. oxalicum* is associated with sorghum seed and upon planting can cause pre-emergence damping-off in climates with short-season growing conditions. Edmunds (1970) noted that *Penicillium* caused seed rot at 15°C and blighted seedlings at slightly higher temperatures (15-25°C). Seedling blights and damping-off were also found to occur at low soil temperatures (15°C) by *R. stolonifer* (Leukel and Martin 1943).

Alternaria appears to be a better seedling pathogen than a grain mold pathogen *per se*. Thus, the presence of *A. alternata* in seed is a concern in hybrid seed production, for example, when seed will be produced for planting. Girish *et al.* (2004) found that *A. alternata* did not infect the seed coat, endosperm or embryo as *C. lunata* did. However, *A. alternata* was associated with seed rot and reduced germination. Earlier studies by Arif and Ahmad (1969) and Narasirnhan and Rangaswami (1969) showed reduced germination when seeds were externally inoculated with *A. alternata*. Konde and Pokharkhar (1979) observed that *A. alternata* could cause post-emergent damping-off of sorghum seedlings.

According to Girish *et al.* (2004), several species of seedborne fungi associated with the grain mold complex can cause seed rot and reduce seed germination. These include *A. alternata*, *Bipolaris sorghicola*, *C. lunata*, *Exserohilum rostratum*, and *Phoma sorghina*. Among these, *C. lunata* was the most important species. Somani *et al.* (1994) showed that *C. lunata* could form several "toxic metabolites" and hydrolytic enzymes (amylase, cellulase) on media. These compounds were hypothesized to cause reduced germination, root and shoot length, and seedling vigor in sorghum cultivars CSH 1 and CSH 14. However, this could also be due to the fact that, unlike *A. alternata*, *C. lunata* can actively colonize many parts of the sorghum caryopsis, including the pericarp, endosperm, and embryo (Girish *et al.* 2004).

Various *Fusarium* spp. have been used to inoculate sorghum seedlings in pathogenicity tests. Results show that there are relative levels of pathogenicity exhibited by various *Fusarium* pathogens that are associated with sorghum caryopses. Leslie *et al.* (2005) demonstrated a relative gradient of pathogenicty among *Fusarium* strains upon sorghum seedlings. In their tests, "*F. thapsinum* > *F. andiyazi* > *F. verticillioides* > *F. nygamai* = *F. pseudonygamai*". However, Jardine and Leslie (1992) showed that there was no difference between *F. thapsinum* and *F. verticillioides* as far as their ability to cause stalk rot disease on adult plants in the greenhouse.

Mycotoxins are secondary metabolites produced by fungi. These compounds are produced when caryopses are produced, or grains and grain products are stored, under high humidity and warm temperatures that encourage asexual fungal growth. Mycotoxins are more problematic on maize than on sorghum because the fungi that produce the most serious mycotoxin problems do not occur as frequently on sorghum grain. However, it is important to note that the move away from traditional grain foods, such as sorghum and millets, and the move towards mycotoxin-prone maize in parts of Africa is problematic in this regard. Increasing levels of esophogeal cancer and other mycotoxicosis-related illnesses in subsistence agricultural communities is a warning sign (Leslie *et al.* 2005). Regulated mycotoxins include aflatoxins, fumonisins, deoxynivalenol, patulin, non-fumonisin trichothecenes, zearalenone, the ergot alkaloids, and ochratoxins. Legal limits vary from country to country, food and feed product type or usage, and whether or not more sensitive individuals, such as infants, children, and the elderly, will consume contaminated grains, products, or milk. Comaparative regulation can be obtained from the following web site: http://www.knowmycotoxins.com/regulations.htm

Although the fungi that attack maize are more rare on sorghum grain, this does not mean that many of the species cannot be present on sorghum and produce mycotoxins under similar conducive environmental conditions. For example, Martin *et al.* (1974) and Tripathi (1974) found aflatoxins (B_1, B_2, G_1, and G_2) in moldy sorghum grain and products. Silva *et al.* (2000) found a predominance of *Aspergillus* spp. (CFUs) in Brazilian sorghum grain. In these samples, *A. flavus* Link was the most frequently encountered species and aflatoxin was formed on 12.8% of the grain samples evaluated. Banchero *et al.* (1984) also showed that aflatoxin was formed on stored grain samples

in Argentina.

A. alternata causes black spot of grains and produces mycotoxins (Logrieco *et al.* 1990). In addition, *Alternaria* spp. produce numerous mycotoxins and phytotoxins, which may contribute as virulence factors in disease or provide a competitive benefit. These include altuenes (dibenzo-a-pyrones) (Sauer *et al.* 1978) and *A. alternata* produces alternariol and alternariol monomethyl ether on stored sorghum grain samples, which are both toxic secondary metabolites (González *et al.* 1997). Other storage fungi, including *Penicillium* spp. such as *P. ochreaceus* Westling are important producers of ochratoxin (Bandyopadhyay *et al.* 2000). Patulin is produced by several species of fungi. However, *Penicillium* is the most important patulin producer on sorghum (Husseini *et al.* 2009).

Host resistance

In India, many modern cultivars are short or medium duration and mature during the rainy season in semi-arid and tropical production systems. These are particularly prone to infection by grain mold fungi (Sharma *et al.* 2010b). Thus, adjusting planting dates or using longer duration, photoperiod sensitive cultivars that ensure that flowering and grain maturity occurs during drier periods is can be a viable avoidance strategy for grain mold (Singh and Bandyopadhyay 2000; Navi *et al.* 2005; Thakur *et al.* 2006).

Large cohorts of sorghum accessions have been screened for traits such as flowering time (early, medium, late) and panicle shape (compact, semi-compact, loose, very loose) (Menkir *et al.* 1996; Sharma *et al.* 2010b). There is extensive variability in the collections for many of the physical traits thought to have some influence on grain mold. Unfortunately, flowering time, e.g., "days to 50% flowering", and panicle shape have shown low or non-significant correlations with grain mold severity scores in various sorghum types (Williams and Rao 1981; Ibrahim *et al.* 1985; Mukuru 1992; Menkir *et al.* 1996). These results are non-intuitive, since one would expect that a plant that can escape the most conducive periods of moisture or produce a panicle that does not favor a conducive microclimate would be able to avoid grain mold infection (Hall *et al.* 2000), and cases can be cited. For example, Glueck *et al.* (1977) and Mansuetus *et al.* (1988) observed that "lax panicles" contributed to grain mold avoidance. Also, in a recent study, Sharma *et al.* (2010a) found a significant, positive correlation between grain mold severity and panicle compactness ($r = 0.47$).

Although avoidance can be a strategy for grain mold control, host resistance is the best means to reduce fungal damage. However, as described below, grain mold resistance is a complex trait characterized by many preformed plant structural characteristics and constitutive and inducible physiological mechanisms that work in concert, but none of which appear to be a "silver bullet" for eliminating fungal infection and subsequent grain damage. For example, open panicles, increased glume coverage (including glume length and surface area), and endosperm hardness (e.g., the proportion of corneous endosperm) are pre-formed structural features which can be associated with grain mold resistance (Mansuetus 1990; Audilaksmi *et al.* 1999; Chandrashekar *et al.* 2000) but may have negative effects on yield or food quality.

Glueck *et al.* (1977) and Mansuetus (1990) indicated that long glumes (*sg*) and glumes that covered a larger portion of the caryopsis surface (broad, truncate glumes) are correlated to grain mold resistance. This phenomenon has been documented previously (Audilakshmi *et al.* 1999; Thakur *et al.* 2006). Glueck *et al.* (1977) and Mansuetus (1990) indicated that increased glume coverage resulted in decreased grain mold severity. More recently, Sharma *et al.* (2010a) found that grain mold was negatively correlated ($r = -0.32$) with glume coverage. This relationship was complicated by the presence of glume pigments, which enhanced resistance in red- (PPq^rq^r) and purple-pigmented ($PPqq$, $PPQQ$) sorghum genotypes. However, both Menkir *et al.* (1996) and Audilakshmi *et al.* (1999) tested numerous sorghum accessions and did not find a significant relationship between grain mold resistance and glume coverage. Earlier reviews by Williams and Rao (1981) and Williams and McDonald (1983) echo this conclusion.

The presence of grain surface wax (deposited on the outermost epicarp), and the thickness thereof, is a preformed trait that functions to provide a barrier for saprophytic and facultatively pathogenic grain weathering fungi (Waniska 2000). In maize, pericarp wax contributes to *A. flavus* and *F. verticillioides* resistance. Further, removal of the pericarp resulted in increased fumonisin accumulation, suggesting that contents of the waxy layer and pericarp contribute resistance to mycotoxin accumulation (Sampietro *et al.* 2010).

The sorghum caryopsis is composed of two types of endosperm: soft (chalky) and hard (corneous). The ratio and distribution of soft to hard endosperm within the sorghum caryopsis is a highly variable trait that differs among sorghum accessions (Waniska *et al*. 2001). Typically, larger proportions of corneous endosperm are related to grain mold resistance. Hard endosperm is derived from the presence of α-, β-, and γ-kafirins in the corneous endosperm (Waniska 2000; Waniska *et al.* 2001). However, numerous antifungal proteins are associated with hard endosperm in sorghum, thus this is a potentially confounding factor that underlies this preformed source of structural resistance (see below) (Waniska *et al.* 2001).

