Fungal Invasion of Kernels and Grain Mold Damage Assessment in Diverse Sorghum Germ Plasm

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ABSTRACT

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Use of resistant cultivars is the most feasible way to minimize crop damage from grain mold when sorghum (*Sorghum bicolor*) is grown in a climate conducive to fungal invasion. An experiment was conducted to assess relative contribution of fungal species to grain mold damage and to evaluate extent of variation in sorghum for resistance to grain mold. A large and diverse set of landraces were evaluated for grain mold resistance at different stages of grain maturity. Fungal species infecting sorghum kernels were isolated and counted. Significant differences in the percentage and severity of kernel infection were observed among accessions at all stages of kernel development. The predominant fungal species isolated from sorghum kernels collected from field-grown panicles did not change across different sampling dates and years. Although visual rating identified highly susceptible accessions as early as 40 days after flowering, rating a few weeks after physiological maturity more reliably identified genotypes with higher levels of resistance to kernel damage. A multiple regression model involving all the fungal species isolated from sorghum kernels accounted for 64% of the variation in the final visual grain mold damage rating. *Gibberella zeae* and *Fusarium moniliforme* each accounted for 46 and 16%, respectively, of the variation in the final visual grain mold damage rating. Sorghum accessions free from colonization by one or more fungal species across three sampling dates were identified. Thus, it should be possible to establish differentials for each fungus or group of fungi to facilitate screening of germ plasm for resistance to grain mold.

Grain mold is a significant constraint on sorghum (*Sorghum bicolor* (L.) Moench) improvement and production in many parts of the world. It is of particular concern in areas where the period between anthesis and harvest coincides with high humidity and warm temperature (5,14). Grain mold is caused by a number of fungal species belonging to different genera (2,5,14). Most of the fungi isolated from molded grain are facultative parasites, and the predominant species differ among locations and across years (4). Because of the presence of abundant, naturally occurring inoculum of the various fungal species, the development of grain mold on sorghum depends heavily on the climatic pattern at flowering and grain filling stages of the crop (12).

Fungal damage to kernels may be limited to the discoloration of the pericarp or may involve extensive internal invasion (5, 11). Grain mold reduces yield, seed viability, kernel weight, nutritional quality, and market value (5,14). Molded grain may also contain mycotoxins and present health hazards to animals and humans (2).

Genetic resistance in sorghum is considered to be the only feasible means to mitigate damage by grain mold in many areas where the crop is grown in a climate conducive to fungal invasion. Visual appraisal of the extent of kernel deterioration has been commonly used to screen germ plasm for resistance to grain mold in sorghum breeding nurseries (1,5,14). However, visual rating per se may not adequately reflect the degree of damage caused by fungal invasion of the kernels (11). Measuring the incidence of the different fungi on sorghum seeds may readily differentiate genotypes with otherwise similar kernel appearance (11). It also is useful to determine fungal species that are more important in grain molding in a particular environment.

The objectives of these experiments were to (i) assess the relative incidence of various fungal species and their correlation with kernel discoloration, and (ii) evaluate the extent of variation for grain mold resistance at different stages of maturity in a large and diverse sorghum accession.

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MATERIALS AND METHODS

Genetic material. A diverse array of 231 photoperiod-insensitive sorghum accessions (referred to hereafter as the working collection) representing different cultivated races of sorghum from a number of countries were evaluated for grain mold resistance in West Lafayette, IN. Forty-three resistant accessions (with grain mold scores of 1 and 2 at harvest) and nine susceptible accessions (with grain mold scores of 4 and 5 at harvest) based on the 1984 test were selected as a subset of the working collection to confirm their reaction to infection by grain-mold-causing fungi in 1985.

Experimental design. The 231 accessions in the working collection were planted on 18 May 1984 in unreplicated plots at the Purdue Agronomy Research Center. Each accession was planted in three 6-m-long rows spaced 75 cm apart. The 52 accessions in the subset of the working collection were planted at the same site on 23 May 1985 in a randomized complete block design with three replications. Each accession was planted in a single row plot 5 m in length; plots were spaced 75 cm apart.

