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Grain Molding Fungi Association in Food Type Sorghum Kernels And Effects on Germination

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Abstract: A series of experiments was conducted to assess the relationship among grain molding fungi species within each grain mold rating. Seed samples from a field evaluation of 131 F₂-derived F₃ recombinant inbred families at eight environments were collected to isolate grain mold fungal flora. At each environment grain mold was recorded on a 1 to 5 scale at 40-45 days after flowering. The most prevalent molding fungi species isolated was *Fusarium verticillioides*, which accounted for 46% of the total fungi species recovered. *C. lunata* was negatively correlated with *Alternaria* spp. and *Dreschlera* sp., *F. verticillioides* was negatively related with *Alternaria* sp. and positively related with *C. lunata*. A positive correlation was observed between the predominance of *F. verticillioides* and *C. lunata* with germination. A Spearman rank-order correlation determined negative relationship between scoring rates and seed germination and a positive relationship between seed germination and fungal species diversity. Principal component analysis indicates that as grain mold ratings increase, so does fungi interaction complexity. Knowledge about fungal flora present in different grain mold rating scores allows making inferences about the most predominant fungi species, the most important interactions among those species and may help to enhance selection process for resistant varieties.

Key words: *Sorghum bicolor* L., principal components, fungi diversity

INTRODUCTION

The major constraint to production of consistently high quality sorghum grain is the destruction or severe damage caused by grain molds. Molds discolor the grain, break down the endosperm and significantly deteriorate processing qualities. Mold damaged or weathered grain cannot be decorticated; the flour or grits are badly discolored and cannot be used for food (Prom *et al.*, 2005). Molded grains have lower weight which is related to the breakdown of endosperm structure and lower endosperm density (Rooney and Serna-Saldivar, 1991). Grain mold is a world wide disease of sorghum that affects yield quality and quantity. Thus, resistance to grain mold is considered to be the most important factor in food quality of sorghum grain (Rosenow *et al.*, 1995).

About 40 genera of fungi have been detected in moldy sorghum grain, some of which are capable of producing mycotoxins. Incidence of grain mold varies annually, however the highest increase of disease incidence has been observed when weekly mean

temperature range between 20-24 °C (Montes *et al.*, 2003). A complex of fungi including *Fusarium thapsinum* (Montes *et al.*, 2003), *Curvularia*, *Cladosporium*, *Alternaria* sp. (Stack and Pedersen, 2003) and *Fusarium verticillioides* (Funnell and Pedersen, 2004). cause grain mold. Most of these fungi are unspecialized or facultative parasites and the predominant species vary with location, year and season. Weak pathogenic organisms or facultative parasites may invade sorghum grain under rainy or humid conditions and warm temperatures (Bandyopadhyay *et al.*, 1991). Sorghum lines and hybrids have been developed that have improved tolerance to grain molding and weathered; but no commercial hybrid is resistant in hot, humid environment (Bejosano *et al.*, 2001). The complex genetics of grain mold resistance is due the presence of different mechanisms of inheritance from various sources and interfungal competition (Audilakshmi *et al.*, 2005).

Some pathogens that are associated with grain molding may be toxic when sorghum is used as food or feed. Species of *Fusarium* produce a variety of

mycotoxins such as zearalenone and toxic trichothecenes. The former is associated with reproductive problems in swine (Bowman and Hagler, 1991) and the latter metabolites are associated with the hemorrhagic syndrome and alimentary toxic aleukia in farm animals and humans (Bhavanishankar and Shantha, 1978). *F. verticillioides* has been associated with leukoencephalomalacia (LEM) in horses (Mills, 1989). Another metabolite associated with *Fusarium* is deoxynivalenol, which causes feed refusal or emesis in feeder swine (Bowman and Hagler, 1991).

In reviewing the literature on sorghum grain mold one finds lists of fungal species isolated from or associated with sorghum grain mold. Many of these reports can be classified as purely seed mycoflora studies and in many of them market samples or stored grain were used. Few studies have determined the relative roles of the various fungal species in the etiology of sorghum grain mold. Menkir *et al.* (1996) report that *Gibberella zeae* and *Fusarium verticillioides* accounted for 46 and 16%, respectively, of the variation in the final visual grain mold rating. The aim of the present study was 1) To establish the extent of the relationship between grain molding fungi; 2) To establish which of the molding fungi species had the most significant effect on seed germination; 3) To determine if there is any relation between grain mold rating, germination and fungi species diversity; and 4) To determine the relative role of each molding fungi species within each grain mold rating.

MATERIALS AND METHODS

This study was carried out during 1996-2000 in the sorghum pathology laboratory, Plant Pathology Department at Texas A and M University, College Station Tx. USA.

Germplasm development: The experimental material consisted of 131 recombinant inbred lines (RIL). These RIL were developed by single-seed descent from the cross between Sureño and RTx430. Sureño is a dual-purpose grain and forage variety, with moderate resistance to grain molding. The pedigree of Sureño is [(SC423 × CS3541) × E 35-1]-2 (Meckenstock *et al.*, 1993). It is photoperiod insensitive, has tan plant color (ppqq) with tan-colored glumes and awnless lemma. The seed has a white translucent pericarp. The genotypes of pericarp related traits of Sureño are *RR yy ZZ II b₁ b₁ B₂ B₂* and *SS*. Its genotype for height is *dw₁ Dw₂ Dw₃ dw₄*. RTx430 is a widely adapted inbred line with excellent combining ability and is a common restorer line in many U.S. grain sorghum hybrids. It is highly susceptible to grain mold. The pedigree of RTx430 is (Tx2536xSC-170-6-5-1-E2)-10-4-4-1-4 (Miller, 1984).