Menkir *et al.* (1996) found that greater levels of corneous endosperm enhanced grain mold resistance in sorghum accession, however this was generally associated with the presence of a pigmented testa. Also, the amount of corneous endosperm did not relate to grain mold resistance in brown, or high-tannin, sorghums. However, Jambunathan *et al.* (1992), Ghorade and Shekar (1997), and Audilakshmi *et al.* (1999) showed direct, positive relationships between endosperm hardness and grain mold resistance. But, a trade-off is required for endosperm hardness, since grain that has too much corneous endosperm may not possess processing characteristics that are required for food grade applications when softer textures are often preferred.

The ability of grains to absorb water is also a key factor for the production of clean and healthy caryopses. Rana *et al.* (1977) tested tan plants and found low water absorption capacity due to increased grain hardness. Softer grains occasionally exhibit pre-harvest sprouting while still attached to the panicle, however this is a trait that is also genetically controlled (Hall *et al.* 2000).

Grain hardness in sorghum is associated with several grain quality factors including milling yield, particle size index, test weight, kernel and percent vitreousness (Riechert *et al.* 1988; van Loggerenberg 2001). In addition to a "high proportion of corneous to floury endosperm", and pericarp surface wax, kernel density has been associated with grain mold resistance (Glueck and Rooney 1980; Menkir *et al.* 1996).

Glume color and grain mold resistance have been strongly associated in studies by Audilakshmi *et al.* (1999) and Sharma *et al.* (2010a). In these cases, purple or black glumes (that also covered a greater amount of caryopsis surface area and are a function of plant color genotype; *see above*) provided the greatest resistance to grain mold. Combining soft, white endosperm food-grade types with pigmented glumes is viewed by some authors to be a strategy for controlling grain mold in sorghum types that would normally be very susceptible to fungal colonization and damage (Audilakshmi *et al.* 1999; Hall *et al.* 2000).

Colored pericarps tend to resist grain mold much better than caryopses with white or lighter pericarp colors (Menkir *et al.* 1996; Sharma *et al.* 2010a). This difference is due, in part, to higher amounts of free phenolic compounds that occur in the pigmented pericarps (Doherty *et al.* 1987). However, like so many other traits, grain mold resistance does not only depend upon pericarp color. Menkir *et al.*

(1996) identified grain mold resistance in accessions that had white, straw, yellow, orange-red, light brown, brown, red-brown, gray, and purple pericarp colors. One key factor varied, however; the concentrations of key sorghum phytoalexins, apigeninidin and luteolinidin, which contributed more to grain mold resistance in lines with darker pericarp colors than light.

Ratnavathi and Sashidhar (2000) found significant changes in grain composition upon degradation by toxigenic isolates of *A. parasiticus*. Sorghum grain with red pericarps proved to be a poorer substrate for colonization and aflatoxin production than did white pericarp genotypes where maximum colonization and aflatoxin accumulation were observed. Red pericarp varieties accumulate flavan-4-ols, which can contribute to grain mold resistance (Waniska *et al.* 2001). Esele *et al.* (1993) found that the combination of red pericarp and pigmented testa provide additive resistance to grain mold. However, the presence of red pericarp (or an associated pigmented testa) is not favored by grain processors for all end-uses (Hall *et al.* 2000). Red pericarp color (as enhanced by an intensifier gene, *I*), pigmentation of the testa layer, tannin content, flavan-4-ol concentration, and the presence of antifungal proteins are all traits that have been associated to some degree with grain mold (Mukuru 1992; Esele *et al.* 1995; Seetharaman *et al.* 1997; Audilakshmi *et al.* 1999; Rodriguez-Herrera *et al.* 1999; Chandrashekar *et al.* 2000; Bueso *et al.* 2000; Waniska *et al.* 2001), with differences perhaps reflecting the large number of different fungal species that can be involved.

Monomers of the esterified phenolic acid, gallic acid, may be linked together to form "gallotannins", which make up the *hydrolyzable tannins* in plants (Dai and Mumper 2010). Gallotannins have often been asssociated with fungistatic activity (Vega *et al.* 1997). Forbes (1986) examined gallic acid levels in grain of inoculated and control lots of sorghum and concluded that there was little accumulation in a grain mold resistant cultivar ('SC0103-12'). On the other hand, *condensed tannins* are derived from *p*-coumaric acid and flavonoid biochemistry (Vogt 2010). Although high tannin levels confer grain mold resistance (and reduce bird feeding damage), higher condensed tannin levels in sorghum grain are associated with poor processing quality and reduced protein digestibility (Waniska *et al.* 2001). White grained, non-pigmented sorghums, which are optimum for food-grade uses, typically have trace levels of tannins (or other chemicals such as flavan-4-ols and phytoalexins), but are more palatable to livestock and more amenable to processing (Menkir *et al.* 1996).

The PAL (phenylpropanoid) pathway occurs in every plant species that has been investigated (Vogt 2010), leading to the synthesis of numerous secondary metabolites including, coumarins, isoflavonoids, stilbenes, aurones, catechins, and proanthocyanidins (tannins), phenylpropanoid esters, and lignins (Vogt 2010). In sorghum, the flavonoid derived phytoalexins, apigeninidin and luteolinidin (Lo and Nicholson 1998; Liu *et al.* 2010) are PAL pathway derivatives. Besides being made and stored as a deterrent to molds, infection can also serve to induce expression of genes in the PAL pathway (Little and Magill 2003).

Free phenolic acids are inducible in panicles inoculated at anthesis in 'Tx2536' (grain mold susceptible) and 'SC630-11E' (grain mold resistant) with *F. moniliforme sensu lato*. The percent change over time in free phenolic acids in the spikelets of 'Tx2536' was significantly less than that of 'SC0630-11E'. In addition, levels of free phenolic acids obtained from mature caryopses across resistance levels remained about the same, including cinnamic acid, the first phenolic acid in the phenylpropanoid pathway. However, there were subtle differences between non-inoculated highly resistant, resistant, moderately susceptible, and susceptible lines at maturity (Mansuetus 1990).

Bound phenolic compounds include those that are glycosylated, alkylated or esterified in some manner and may be associated with the plant cell wall fraction (Dykes and Rooney 2007). Several phenolic acids are produced in sorghum and have been measured in relation to grain mold. These include gallic, gentisic, *p*-hydroxybenzoic, caffeic, syringic, cinnamic, ferulic, synapic, salicylic, and coumaric acids (Forbes 1986; Mansuetus 1990). In artificially inoculated sorghum panicles, bound phenolics show different patterns of accumulation than free phenolic acids (Forbes 1986; Mansuetus 1990). Mansuetus (1990) looked at levels of a number of organic acids produced in response to *F. moniliforme sensu lato* infection of spikelet tissues. He observed that free syringic acid generally increased, but only slightly, in inoculated treatments. However, bound syringic acid increased at a much greater rate than free syringic acid. Levels of free and bound caffeic acid in *F. moniliforme sensu lato* both increased, while levels of free coumaric acid showed an overall decrease across inoculated treatments. Levels of bound coumaric acids seemed to be greatly increased in comparison to control under inoculated conditions. Mansuetus (1990) also measured ferulic acid levels and observed a decrease after infection of sorghum spikelets by *F. moniliforme sensu lato* in some grain mold resistant lines but increases in moderately resistant and susceptible lines. Bound ferulic acid levels either increased or decreased; however, there was no strict segregation of this effect between resistant and susceptible cultivars.

Pigmentation of the testa layer has long been associated with grain mold resistance and recognized as playing an additive or synergistic role in regards to other traits, such as red pericarp, flavan-4-ols, and the presence of condensed tannins (Glueck *et al.* 1977; Esele *et al.* 1993; Menkir *et al.* 1996; Audilakshmi *et al.* 1991; Thakur *et al.* 2006; Sharma *et al.* 2010a). However, a pigmented testa is generally undesirable for food quality sorghum as it is often associated with high amounts of bitter tannins (Hall *et al.* 2000). Menkir *et al.* (1996) indicated that sorghum accessions that did not have a pigmented testa generally contained lower levels of tannin. In brown sorghums, the presence of a pigmented testa (and associated high tannin levels) is one of the most important features conferring grain mold resistance (Glueck and Rooney 1980; Weitz *et al.* 1983; Bandyopadhyay *et al.* 1988; Menkir *et al.* 1996).

Overall, there is relatively little information suggesting that induced expression of genes in the PAL pathway has a significant role in grain mold resistance, at least for the few cultivars that have been examined. As early as two days after inoculation, Mansuetus (1990) observed that red-pigmented compounds (consistent with apigeninidin and luteolinidin) accumulated in 'Tx2536' glume epidermal cells. This accumulation appeared to occur prior to hyphal penetration or cellular degradation by *Fusarium moniliforme sensu lato*. In more recent work, while Northern blots showed an increase in PAL mRNA levels in floral tissues following inoculation with *Fusarium* or *Curvularia* spores, the response occurred both in susceptible and resistant cultivars, with no clear distinction over time (Little and Magill 2003). Similar results were obtained using real time reverse transcriptase PCR to quantify mRNA levels for PAL in glumes or gynecia (Katilé 2008).