Sampling procedure. Twelve randomly selected plants were tagged at anthesis in each unreplicated plot in 1984 and in each replicated plot in 1985. Three tagged panicles were harvested at 40, 50, and 60 days after flowering (DAF). The remaining three panicles were harvested at the end of November (referred to as "final").

Grain mold damage assessment. The warm and humid weather at the Purdue Agronomy Research Center was conducive to the development of a uniform level of grain mold without artificial inoculation. For each sampling date, we visually estimated grain mold severity in three harvested panicles, using a rating system described by ICRISAT (7) and subsequently used by others (1,5,14). Ratings were based on a 1 to 5 scale in which 1 = no visible mold, 2 = 1 to 10%, 3 = 11 to 25%, 4 = 26 to 50%, and 5 = more than 50% of the kernels in the panicle molded.

Fungal isolation and colony count. Two of the three panicles harvested from each replication at each sampling date were threshed separately, and randomly selected 50-kernel samples (one in 1984 and two in 1985) were drawn for fungal isolation. Each 50-kernel sample was surface sterilized in a 1% NaOCl solution for 3 min, rinsed in sterile water, and then plated in petri plates (10 seeds per plate) containing 10 ml of acidified (2.5 ml of 25% [vol/vol] lactic acid per liter of me-

dium) potato dextrose agar (APDA). Thus, for each harvesting date, two samples from two panicles were assayed in 1984 and four samples (two samples per panicle) for each replication were assayed in 1985. The plates were incubated at room temperature for 6 to 7 days. Fungi growing from each 50-kernel sample were identified and colonies were counted. Fungal incidence was calculated as an average of percent colonies recovered from the two samples for each harvesting date.

Statistical analysis. In 1985, means for each of the 52 accessions in the subset were calculated from three replications for grain mold damage scores and two replications for percent incidence of each fungus. The 1985 means were then combined with the 1984 values to calculate 2-year averages for each accession. Subsequent statistical analyses for the subset of 52 accessions were based on combined values. Mean percent incidence of each fungus and the corresponding standard error for the working collection and the subset were calculated by the univariate procedure of SAS (SAS 6.03, SAS Institute, Cary, NC). Spearman's rank correlation and Pearson's simple correlation coefficients were computed for the 1984 data from the working collection and for the combined data for the subset. A multiple regression analysis was computed with the 2-year mean final visual grain mold score of the 52 accessions as a dependent variable and the 2-year mean parentage incidence of fungi recovered from seeds harvested 60 DAF as independent variables. The maximum *R*-square improvement method of SAS was used to determine the relative importance of the different fungal species in kernel discoloration. We computed Pearson's correlation between number of days from planting to flowering (DPF) and grain mold damage scores (GMDS) at different stages of kernel development.

RESULTS AND DISCUSSION

Incidence of fungal species at different stages of maturity. Fungal species isolated from kernels of diverse sorghum accessions are listed in Table 1. The predominant fungal species isolated from kernels in both the working collection and the subset were *Alternaria* spp. and *Fusarium moniliforme* J. Sheld. *F. moniliforme* is an important grain pathogen worldwide (5). Accessions from the working collection and the subset exhibited large differences in levels of kernel infection by each fungal species at all stages of kernel development (data not shown). Mean percent incidence of fungi increased with delay in time of sampling for almost all species (Table 1).