A number of other defense response genes have also been identified in sorghum and can be induced by grain mold fungi, but links to a role in grain mold resistance favor developmental differences in cultivars over induced responses. One possible exception is significantly higher levels of PR10 mRNA induced in glumes of resistant cultivars ('Sureno' and 'Tx2911') 48 h after inoculation with *Fusarium thapsinum* or *Curvularia lunata* when compared to two susceptible cultivars (Katilé *et al.* 2010). Previous studies have shown that β-1,3-glucanases, chitinases, RIPs, and permatins are elevated during grain development from flowering to physiological maturity (Darnetty 1993; Krishnaveni *et al.* 1999a; Krishnaveni *et al.* 1999b; Kumari *et al.* 1992; Kumari *et al.* 1994; Rodriguez-Herrera *et al.* 1999). Proteins inhibitory to fungal growth *in vitro* that have been identified in sorghum include chitinases, glucanases, and sormatin/zeamatin (Roberts and Selitrennikoff 1986; Roberts and Selitrennikoff 1990). Kumari *et al.* (1992)

tested extracts from hard and soft endosperm sorghum seed and observed that these were inhibitory to *F. moniliforme sensu lato*. Kumari *et al*. (1994) found proteins of similar size in pearl millet, maize, and sorghum that were inhibitory to fungi. Seetharaman *et al.* (1997) found that antifungal proteins from sorghum could inhibit hyphal elongation and lyse hyphal tips. Inhibition of fungal growth in culture may indicate a direct inhibitory role by these proteins in early defense interactions.

Rodriguez-Hererra *et al.* (1999) observed that glucanase content in developing seed accrued in the field. 'Sureno', a line resistant to grain mold, accumulated larger amounts of glucanases than 'Tx430' (susceptible) in several environments.

Chitinases cleave β-1,4-N-acetylglucosamine linkages and are involved in the degradation of fungal cell wall chitin (Yun *et al.* 1997). No substrate for chitinase activity has been identified in plants (Roberts and Selitrennikoff 1986). The work of Punja and Zhang (1993) clearly illustrate that resistant plants may accumulate chitinases more quickly and to higher levels than susceptible plants. Rodriguez-Herrera *et al.* (1999) found that chitinase levels in resistant ('Sureno') sorghum caryopses were consistently higher than those of susceptible ('Tx430') caryopses throughout the maturation process in multiple environments. F1 hybrids of 'Sureno' *x* 'Tx430' showed significantly higher levels of chitinase activity than either of the parents under high grain mold pressure. This study also showed that chitinase levels were 1.5-15 times higher in grain mold resistant sorghums than grain mold susceptible. Rodriguez-Herrera *et al.* (1999) also showed that in genotypes where pericarp color or testa pigmentation do not contribute, resistance was correlated to high levels of chitinase accumulation in the sorghum kernel during seed development under grain weathering conditions. This was especially true in the white-grained, resistant genotype, 'Sureno'.

Chitinases appear to have the most promising potential as a primary source of resistance to grain mold fungi. Several experiments have shown that purified chitinases inhibit fungal growth *in vitro*. Three chitinases, CH1, CH2, CH3 (Krishnaveni *et al.* 1999) were shown to inhibit *F. moniliforme sensu lato* at 500 ng/µl. Seetharaman *et al.* (1997) showed hyphal tip rupture and spore germination inhibition of *F. moniliforme sensu lato* and *C. lunata* by mixtures of chitinases, RIPs (ribosome-inactivating proteins), permatin, and glucanase at 360 ppm.

The thaumatin-like proteins (TLPs) are categorized with the PR-5 group of pathogenesis-related proteins and have been shown to have antifungal activity (Roberts and Selitrennikoff 1990; Vigers *et al.* 1992; Ye *et al.* 1999; Jayaraj *et al.* 2004). These sweet-tasting proteins are homologous to thaumatin, which gets its name from a West African shrub, *Thaumatococcus danielli* (Velazhahan *et al.* 1999). These proteins permeabilize cell membranes and may be able to interact with ligands due to the presence of receptor motifs (Koiwa *et al.* 1994). Jayaraj *et al.* (2004) isolated TLP-D34 from rice and used a protein obtained from an *E. coli* expression system to inhibit hyphal growth of the grain mold fungi, *F. graminearum* and *C. lunata*. Radhajeyalakshmi *et al.* (2003) were able to express the 23 kDa TLP in sorghum seeds. Sormatin is a 22 kDa TLP found in sorghum seeds (Vigers *et al.* 1991). Sormatin functions to permeabilize fungal membranes. In a study by Bueso *et al.* (2000) resistant lines had higher levels of sormatin one or two weeks after being stressed with grain mold fungal species than susceptible lines.

Molecular markers for grain mold resistance

Klein *et al.* (2001) analyzed 125 F_5 RILs from a 'Sureno' (grain mold resistant) × 'RTx430' (grain mold susceptible) cross in multiple Texas locations. As a result, these authors mapped QTLs associated with grain mold resistance (from the 'Sureno' "allele") on linkage groups D, E, F, G, and I using 130 mapped markers (44 microsatellites markers, 85 AFLPs, and one morphological trait locus). Interestingly, QTLs associated with anthracnose, zonate leaf spot, and bacterial leaf stripe also flanked the QTL for grain mold resistance on linkage group I. Franks (2003) followed this study by establishing five new breeding populations by crossing 'RTx430', 'RTx436', 'BTx631', 'BTx635', and 'Tx2903' with 'Sureno'. As a result, this author found results that matched the Klein *et al.* (2001) study in the 'Sureno' × 'RTx430' RIL population, but marker assisted selection was not applicable to the other RIL populations. Sharma *et al.* (2010a) found 44 polymorphic SSR markers (7.55 alleles per locus, 0.54 polymorphic information content) associated with grain mold resistance across 92 genetically diverse sorghum lines (74 grain mold resistant and 18 grain mold susceptible). Grain mold susceptibility and resistance accounted for 12% of variability between the lines, but panicle type, glume coverage, and grain color influenced clustering.

Future directions

The identification and characterization of resistance genes for sorghum grain mold has been an elusive target. Thus, a strategy to look for the presence of common resistance gene motifs is the correct course of action. However, because of the diverse number of fungi that cause grain mold, future efforts in finding resistance genes should be focused towards gene discovery for particular pathogens. Thus, finding specific resistance genes for *F. thapsinum* and *C. lunata* should be a high priority, if they exist. It is likely that resistance to other species of *Fusarium*, for example, could be conditioned by other members of a particular resistance gene family for those in the *G. fujikuroi* complex. Unfortunately, a completely different set of resistance genes or mechanisms may be required to condition tolerance or resistance to the *Fusarium incarnatum/equiseti* species complex (FIESC), and other *Fusarium* spp. not in the *G. fujikuroi* species complex that behave as facultative saprophytes or weak grain mold pathogens. All of this demands continued effort to better understand grain mold pathogens and their diversity. With the ability to identify and track alleles of genes that confer resistance to a specific pathogen with easily screened PCR-based markers, the task of combining multiple genes into cultivars with broad grain mold resistance will be greatly simplified. However, due to the many host genes and fungal species involved, it seems likely that selection of locally adapted varieties will remain the most effective breeding tool for some time.

Caryopses that are colonized by grain mold fungi generally show reduced nutritional properties when compared to healthy grain (Waniska *et al.* 2001). In addition, some fungi produce mycotoxins that are chronically or acutely toxic or capable of reducing feeding value and palatability, thus directly impacting the nutritional quality of grain (Leslie *et al.* 2005). Mold infestation has been linked to changes in grain composition and nutritional properties such as crude protein and starch content (Waniska *et al.* 2001). These properties can be altered when grain is infected with mold. Furthermore, the same factors, i.e., the physical and chemical composition of sorghum grain, that are the principal source of resistance to mold and pests are also linked to the end-use quality of sorghum. This creates a complicated series of interrelationships between resistance factors, grain structure, and end-use quality. Future efforts need to be directed towards developing grain mold resistance in white, food-use sorghums that have high nutritional quality, long-term storability, are mycotoxin free, and are of optimum texture for end-use processing.

HEAD SMUT

Introduction

Head smut is caused by the soilborne facultative biotroph, *Sporisorium reilianum* (Kühn) Langdon & Fullerton (syns. *Sphacelotheca reiliana* (Kühn) G. P. Clinton and *Sorosporium reilianum* (Kühn) McAlpine). Head smut is an important sorghum disease because of its presence in all parts of the world where sorghum is grown, its potential to cause yield losses and due to its natural variability. Africa has been suggested as the origin of the pathogen, although different races infect sorghum, corn and sudangrass over a wide geographical area, including Europe, North and South America, Mexico, Africa, Asia, Australia, New Zealand, and the West Indies. Although *S. relianum* also causes head smut of maize, since isolates have been shown to be host specific (Ghareeb 2011; Martinez 2002) and have been demonstrated to differ in chromosomal composition (Naidoo, 1992), recent papers have accepted the concept that the maize pathogen is a "*forma specialis*" if not a separate species.

Pathogen biology (*Sporisorium relianum*)

While rare solopathogenic diploids have been described (Sabbagh *et al.* 2010), *S. relianum* normally requires a combination of haploid nuclei of opposite mating types to cause infection and grow through the plant as a dikaryon (Morrow 2009). Typically, there are two genes involved in defining mating type; an "*a*" locus with two alleles and a "*b*" locus, which often has multiple alleles. However, in *S. relianum*, at least for the maize form, molecular sequence data have identified three alleles at the *a* locus and five at the *b* locus (Schirawski 2005). At the time of flowering, a diploid phase leads to the production of teliospores in sori, essentially replacing seed production in the host (Prom *et al.* 2011) (**Figs. 4-6**). Teliospores overwinter in the soil and crop debris to provide primary inoculum for the next cycle of infection. When sorghum seed is planted in the spring, the smut spores (9 to 14 μm in diameter) already in the soil germinate along with the seed to form a 4-celled or branched promycelium that bears sporidia terminally and near the septa. As a product of meiosis, sporidia may sprout to form yeastlike secondary sporidia or may germinate to form a germ tube or fuse with the opposite mating type to create dikaryotic hyphae that penetrate meristematic tissue in the sorghum seedling. Germination is highest in moist soil where the temperature is 81 to 88°F (27 to 31°C). Often, no sign of infection is evident until heading time. The fungus develops only in actively growing meristematic tissue. The smut spores also may cling to the surface of sorghum seed, thus introducing the smut fungus into the soil of fields not previously infested. Apparently, seedborne spores are not important in causing infection. Once present in a cropping area, the pathogen is very difficult to remove from the soil, even by adopting crop rotation to prevent annual soil reinoculation.

Disease symptoms and losses

As indicated, *S. reilianum* infects at the seedling stage but obvious symptoms are not expressed until flowering. Disease results in the inability of infected plants to produce grain. *S. reilianum* is one of the few smuts that cannot be controlled by seed treatment, leaving host resistance as the only practical control mechanism (Frederiksen 2000). Infection first appears when the young head, enclosed in the boot, is typically replaced by a large smut gall covered by a thick white-grey membrane (**Fig. 5**). The membrane soon ruptures, often before the head emerges, exposing a mass of dark brown to black, powdery teliospores intermingled with a network of long, thin, and dark broomlike filaments of vascular tissue. The head may be totally smutted with characteristic "witches' brooms," i.e., many small, rolled leaves protruding from the heads of suckers at the nodes or joints of some sweet sorghums and sudangrass cultivars (**Fig. 6**). Some cultivars are dwarfed; others are stunted due to a lack of peduncle elongation (**Fig. 4**). Wind and rain quickly scatter the smut spores to the soil and plant debris, where they live through the winter. Parts of an infected panicle not included in the smut gall or sorus usually show a blasting (sterility) or proliferation of individual florets. Smut galls may occasionally develop on the leaves and stems in some sweet sorghums and sudangrass cultivars. Marley and Aba's (1999) survey of farmers' fields in the major sorghum growing areas within the four climatic zones of the Nigerian savanna established changing patterns in the incidence, severity and distribution of sorghum smuts. Covered smut (*S. sorghi*), although widely distributed, was found to be highly predominant in the Sudan (24.8%) and the northern Guinea (29.5%) savannas. Loose smut (*S. cruentum*) and long smut (*Tolyposporium ehrenbergii*) were most prevalent in the Sahel savanna (21.8 and 15.5%, respectively). Head smut (*S. relianum*) was absent in the Sahel and Sudan, low in the northern Guinea savanna zones, but was most predominant in the southern Guinea savanna. These smuts are economically important and continue to be a major biotic constraint in the effort to sustain high sorghum production levels. Disease severity of head smut (*S. reilianum*) was observed in 73 to 75%, covered kernel smut (*S. sorghi*) 42 to 43% and loose smut (*S. cruentum*) 14 to 24% of fields in Kenya (Ngugi *et al.* 2002). A study was conducted by Jalali and Behdad (2003) to identify the species of *Sporisorium* attacking sorghum in Ardestan (Iran). On the basis of morphology and infection type, *Sporisorium sorghi*, the causal agent of covered kernel smut, with frequency of 31.5%, *S. ehrenbergii* [*Tolyposporium ehrenbergii*], the causal agent of long smut, with frequency of 28% and *S. reilianum*, the causal agent of head smut, with frequency of 7.5% were identified from two regions for the first time. Silaev (2005) analyzed the data on prevalence of sorghum smut diseases in the Volga region (Russia) collected through field experiments during 1975-2004. The most harmful smut pathogens included *Sporisorium sorghi* (covered kernel smut), *Sorosporium reilianum* (head smut) and *Sporisorium cruentum* (loose kernel smut). In the United States, head smut incidence has recently increased in sorghum fields in the coastal bend area of Texas (*personal communication*; Gary Odvody, Department of Plant Pathology and Microbiology, Texas A&M University, Corpus Christi, TX). In northern Tamaulipas, Mexico, head smut may affect susceptible genotypes up to 80% (Williams-Alanis *et al.* 2009).

Pathogen races

In the United States, four pathogenic races (1 to 4) were defined in 1980 among sorghum isolates of *S. reilianum* on the basis of reactions to a series of host differentials (Frederiksen and Reyes 1980). Races 1 and 3 were also found on cultivars/hybrids grown in Queensland, Australia by Dodman *et al.* (1985). In Corpus Christi, Texas, Palma *et al.* (1993) studied a screening nursery (field infection) including most entries of the Uniform Head Smut Nursery (UHSN) and controls. Selected parental lines and their hybrids were evaluated with 4 A1 inbred lines and 10 restorers. Data indicated that races 1 and 2 of *S. reilianum* was disappearing or losing pathogenicity on susceptible differentials ('RTx7078' and 'Early Hegari'). Data were similar to those collected during 1987-1992 in the UHSN at Corpus Christi. The same nursery was evaluated at College Station in 1992 (artificial inoculation) using 4 isolates representing race 4 (Victoria), races 1 and 3 (Taylor), races 1 and 4 (College Station) and races 1, 3, and 4 (Corpus Christi). Analysis of variance for percentage of resistant progenies indicated highly significant differences among F1 hybrids, parents and races, as well as interactions for F1 hybrids × races and parental lines × races. The Corpus Christi isolate was more virulent in F1 hybrids (41% susceptible) and parental lines (38% susceptible) than the Taylor isolate, but

Fig. 4 Characteristic symptoms of sorghum head smut caused by *Sporisorium reilianum*. (**A**) Stunted growth due to a lack of elongation of the peduncle and rupture of the thick, white peridium membrane. (**B**) Sorus covered by remnants of the peridium.
Fig. 5 Tissue deformation and panicle sterility associated with *S. reilianum* infection of the apical meristem. (**A**) Phylloidal miniature leaves with smut gall. (**B**) Bleached sterile panicle with smut gall. (**C**) Smutted panicles with characteristic phylloidy.
Fig. 6 Smutted plants in senescent plants showing (**A**) deteriorated sori and (**B**) remaining vascular strands. (**C, D**) Large-scale field infestation of head smut with primary and secondary tiller (late) panicles completely replaced with sori.

with certain hybrids ('ATx635 × BTx399' and 'ATx399 × BTx635') and parents ('ATx635' and 'ATx399'), the Taylor isolate was more virulent than those in Corpus Christi.

Recent increases of reports of *S. reilianum* in the grain sorghum producing areas of the United States may be due to the introduction of susceptible sorghum hybrids, the appearance of more virulent races, or both. In a recent study by Prom *et al.* (2011), inoculations of sorghum host differentials 'BTx643', 'BTx7078', 'BTx635', 'SC170-6-17' (TAM2571), 'SA281' (Early Hegari), and 'Tx414' showed 23 of 32 Texas isolates were race 4. Two isolates from College Station, Texas were classified as race 1, but no race 2 or 3 isolates were found. New virulent races 5 and 6 were identified among isolates from south Texas.

The relationship of cytoplasm type and field resistance to head smut (*S. reilianum*) was studied by Pecina-Quintero *et al.* (2004) in grain sorghum. Disease incidence at physiological maturity was registered on sixteen hybrids in two different cytoplasm backgrounds (A1 and A2) for a total of 32 hybrids. Sorghum hybrids were grown in El Tapon, Tamaulipas, Mexico, under rainfed conditions from 1990 to 1994 in fields with natural infestation by *S. relianum* and physiological races of the fungus were determined by differential sorghum lines. Significant differences in disease incidence were found between cytoplasm types in 1991 and 1994 with disease incidence lower in hybrids with A1 cytoplasm. In 1991, only physiological race 3 was present, being the most prevalent and virulent. Results suggest that sorghum hybrids with A2 cytoplasm may be more susceptible to head smut race 3.