Pearson's simple correlations between mean percent fungal incidence at each sampling date were calculated for the subset of 52 accessions to determine if extent of colonization by each fungal species was consistent from early to late stages of kernel development. Correlations between fungal incidence at 40 and 50 DAF for *Alternaria* spp. ($r = 0.81$), *F. moniliforme* ($r = 0.75$), *Gibberella zeae* (Schwein.) Petch ($r = 0.80$), *Colletotrichum graminicola* (Ces.) G. W. Wils. ($r = 0.30$), and other, unidentified, fungi ($r = 0.52$) were significant ($P < 0.05$) and positive. The correlation coefficients between incidence at 50 and 60 DAF for these fungal species were 0.31, 0.73, 0.89, 0.60, and 0.40, respectively. These results suggest that some accessions were consistently less extensively colonized by the major fungal species than others were, at all stages of kernel development. The mechanism of resistance to fungal pathogens by these accessions is not known. Several mechanisms for resistance in sorghum have been proposed. For example, some genotypes restrict fungal growth by responding to fungal invasion through rapid increase in levels of antifungal phenolic compounds in their kernels (5, 8,13). Others may employ physical kernel properties such as corneous endosperm, thin mesocarp, and thick surface wax to minimize fungal penetration and colonization (13), or depend on antifungal proteins for their defense (3,10).

Visible grain mold damage at different stages of maturity. Visual assessment of the extent of kernel discoloration has been the most commonly used method to quantify grain mold severity (4). Genotypes in the working collection and the subset exhibited large differences in visual grain mold damage scores at different stages of maturity (Table 2). The proportion of accessions with ratings of 1 or 2 dropped from over 80% at 40 DAF to less than 55% on the final date of sampling. Despite increases in the number of accessions rated as susceptible (>3.0) from first to last sampling dates, visual grain mold scores at all stages of maturity were sig-

Table 1. Incidence of fungal species isolated from seeds of sorghum accessions sampled 40, 50, and 60 days after flowering in 1984 and 1985

	Working collection[a]			Subset of working collection		
	Days after flowering					
Fungi	40	50	60	40	50	60
Penicillium spp.	2.6 (± 0.2)[b]	1.4 (± 0.2)	0.7 (± 0.1)	1.6 (± 0.2)	1.2 (± 0.2)	0.6 (± 0.1)
Alternaria spp.	39.4 (± 1.6)	55.8 (± 1.2)	62.4 (± 0.8)	26.1 (± 2.1)	43.4 (± 2.0)	57.0 (± 1.6)
F. moniliforme	3.9 (± 0.4)	6.6 (± 0.5)	9.0 (± 0.6)	3.1 (± 0.5)	6.8 (± 0.9)	8.3 (± 0.8)
G. zeae	0.9 (± 0.1)	1.8 (± 0.2)	2.8 (± 0.3)	0.7 (± 0.2)	1.5 (± 0.3)	2.4 (± 0.3)
C. graminicola	0.4 (± 0.1)	1.0 (± 0.1)	1.5 (± 0.2)	0.4 (± 0.1)	0.9 (± 0.1)	1.3 (± 0.2)
Helminthosporium spp.	3.7 (± 0.3)	5.9 (± 0.4)	7.7 (± 0.4)	...	...	...
Cladisporium spp.	1.7 (± 0.1)	3.6 (± 0.2)	4.9 (± 0.3)	...	...	...
Others	0.9 (± 0.1)	1.6 (± 0.2)	1.5 (± 0.1)	4.3 (± 0.4)	7.3 (± 0.6)	11.4 (± 0.7)

[a] The working collection comprised seeds from 231 sorghum accessions, the subset of the working collection comprised 52 accesssions.

[b] Values are means derived from assays of 100 seed per accession in 1984 and 200 seed per accession in 1995. Values in parentheses are standard errors of the mean.

Table 2. Percent distribution of sorghum accessions in each mold damage rating category at four sampling dates

	Subset of working collection[b]				Working collection			
	Days after flowering							
Mold rating[a]	40	50	60	Final[c]	40	50	60	Final
1	49.1	26.7	9.2	10.8	80.8	57.7	34.6	19.2
2	31.9	28.0	15.7	20.3	13.4	26.9	44.2	34.6
3	17.2	34.5	39.7	37.2	5.7	5.7	7.7	21.2
4	1.7	10.3	32.3	27.3	0.0	9.6	11.5	11.5
5	0.0	0.4	3.1	4.3	0.0	0.0	1.9	13.5

[a] Ratings: 1 = no visible mold; 2 = 1 to 10%; 3 = 11 to 25%; 4 = 26 to 50%; and 5 = more than 50% of kernels in the panicle molded.