In order to effectively utilize host resistance to control *S. reilianum* in sorghum, it is necessary to monitor changes in disease dynamics and the virulence of the pathogen. An outbreak of head smut was recently observed in a sorghum field near Gaoping, Shanxi Province, China, and research was undertaken by Zhang *et al.* (2011) to characterize a putative new race of *S. reilianum*. A set of differential sorghum lines with resistance to several conventional races was used to characterize the newly collected isolate of *S. reilianum*. The reactions of differential cultivars/germplasm lines to the new isolate indicate that it is a new physiological race of *S. reilianum*. The new race is highly virulent on sorghum line 'A(2)V4' and its hybrid, 'Jinza 12', that are known to be resistant to all existing Chinese races of *S. reilianum*, including races 1, 2, and 3. The new isolate of *S. reilianum* is different from the described races of the pathogen; thus, it is designated as race 4 of *S. reilianum*. A collection of 34 sorghum genotypes including commercial cultivars and germplasm lines was evaluated for disease reaction to the newly described race and the three known races of the pathogen.

Disease screening techniques

The use of genetic resistance to prevent head smut in sorghum has been hampered by the lack of efficient and effective inoculation methods for identification of resistant genotypes. Field screening under natural infection at hot spots with high infection frequencies requires repeated trials for reliable results because of fluctuating soil temperatures unfavorable for disease development. Soil temperatures below 21°C and above 31°C seriously reduce infection efficiency (Ramasamy *et al.* 2007). Also, erratic distribution of inoculum in the soil leads to high rates of disease escape, even in fully susceptible cultivars. An inoculation method developed by Edmunds (1963) using a hypodermic syringe

also may result in inconsistent disease expression. Craig and Frederiksen (1992) developed a soil-based inoculation protocol for inducing symptoms of infection by *S. reilianum* in sorghum seedlings and compared the relationship of these symptoms to known head smut responses using a small sample of the sorghum germplasm collection. It is not clear if all susceptible genotypes would be detected by this technique.

Osorio and Frederiksen (1998) studied the practical value of dry teliospores of *S. reilianum* as inoculum in head smut infection using three different isolates of the pathogen. Overall germinability of teliospores ranged from 8.0 to 28.0% on culture media after 48 h of incubation. Teliospores germinated at slower rates in the vicinity of sorghum seedlings growing on germination paper, reaching maximum values of 11.5 to 13.0% after 120 h. These results suggested adaptation in the germination strategy of *S. reilianum*, probably modulated by the variable soil environment. It is postulated that the increased infection efficiency observed with this inoculation technique results from uniform, timely delivery of higher levels of inoculum under stable soil conditions that also provide the pathogen with an environment more conducive for infection.

Prom *et al.* (2011) used four inoculation techniques: soil and teliospore mixture, seed coating, media placement and syringe injection. Of the four, the syringe injection developed by Edmunds (1963) with modification by Ramasamy *et al.* (2007) was determined to be the most effective. In this technique, the sporidial colonies were grown from *S. reilianum* isolate for four days in potato dextrose broth (PDB) on a rotary shaker set at 150 rpm. The resulting sporidial inoculum (milky color) is filtered through cheesecloth and adjusted to a concentration of 1×10^5 spores ml^{-1}. The resulting sporidial suspensions is used to inoculate by injection below the apical meristem of 18- to 20-day-old seedlings (approx. 10 cm in height) between 4:00 and 7:00 pm to avoid heat-related drying. Fresh inoculum is prepared separately every time, and each plant is injected with 0.5 to 1.0 ml of sporidial suspension using a Precision Glide Needle® #22 G × 1 in. (Becton, Dickinson and Company, Franklin Lakes, NJ) attached to a 30 ml hypodermic syringe. Resistant plants have regular flowering with normal grain development and no sori, whereas the panicle is replaced by sori in susceptible plants. Sori have a variety of appearances that can be seen in the main head and/or tillers (**Figs. 4-6**). To avoid disease escapes and obtain accurate disease scores, plants with a healthy inflorescence at the time of initial flowering are cut and the side tillers that developed are also examined for signs of systemic infection at flowering. Qualitative scoring system (susceptible or resistant) is used to assign the phenotypes without intermediate reaction types.

Host resistance

Host resistance to *S. reilianum* is the only practical means for control of sorghum head smut. Identification of resistance sources is a very slow process due to the difficulty in observing disease expression in late-maturing day length-sensitive sorghums (Craig and Frederiksen 1992) and to a number of plant and environmental factors that can interfere with natural infection events (Edmunds 1963). Sorghum genotypes susceptible to head smut can escape disease due to the uneven distribution of infective propagules of *S. reilianum* in the soil or to environmental factors that prevent spore germination or seedling infection. Inoculation with sporidial suspensions has been considered more reliable than natural infection (Miller 1978), but it bypasses at least one resistance mechanism and is sensitive to poor technical skills and genetic heterogeneity of the plant material. Soil temperatures in the range of 25 to 30°C appear to be the most favorable for infection both in maize and sorghum (Christensen 1926; Foster 1979; Matyac and Kommedahl 1985). Several reports indicate that soil moisture content near 40% of water holding capacity is most conducive to head smut development in sorghum (Miller 1978; Foster 1979). These and other soil factors may play a major role in inoculum survival as well. Plant age appears to be important for the initiation of infection. Most studies indicate that sorghum is susceptible only for a short period during the seedling stage (Metha 1965).

Head smut resistance in sorghum results from the action of one or more different host mechanisms and represented four classes of reaction to *S. reilianum* based on susceptibility to natural infection, susceptibility to artificial inoculation and differential reaction to Texas races of the pathogen referred to as R1, R2, R3, and S1 (Frederiksen and Reyes 1980; Craig and Frederiksen 1992).

In a series of papers, Frederiksen *et al.* (Frederiksen and Rosenow 1971; Frederiksen 1978; Frederiksen 1986; Frederiksen and Reyes 1980) defined and identified different types of resistance. Horizontal (non-race specific) resistance to natural infection and horizontal susceptibility to inoculation with hypodermic syringe was designated "R1". Presumably, the resistance factors involved inhibit the progress of the pathogen through the exterior plant tissues surrounding the apical meristem. R1 resistant lines identified include 'TAM428' and 'TX430' (Frederiksen and Rosenow 1971; Frederiksen 1978; Frederiksen 1986; Frederiksen and Reyes 1980). Vertical (race-specific) resistance, "R2", was characterized by resistance to some races of *S. reilianum* and susceptibility to others, with the same response to natural infection as to artificial inoculation. Apparently, the resistance factors are expressed in the apical meristem as an incompatible host/pathogen interaction and do not provide the horizontal resistance to natural infection postulated for the R1 reaction class. The R2 entries orginally identified were 'SA281', 'SC170-6-17', and 'TX414'. Horizontal resistance to natural infection and inoculation by injection was designated "R3". Resistance to artificial inoculation suggests that factors in the apical meristem presumably produce an incompatible host/pathogen interaction. The R3 entries identified in the original study were 'FC6601' and 'Lahoma' sudangrass although these may lose that designation if new virulent races are tested. Horizontal susceptibility to natural infection and inoculation is designated "S1". Presumably, none of the factors that confer resistance are present. S1 examples included 'SC324-12E' and 'TX7078' (Frederiksen 1986; Frederiksen and Reyes 1980).

Head smut resistant sorghum lines 'TX2962' through 'TX2978' were developed and released by the Texas Agricultural Experiment Station, the Texas A&M University System, Lubbock, Texas in 2006. These lines are generally earlier in maturity and shorter in plant height than the standard check 'TX278'. The head smut resistant line 'BTX635' is one of the most popular resistant lines in the production of hybrid sorghum. This line possesses all three types of resistance against head smut.

Xu *et al.* (1997) developed two sorghum hybrids, 'Kang 4' and 'Kang 7', in China from crosses between the male-sterile line 'Tx622A' and the restorer lines 'Jinliang 5' and 'Xinliang 7' and recorded high yields, high resistance to head smut and extensive adaptability.

In Mexico, Williams-Alanis *et al.* (2009) studied the reaction of 49 experimental and commercial grain sorghum hybrids to *S. reilianum*, with the objective of identifying resistant hybrids (0-3% disease incidence), which could be used as parental lines. The evaluation was conducted under irrigation and natural infection in Empalme County in Matamoros, Tamaulipas, Mexico during the fall and winter seasons of 2005-2006 and 2006-2007. The experimental design was a duplicated simple latice 7 × 7 with four replications. Commercial and experimental resistant hybrids with acceptable grain yield (4 ton ha^{-1}) were detected; the most outstanding were 'Pioneer 82G63', 'RB-5x204', 'RB-118x437', 'Asgrow Ambar', 'RB-118x204' and 'RB-119x430' with 0, 0.8, 1.1, 1.2, 1.6, and 2.1% incidence, respectively. The parental lines that produced the most resistant hybrids were the female '46038' and males 'Tx-437' and 'LRB-204'. They generated the most resistant experimental hybrid

'46038x437' with only 0.2% incidence of head smut.