[b] The working collection comprised seeds from 231 sorghum accessions, the subset of the working collection comprised 52 accesssions.

[c] Remaining panicles were harvested at the end of November.

Table 3. Spearman's rank correlation coefficients between visual grain mold damage ratings at 40, 50, and 60 days after planting (DAF) and Final (late November)

Rating combinations	Subset of working collection[a]	Working collection
40 DAF vs 50 DAF	0.46**[b]	0.65**
40 DAF vs 60 DAF	0.42**	0.73**
40 DAF vs Final	0.46**	0.63**
50 DAF vs 60 DAF	0.54**	0.74**
50 DAF vs Final	0.62**	0.76**
60 DAF vs Final	0.65**	0.78**

[a] The working collection comprised seeds from 231 sorghum accessions, the subset of the working collection comprised 52 accesssions.

[b] ** = Significantly different from zero at $P = 0.01$.

nificantly correlated ($P < 0.01$) (Table 3). For each sampling date, Spearman's rank correlation coefficients ($r = 0.54$ to 0.73) between 1984 and 1985 mold scores of the subset of 52 accessions were significant ($P < 0.001$) and positive. These results indicate that visual mold scores of accessions were consistent across sampling dates and years. The time between flowering and assessment of mold damage was critical in separating resistant and susceptible genotypes more clearly. Although visual rating identified highly susceptible accessions as early as 40 DAF, rating at or after 50 DAF was found to be more reliable in identifying genotypes with higher levels of resistance to grain mold.

In order to make meaningful comparisons between accessions for resistance or susceptibility to grain mold damage, it is necessary to know the relationship between visible kernel discoloration and days to maturity. Although differences among accessions for DPF were very large in both the working collection (62 to 110 days) and the subset (70 to 98 days), correlations between GMDS at 40, 50, and 60 DAF and DPF were very low ($r = -0.23$ to -0.05). Correlations between DPF and GMDS at the final sampling date were also low in both the working collection ($r = -0.34$, $P = 0.05$) and the subset ($r = -0.04$, $P = 0.98$). Such low correlations between DPF and GMDS suggested that length of maturity in these genotypes had little influence on visible kernel discoloration.

Relative importance of fungal organisms. To identify the major fungi associated with kernel discoloration at different stages of kernel development, Pearson's correlations between mean fungal incidence and mean visual grain mold damage scores were calculated for the subset of 52 accessions. Incidence of *G. zeae* was significantly and positively correlated with grain mold damage scores at 40 ($r = 0.66$), 50 ($r = 0.68$), and 60 ($r = 0.68$) DAF. The extent of infection by *F. moniliforme* was significantly correlated ($r = 0.32$) with levels of visible mold on the grain only at 60 DAF. The relationship between kernel colonization by the remaining fungi and grain mold damage scores was not significant ($r < 0.35$). These results indicated that *G. zeae* was the most important fungus associated with visible kernel discoloration in all stages of kernel development at our location, while *F. moniliforme* also became important later in the season. The importance of these organisms to sorghum grain mold development in humid environments has long been established (9).

A multiple regression analysis was conducted to further identify combination of two or more fungal species that contributed the most to visible kernel discoloration at the end of the season. Again, *G. zeae* and *F. moniliforme* made significant contributions to the final visual grain mold scores of accessions. These two fungi individually explained 46 and 16% of the total variation in mold scores, respectively (Table 4). Combining *G. zeae* with a group of unidentified fungi (others) accounted for 61% of the variation in visible kernel discoloration. Incorporating three or more fungi into the regression equation did not substantially improve the R^2 value. *Alternaria* spp., the most common fungi at our location, contributed very little to the visible kernel discoloration.