The sorghum cytoplasmic male sterile (CMS) line 'A_1Tx3197' was used widely in China. At the end of 1970s, this line and its hybrids gradually lost resistance to sorghum head smut because of race shift. Meanwhile, floret abortion in this line led to low yield. Since the maintainer of A_2 type CMS can restore the fertility of A_1 type CMS, 'BV_4' with genes conferring head smut and floret abortion resistances was used as a donor, and 'A_1Tx3197' was used as a recurrent parent. After five generations of backcrossing, an intermediate material 'BSx3197' carrying head smut and floret abortion resistance genes, a maintainer for A_2-CMS, was acquired. Then 'BSx3197' was used to replace the nuc-lear genes of A_2 type CMS line. Finally, a novel A_2 type CMS line 'A_2Sx3197' with its maintainer 'BSx3197' ($MS_1MS_1ms_2ms_2$) was bred. The line 'A_2Sx3197' was restorable in A_1 and A_5, but sterile in A_2, A_3, A_4, A_6, and 9E cytoplasmic backgrounds. The average disease incidence of 'A_2Sx3197' was 0-0.8%, and the average abortion incidence was 0-8.4%. 'A_2Sx3197' had significantly higher resistance to head smut and floret abortion than 'A_1Sx3197', which was similar to 'BV4'. The main agronomic traits of 'A_2Sx3197' and 'A_1Tx3197', such as days of heading, plant height, spike length, spike width, 1000-grain weight, grain weight per spike, grain color, shell color, spike shape, and spike type were not significantly different (Cheng *et al.* 2010).

DNA-based diversity and molecular tags

Ten isolates of sorghum head smut fungi (*S. reilianum* from different hosts and geographic origins, and of races 2 and 3 were studied by Xu *et al.* (2003) using random amplified polymorphic DNA (RAPD). Rich genetic diversity was recorded among the 10 isolates. UPGMA analysis divided the 10 isolates of *S. reilianum* into two groups, depending on the geographical origin and physiological race. The isolates from Qingyuan and Suihua formed one group. Eight isolates from sorghum from Shenyang, Fuxin, Yingkou, Yuci, Siping and Harbin, and one isolate from maize from Shenyang formed another group. Marked differences between races 2 and 3 of *S. reilianum* of sorghum existed at a molecular level.

The genetic structure of 459 isolates of *S. reilianum* from field populations in Mexico, Niger and the United States was previously characterized in a limited fashion using restriction fragment length polymorphism (RFLP) and randomly amplified polymorphic DNA (RAPD) techniques (Naidoo *et al.* 2002). Advances in technology since that time offer opportunities to more effectively sample genomic differences. For example, the use of amplified fragment length polymorphisms (AFLP). AFLP fingerprinting has wide taxonomic applicability, as demonstrated by its effective use in a variety of taxa including bacteria (Huys *et al.* 1996) and fungi (Majer *et al.* 1998). Recently, AFLP-based genetic diversity was assessed by Prom *et al.* (2011) in DNA samples from 49 *S. reilianum* isolates, including 44 sorghum isolates from Texas, USA, two from Uganda, and one from Mali, and two maize isolates from Mexico. Single-base extensions with *Eco*RI and *Mse*I primers in the selective amplification increased the number of informative polymorphic bands. High genetic dissimilarity (50%) was observed between isolates originating from maize and those originating from sorghum. The resultant dendrogram, constructed using cluster analysis, grouped 82% of the sorghum *S. reilianum* isolates into four small clusters with $\geq$ 82% similarity. Other than for two race 6 isolates from Weslaco, Texas, no evidence for geographical or other restrictions on gene flow was evident.

Restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) methods were used by Oh *et al.* (1994) to find markers linked to a head smut resistance gene (*Shs*) in sorghum. To select parents for a mapping population, RFLPs were identified between four resistant accessions ('Lahoma Sudan', 'White Kafir', 'SC325', and 'CS3541') and four susceptible accessions ('RTx7078', 'SC170-6-17', 'BTx399', and 'BTx623') for three restriction enzymes (*Eco*RI, *Eco*RV, and *Hind*III) with 43 maize genomic clones. Since 'SC325' (resistant) and 'RTx7078' (susceptible) showed the maximum RFLP frequency, these accessions were selected for mapping. Fifty-two F_2 progeny from a selfed cross between accessions 'SC325' and 'RTx7078' were used to map the *Shs* locus. One hundred twenty-four sorghum genomic clones hybridized to DNA fragments produced by each of five restriction enzymes (*Bam*HI, *Eco*RI, *Eco*RV, *Hind*III, and *Xba*I) and 326 RAPD markers were used for linkage analysis with *Shs*. Linkage of RFLP and RAPD loci with *Shs* was verified by using F_3 lines to determine the *Shs* genotypes of the F_2 plants after inoculation with a race 5 isolate of *S. reilianum*. RFLP and RAPD analyses revealed that RFLP loci detected by probes pSbTXS560 and pSbTXS1294 and one RAPD locus from primer OPG5 were linked to *Shs*.

Zou *et al.* (2010) studied two mapping populations: restorer line population (R population) '2381R/Aisi' and maintainer line population (B population) 'Tx622B/7050B' to identify SSR molecular markers of a sorghum resistance gene that confers resistance to head smut physiological race 3. Two mutually independent non-allelic genes, which interact, controlled resistance to sorghum head smut physiological race 3. Molecular markers were easier to find in the B (maintainer) population than the R (restorer) population during the screening of SSR markers and showed that different resistance mechanisms are present in the restorer line and maintainer lines. SSR markers *Xtxp*13 and *Xtxp*145. *Xtxp*13 located in linkage group B in the R population, and *Xtxp*145 located in linkage group I in B population were linked to resistance. The recombination percentage between marker and resistant gene are 9.6% and 10.4%, respectively. Li *et al.* (2011) used restorer segregation population ('2381R/Aisi') to identify closely linked SSR molecular markers resistance gene to head smut physiological stain No. 3. Of the 150 pairs of SSR primers used, one pair of primers IS10 264, when tested on 114 F_2 resistant and 60 F_2 susceptible individuals, showed consistent polymorphism bands between parents and bulks located in linkage group I, with a recombination percentage between marker and resistant gene of 9.8% and the genetic distances to resistant gene are 9.9 cM.

Future directions

While the number of pathogenic races of *S. reilianum* is not large, new races are being revealed (Prom *et al.* 2011; Zhang *et al.* 2011). Thus, screening and identification of resistant sorghum must continue. It would be beneficial if a common set of host differentials were used across studies so that a common classification could be employed. (It is unlikely that the race 4 isolates identified in Texas and China are actually the same.) Simple and accurate DNA-based protocols such as PCR can now be devised for rapid species-specific identification of the sorghum-infecting smut fungi. Even though the smut pathogens can be grown in culture, crosses require a compatible mating which still requires passage through the host. Now that the DNA sequences of mating type alleles are being determined and DNA polymorphisms identified that can serve as markers it should soon also be possible to map and identify genes that contribute to pathogenicity and race specificity.

ERGOT

Introduction

Sorghum ergot or sugary disease was confined to Asia and Africa until 1995 when it was diagnosed in Brazil (Casela *et al.* 1999). Subsequently, the disease was found in sorghum in Australia in 1996 (Ryley and Henzell 1999) and in the United States, the disease was first observed in 1997 on

tillers of ratooned sorghum in the lower Rio Grande Valley of Texas (Isakeit *et al.* 1998). Presently, ergot is endemic and is found in all major sorghum growing regions in the US. Globally, three *Claviceps* species cause ergot in sorghum. *Claviceps africana* Frederickson, Mantle, & de Milliano is the most prevalent occurring throughout the Americas, Australia, Asia, and Africa, while *C. sorghi* P. Kulkarni *et al.* is limited to Asia and *C. sorghicola* Tsukiboshi, Shimanuki, & Uematsu is confined to Japan (Bandyopadhyay *et al.* 1998; Pažoutová 2000; Pažoutová *et al.* 2000; Tooley *et al.* 2000).

Pathogen biology (*Claviceps africana*)

The genus *Claviceps* belongs to the family *Clavicipataceae* under the subdivision *Ascomycotina*. The species *C. africana* and *C. sorghi* share the same anamorph *Sphacelia sorghi*, which is different from that of *C. sorghicola* (Mantle and Bogo 2002). The different species also are distinguished by several morphological features such as color, size, and texture of the sclerotia and stromata, and in their nucleotide sequences of transcribed spacer 1 and 5.8S rDNA (Frederickson *et al.* 1991; Pažoutová *et al.* 2000; Tsukiboshi *et al.* 2001). All the ergot pathogens infect the unfertilized ovaries by penetrating primarily through the stigma, and sometimes via the style and ovary wall (Futrell and Webster 1965; Bandyopadhyay *et al.* 1998). After penetration, infective hyphae grow intercellularly through the stigma and ovary to the inner ovary wall near the ovule and finally reaches the vascular bundles. The fungus colonizes both ovary and ovule tissues acrop*etal*ly. Finally, the fungus replaces the ovary forming a deeply involuted soft mass called a *sphacelium* (Bandyopadhyay *et al.* 1998) (**Fig. 7**). The sphacelia secrete a clear liquid, which becomes opaque called *honeydew* (Bandyopadhyay *et al.* 1998). Ergot inoculum comes from infected panicles as sphacelia containing macroconidia and microconidia, which act as the primary sources for disease development (Frederickson *et al.* 1989, 1991). Upon development of the sphacelium, certain changes in host physiology appear, and this host-parasite relationship may be important in triggering the formation of the sphacelium and sclerotium (Mower and Hancock 1975).

The sphacelium is transformed into another structure that provides protection to the fungus during the winter period (Frederickson and Odvody 1999; Frederickson *et al.* 1999). Transformation of sphacelial tissue into sclerotial tissue is favored by cool temperatures and low relative humidity (Odvody *et al.* 2002b). Townsend and Willets (1954) noted three stages in sclerotia formation and development: 1) initiation, appearance of small distinct initials formed by the interwoven hyphae, 2) development and increase in size, and 3) maturation, characterized by surface delimitation, internal consolidation, pigmentation, and often honeydew droplet excretion. Active vegetative mycelial production always precedes the initiation of sclerotia, and nutrients are absorbed by the ergot pathogen from the substrate (Willets and Bullock 1992). Sclerotial tissue of *C. africana* consists of a mass of whitish fungal mycelium surrounded by a protective brown-reddish rind (Alderman *et al.* 1999; Frederickson and Odvody 1999). One characteristic feature of sclerotium rind formation is a change of color from white to buff to dark brown or black. Accumulation of melanin is responsible for the color change (Willets and Bullock 1992). The rind consists of thickened, pigmented, parenchyma-like cells that usually develop on the outer surface (Willets and Bullock 1992) (**Figs. 8, 9**).

Ergot sclerotia can survive for years. Sclerotia are hard, rugose and orange-brown colored structures that can survive the winter conditions and in the spring produce ascomata (Alderman *et al.* 1999; Frederickson and Odvody 1999; Frederickson *et al.* 1999). In addition to sclerotial tissues, ergot sclerotia also may contain sphacelial tissues. Sclerotia are oval to spherical, tapering at the distal end (4.6 × 2-3 mm) with a furrowed, orange-brown surface (Odvody *et al.* 2002b). Information is limited as to the role of sclerotia-like bodies in the life cycle of *C. africana* due to the few attempts that were successful in the germination of sclerotia (Bandyopadhyay *et al.* 1996; Bandyopadhyay *et al.* 1998; Frederickson 1999). Sclerotial tissue development is very important because it ensures survival as a generative fungal propagule and is a potential source of inoculum (Ryley *et al.* 2002), serves as a vehicle to introduce new pathogen genotypes via sexual reproduction.

Disease symptoms and losses

C. africana is a tissue-specific pathogen infecting unfertilized ovaries with the stigmas as the primary site of infection (Futrell and Webster 1965; Bandyopadhyay *et al.* 1998; Frederickson and McLaren 2000). There is a similarity between ergot pathogen-host interaction and pollen-stigma interaction. Hyphae from germinating spores grow through the interstylar tissue in a similar manner as germinating pollen to reach the ovule. Within days after colonization of the ovaries, the pathogen produces structures calle sphacelia, which secretes a clear to opaque liquid, the honeydew (**Fig. 7**). This viscous fluid matrix consists of high concentrations of sugar and massive amounts of conidia that contribute to the secondary spread of the fungus within the field or long distance (Mower and Hancock 1975; Bandyopadhyay *et al.* 1998; Frederickson and McLaren 2000). Also, the high sugar content of the honeydew makes it attractive to insects, which in turn may contribute to the spread of the fungus (Mower and Hancock 1975; Bandyopadhyay *et al.* 1998; Prom *et al.* 2003).

Although ergot may develop extensively in grain sorghum production fields under favorable environmental conditions, the pathogen poses a more serious threat to the sorghum hybrid seed industry. Male-sterile sorghum lines used in hybrid seed production are highly susceptible, especially when pollination is delayed due to environmental or genetic factors (Futrell and Webster 1965; Bandyopadhyay *et al.* 1998). Yield losses due to ergot in hybrid seed production fields ranging from 10-80% in India and between 12-25% in Zimbabwe have been reported (Bandyopadhyay *et al.* 1998; Sangitrao *et al.* 1999). In addition to the reduction in yield, the sugary exudates produced by the pathogen reduces seed quality and makes harvesting and seed processing much more difficult (Bandyopadhyay *et al.* 1998). The rapid global spread of the pathogen raised concerns about the potential for reintroduction and spread of ergot in seedborne inoculum. Quarantines have been ineffective in preventing an eventual initial entry of the pathogen into new geographic regions and their efficacy has been questioned in regions where the pathogen is already established (Odvody *et al.* 2002a). Sorghum grain contaminated with sclerotes (vegetative fungal propagules) can cause toxicity when fed to livestock (Blaney *et al.* 2000). Sorghum ergot produces dihydroergosine (DHES) and related alkaloids, which cause hyperthermia in cattle (Blaney *et al.* 2010). Even at low concentrations (1.1 mg alkaloids/kg feed) is severely detrimental to the performance of steers (*Bos taurus* Bojanus) in the feedlot (Blaney *et al.* 2011).

Pathogen races

Variation within the sorghum ergot pathogens, especially among isolates of *C. africana* and *C. sorghi*, has been reported by some researchers (Komolong *et al.* 2002; Muthusubramanian *et al.* 2006; Pecina-Quintero *et al.* 2007). Pecina-Quintero *et al.* (2007) identified three distinct groups of *C. africana* affecting sorghum in Mexico based on pathogenicity on several sorghum hybrids, lines, and male-sterile lines. One group of *C. africana* was responsible for causing sorghum ergot in Guanajuato and Jalisco, the second group in Tamaulipas, and the third group in Sinaloa region of Mexico.

Fig. 7 Honeydew production results from *Claviceps* infection of sorghum florets. (**A, B**) Examples of highly severe ergot producing conidia-saturated honeydew droplets early in the morning. (**C**) Healthy sorghum seed encrusted with dried honeydew deposited during combine harvest.

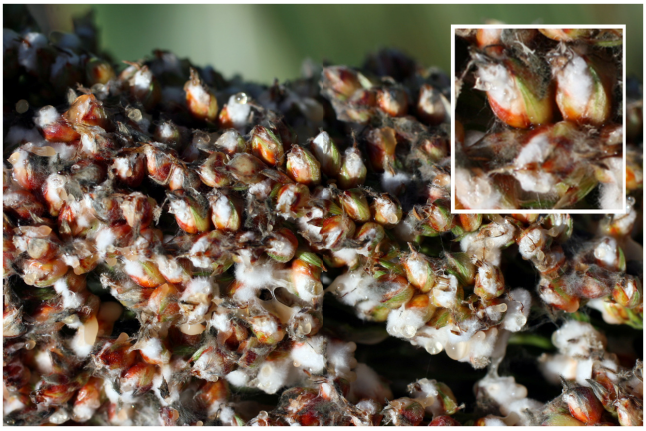

Fig. 8 Newly emerging *Claviceps* sphacelia prior to honeydew expression (close-up, *inset*).

Fig. 9 Sclerotia of *C. africana*. Image courtesy of Noe Montes, Rio Bravo, Mexico.

DNA-based diversity measures

Pažoutová *et al.* (2000) noted that *C. africana* isolates from Texas and Mexico were similar based on their RAPD banding patterns in 65 of 100 primers used. The different species of *Claviceps* affecting sorghum were distinguished by their nucleotide sequences of transcribed spacer 1 and 5.8S rDNA (Frederickson *et al.* 1991; Pažoutová *et al.* 2000; Tsukiboshi *et al.* 2001). Using radiolabelled DNA amplification fingerprints analysis of 110 Australian and overseas isolates of *Claviceps* spp., Komolong *et al.* (2002) were able to distinguish *C. africana* from other *Claviceps* spp., including *C. sorghi* and *C. sorghicola*. Parh *et al.* (2006) reported that many genes control ergot resistance in sorghum and that pollen traits, pollen quantity and pollen viability have moderate genetic correlation with ergot infection frequency. Determining the number and location of genes controlling resistance as well as studying the association of pollen traits with ergot resistance can assist in the rapid and efficient development of new ergot-resistant varieties. Both ergot infection frequency and pollen traits were reported to be influenced by G × E interactions and these traits were under the control of QTL (McLaren 1992; McLaren and Flett 1998; Wang *et al.* 2000). Markers tightly linked to major genes or quantitative trait loci (QTL) in one population can be used for marker assisted selection (MAS) screens if they are polymorphic in another population. Parh *et al.* (2008) constructed a genetic linkage map of sorghum RIL population 'R931945-2-2' × 'IS 8525' (resistance source) using 303 markers including 36 SSR, 117 AFLP™, 148 DArT™ and two morphological trait loci. Composite interval mapping identified nine, five, and four QTL linked to molecular markers for percentage ergot infection, pollen quantity and pollen viability, respectively, at a LOD >2.0. Co-location/linkage of QTL were identified on four chromosomes while other QTL for the three traits mapped independently, indicating that both pollen and non pollen-based mechanisms of ergot resistance were operating in this sorghum population. Of the nine QTL identified for percentage ergot infection, five were identified using the overall data set while four were specific to the group data sets defined by temperature and humidity. QTL identified on SBI-02 and SBI-06 were further validated in additional populations.