Genotypes free from specific fungal species. Although none of the genotypes were free from all organisms, we identified individual accessions with apparently superior levels of resistance to specific fungi. At least five accessions derived from the working collection of 231 entries were free from infection by a particular fungal species across six samples (2 panicles × 3 sampling dates) in 1984 (Table 5). Forty-four entries were free from colonization by two fungal species. Ten entries were free from infection by three fungal species. Kernels of IS9454 were free from infection by *Penicillium* spp., *G. zeae, C. graminicola,* and other, unidentified, fungi. IS15676 was free from infection by *Penicillium* spp., *G. zeae, C. graminicola, Helminthosporium* spp., and other, unidentified, fungi. Of the 52 sorghum accessions tested for 2 years, 16 were consistently free from infection by *Penicillium* spp. across all sampling dates. IS0919, IS2821, IS3441, IS9323, IS9370, and IS16100 had negligible mean number of colonies (<1%) of *F. moniliforme* for 2 years across all sampling dates. Five accessions—IS2821, IS3441, IS4225, IS7579, and IS8612—were not colonized by *G. zeae* and had less than 10% average incidence of *Penicillium* spp., *F. moniliforme, C. graminicola,* and other, unidentified, fungi for 2 years across all sampling dates. We found two accessions—IS4225 and IS10493—that were not infected by *C. graminicola* for 2 years across all sampling dates. Nonetheless, the reactions of these genotypes to the respective fungi need to be verified with a selective media (5,6), which may minimize biases resulting from competition among fungi plated on APDA (5), and by specific tests designed to monitor resistance to known pathogens.

In conclusion, rating sorghum accessions at or after 50 DAF more reliably identified genotypes with higher levels of resistance to grain mold. Of the array of fungal species recovered from kernels of diverse sorghum accessions, *G. zeae* and *F. moniliforme* contributed the most to increased visible mold damage on the grain under the prevailing environmental conditions in Lafayette, IN, in 1984 and 1985. Traditionally, screening for resistance to grain mold in sorghum is focused on identifying genotypes that might be free from damage by all organisms. Genotypes that are free from infection by all organisms have been rare. We report here, however, that several accessions were free from colonization by at least one fungal species across all sampling dates in 2 years of testing. This result suggests that it should be possible to identify sources of resistance to specific fungi in existing sorghum germ plasm collections. It should be possible to pyramid genes for resistance to different fungal species into a single genotype by intercrossing selected accessions in line development programs or via population improvement schemes. These results also demonstrate that the potential exists for establishing differentials for each fungus to facilitate screening of germ plasm for resistance to grain mold.

Table 4. Regression models that best described relationships between final visual grain mold rating and incidence of organisms at 60 days after flowering in the subset of 52 sorghum accessions, using maximum *R*-square technique

Fungi	R^2	F
Gibberella zeae (GIB)	0.46	42.64**[a]
Fusarium moniliforme (FUS)	0.16	9.78**
Others (OTH)	0.07	3.85
Alternaria spp. (ALT)	0.06	2.93
Penicillium spp. (PEN)	0.02	1.18
Colletotrichum graminicola (CG)	0.02	1.14
GIB OTH	0.61	38.32**
ALT GIB OTH	0.64	28.26**
ALT FUS GIB OTH	0.64	20.97**
PEN ALT FUS GIB OTH	0.64	16.52**
PEN ALT FUS GIB CG OTH	0.64	13.58**

[a] ** = Significant at $P = 0.01$.

Table 5. Number of entries free from infection by fungal species across six samples (2 panicles × 3 harvesting dates) in 1984

Fungi	Entries (no.)
Penicillium spp.	44
Fusarium moniliforme	8
Gibberella zeae	50
Colletotrichum graminicola	66
Helminthosporium spp.	9
Cladosporium spp.	5
Others	48

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