Genetic analysis of 12 *C. africana* isolates collected from Mexico using AFLP markers revealed 60% intra-population variation and over 90% genetic similarity (Pecina-Qunitero *et al.* 2007). Tooley *et al.* (2010) used designed PCR primers and probes from the intron 3 region of the β-tubulin gene (for *C. africana* and *C. sorghi*) and the intron 4 region of EF-1α (for *C. sorghicola*) and tested them by real-time (RT) PCR with purified DNA from ergot samples from the field and greenhouse. RT-PCR was able to detect three ergot pathogen species from infected materials as well as from sclerotia of *C. sorghi*.

Disease screening techniques

There exists a need to develop a standardized method of inoculation that could more effectively identify sorghum genotypes with heritable resistance genes. This method should ensure that the pathogen is placed on the stigmas and other infection sites at the time of greatest susceptibility, thereby circumventing the influence of floral morphological and phenological characteristics. The method should be rapid, repeatable, yield consistent results under different environments, and be capable of being used to differentiate between levels of susceptibility. Many inoculation methods for screening sorghum genotypes for ergot resistance have

been adopted in Asia and Africa over the years. The sponge–pressing technique followed by bagging used for ergot resistance screening on pearl millet (*Pennisetum glaucum* (L.) R. Br.) (Sharma *et al.* 1987) was also found suitable for screening sorghum for resistance to ergot in those environments. Inoculation techniques previously used by other workers include: single or multiple spray inoculation at: 1) 10% pollen shed in male-fertile; or stigma emergence in male-sterile non-bagged panicles, 2) anthesis only when the tip of the panicles or 25% or more spikelets had completed flowering followed by bagging for 7-10 days (McLaren 1992; Tegegne *et al.* 1994; Musabyimana *et al.* 1995). Also, bagging of the inoculated panicle increases the humidity and thereby enhances the ability of the pathogen to cause infection, to exclude external pollen (Musabyimana *et al.* 1995), and extends the dew period around the panicles. Musabyimana *et al.* (1995) showed effectiveness of a single inoculation at the beginning of anthesis using a dipping method, in which panicles are immersed in an ergot spore suspension. This method is feasible when dealing with tall sorghum genotypes, but difficult to use with the dwarf grain sorghum breeding lines and hybrids planted in the United States.

Prom *et al.* (2005) have shown that a single spray or sponge inoculation before anthesis followed by bagging for 7 days is more reliable than non-bagging or misting methods for screening sorghum germplasm for resistance to ergot under different environments. This technique tended to obviate the influence of flowering characteristics by ensuring that the non-pollinated stigmas are available for infection. Bagging of the inoculated panicle increases the humidity and thereby enhances the ability of the pathogen to cause infection, exclude external pollen (Musabyimana *et al.* 1995), and extend the dew period around the panicles. When the technique developed by Prom *et al.* (2005) is used, disease assessments can be completed within 15 or 20 DAI. Similarly, research conducted in Africa and Asia by Tegegne *et al.* (1994) and Musabyimana *et al.* (1995) showed that a single inoculation at the beginning of anthesis, followed by bagging, was most suitable in screening sorghum for resistance to ergot in those environments. A conidial suspension was prepared by washing infected sorghum heads containing fresh honeydew collected from a nearby ergot-infected disease plot. The resultant suspension was filtered through a fine mesh cloth and the spore concentration was determined using percentage transmittance by a colorimeter. For inoculation, the concentrated spore suspension was then diluted with water in a 6.0-L sprayer to contain 1×10^6 conidia mL^{-1} (Herde *et al.* 2006; Parh *et al.* 2006).

Host resistance

In certain instances, sorghum lines reported to be resistant in one geographic location or under a particular climatic condition were later found to be susceptible in others (McLaren 1992). A number of studies (McLaren and Flett 1998; Wang *et al.* 2000; McLaren 2002) have extensively examined the role of weather on ergot incidence, and models were developed to predict ergot severity based on weather variables occurring at critical periods of host development. These models were shown to accurately predict ergot severity of a genotype assuming the presence of viable inoculum, but may not be applicable under conditions of natural infection (Wang *et al.* 2000). Montes-Garcia *et al.* (2009) conducted a study at Rio Bravo, Tamaulipas, Mexico, during 2002 and 2003 with an objective to determine the relationship between sorghum ergot severity and weather factors and indicated that minimum temperatures above 10°C increased the risk of ergot development, whereas minimum temperatures above 22.5°C prevented ergot development. Also, values of minimum relative humidity above 30% during anthesis promoted infection indicating that environmental variables play a significant role in both the infection process and severity of the disease. Bandyopadhyay *et al.* (1998) and Mantle and Bogo (2002) reported that resistance to ergot was based primarily on the efficiency of rapid self-pollination and fertilization, thus allowing these genotypes to avoid infection by the pathogen. To date, two physiological mechanisms of ergot resistance have been reported: (1) pollen-based (i.e., efficient pollination and fertilization restrict infection) and resistance to ergot has been reported as pollen-mediated in different crops including sorghum (Watkins and Littlefield 1976; Willingale *et al.* 1986; McLaren 1997; Bandyopadhyay *et al.* 1998). Pollen-based mechanisms of resistance have value in some environments but are limited by the overriding effect of the environment (e.g. ergot favourable conditions) on pollen activity (Frederickson *et al.* 1994; Bandyopadhyay *et al.* 1998). In the past, a number of resistance sources have been reported (Sundaram 1971; Khadke *et al.* 1978; McLaren 1992; Tegegne *et al.* 1994), but it appears that resistance in the reported lines could be a pollen-mediated disease escape or environmental effects overshadowed genetic effects on the phenotype; (2) non-pollen-based, in which a combination of floral characteristics, e.g. least exposure time of stigma to inoculum before pollination, rapid stigma drying after pollination and small stigma are associated with resistance (Dahlberg *et al.* 2001). Several new putative sources of resistance have been identified and found to be effective in male-sterile backgrounds (Dahlberg *et al.* 2001; Reed *et al.* 2002b), suggesting that resistance to ergot is not entirely pollen-mediated. Non-pollen-based mechanisms of ergot resistance were first reported in the sorghum line 'IS8525' (Dahlberg *et al.* 2001). The corresponding A_3 male sterile line also showed moderate resistance but resistance was reported to be unstable across environments (Reed *et al.* 2002b). Moran *et al.* (2002) characterized the relative rate of ergot vulnerability of a set of publically available and commercially used A-, B-, and R-lines, and hybrids and also determined the relationship between the duration of stigma receptivity and ergot vulnerability. The study concluded that stigma receptivity was not only the factor that influenced ergot infection, as some ovules no longer receptive to pollen could still be infected by ergot. Also, inconsistency across environments for genetic effects related to the "resistance" of the genotype evaluated, indicated that "pseudoresistance", which is dependent on the environment, prevails.

Ergot resistance sources have been reported by a number of researchers. McLaren (1992) noted that 'SD1/91', 'RTAM428' and 28 other sorghum lines exhibited varying levels of resistance to ergot at two locations in South Africa. Six Ethiopian sorghum accessions ETS '1446', '2448', '2465', '3135', '4457', and '4927' were reported as resistant to ergot (Tegegne *et al.* 1994). Musabyimana *et al.* (1995) identified 12 ergot resistant accessions, including IS '25533', '25576', and '25583'. Reed *et al.* (2002a) reported that 'IS14131' and 'IS14257' might possess genes for resistance based on evaluation of the resulting progeny when these lines were crossed with male-sterile cytoplasm. Ergot inoculation of 'IS8525' at the start of anthesis resulted in a greater infection response with a decrease in ergot severity when inoculations were conducted at later flowering stages. Seeds of 'IS8525' obtained from R.G. Henzell (Queensland Department of Primary Industries, Warwick, Australia) exhibited 20-80% ergot severity under natural infection in Weslaco, Texas (United States) during the fall of 2003 (Prom, *unpublished data*). Greenhouse and field trials in Texas and Puerto Rico have shown that the ergot response of 'IS8525' was highly variable (Reed *et al.* 2002b; Prom *et al.* 2005; Prom and Erpelding 2006). Complex interactions of variable environmental factors with the infection process and on the severity of the disease greatly complicate development of varieties with resistance to sorghum ergot make more complicated and at present no sorghum genotype with stable resistance has been reported (Bandyopadhyay *et al.* 1998; Frederickson and McLaren 2000; Parh *et al.* 2006).

Future research

So far, management strategies such as the use of fungicides and avoidance aimed at minimizing the impact of the disease have been either unsuccessful or economically expensive. Therefore, focus for future research will include identifying sorghum germplasm with stable genetic rather than morphological resistance. Also, it may be possible to identify defense genes are activated at the early stage of infection and to use the available technology to enhance expression of genes to find the function of those genes inhibitory to pathogenesis. Also of value will be a more comprehensive study of pollen management in minimizing the impact of the disease in hybrid seed production fields in the absence of other control strategies.

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