Evaluation sites: In 1995 the 131 F₅ derived F₂ recombinant lines were planted at Beeville TX (BE95) and at College Station TX (CW95). In the last experiment, sprinkler irrigation was applied weekly during grain development to enhance the grain mold incidence. In 1996, 131 F₅ derived F₂ recombinant lines were planted at College Station, TX under two moisture levels with (CW96) and without (CD96) sprinkler irrigation). In 1997, the test was planted at Beeville, TX (BE97), Corpus Christi, TX (CC97) and College Station, TX under two moisture levels (CW97 and CD97).

Standard agricultural practices for these experiments were used. At all environments, significant levels of grain mold occurred naturally; therefore inoculation was not necessary. In all the experiments, grain mold was rated at 40-45 days after flowering on all RIL. Grain mold was recorded on a 1 to 5 scale, where: 0 = No evaluation possible, 1 = Seed bright, free from mold damage, 2 = Moderately resistant to mold, seed slightly discolored, 3 = Moderately susceptible, considerable discoloration, 4 = Susceptible, extensive discoloration and deterioration of seed, 5 = Very susceptible, seed essentially all dead, embryos dead and endosperm deteriorated.

Grain mold data were transformed before analysis of variance to stabilize the variances (Lentner and Bishop, 1993). Grain mold data were transformed with the log of data + 1.

Sample collection: Seeds from each F_{2.5} family was harvested from each location. Seed from families with the same grain mold rating was bulked at each location to create a total of five bulks for each location. A subsample of grain from each sample was surface-disinfected.

Isolation and identification of fungi: Kernels were placed on a cheese cloth and put under tap water for several minutes. Each sample was washed separately. Washed seeds were placed in a solution of 2 parts of bleach to 3 parts of water for 2 min. Afterwards, the seeds were placed in a sterile beaker and rinsed with sterile distilled water. A 120 seed subsample was taken and transferred to plates (10 kernels per plate) of potato dextrose agar (PDA) medium containing rose bengal. The plates were incubated at 24±2°C for 7-9 days. After incubation, the seeds were checked with a stereobinocular microscope for the presence of molding fungi and the fungal population was determined. Fungal isolation were identified according to illustrations and descriptions given by Gilman (1957). Kernel germination was assessed directly on PDA agar plates after incubation and therefore not according to standard procedures. Positive germination was recorded where both the coleoptile and hypocotyl had emerged without being killed by a fungus or without necrotic tissue in the seedling.

Grain mold fungi association and effect on seed germination: The presence (%) of isolations of molding species from kernels grown at the different locations was used to determine any associations between these fungi. Fungi incidence and germination data (%) were transformed before calculating Pearson product moment correlation coefficient to stabilize the variance. The data (%) were transformed with the arcsine of the square root of the original data.

Fungi species diversity: Simpson's index for diversity was determined for each kernel sample (Ricklefs, 1993). In the Simpson's index the contribution of each species is weighted by its relative abundance. Simpson's index is

$$D = [1/\Sigma(\pi^2)]$$

Where (π) is the proportion of the species (i) in the total sample of individuals. For any particular number of species (S) in a sample, the value of D can vary from 1 to S, depending on the evenness of species abundance. D values were transformed to ranks and Spearman rank correlation analyses were performed to determine the relationship between grain mold rating, seed germination and fungal species diversity.

Fungi relative importance based on scoring rates: Data of the average fungal incidence per each grain mold rating across all locations was analyzed using principal component analysis (PCA). PCA is a technique which reduces the dimensionality of multivariate data by removing intercorrelations among variables. Fungi data was standardized prior to PCA. For each observation on each fungal species, the standardized value was calculated, by subtracting the mean for that fungal specie from the value for the observation on the fungal specie and dividing this by the standard deviation for that fungal specie. All statistical analyses were done using SAS 6.12 (1996).

RESULTS AND DISCUSSION

Grain mold fungal associations: Fungal species isolated from sorghum grains of the 131 RIF are shown in Table 1. The predominant fungal species isolated from sorghum kernels in order of frequency were *F. verticillioides*, *Alternaria* spp, *C. lunata* and *Dreschlera* spp. *Fusarium* and *Curvularia* have been identified as the most important parasitic fungi that cause sorghum grain mold in the tropical as well as temperate countries. *F. verticillioides* produces a pink white mycelium that appears powdery in its early stages and later appears fluffy. *C. lunata* appears as a shiny, velvety

black, fluffy growth on the grain surface. *Alternaria* and *Dreschlera* have also been reported as predominant species in molded grains, *Alternaria* spp. was the most abundant genus isolated from kernels of 231 photoperiod insensitive sorghum accessions (Menkir *et al.*, 1996).

The environmental conditions that have the most influence on microorganism-related seed deterioration are moisture, temperature and oxygen. However, in most systems, moisture is the factor of overriding importance. Under field conditions, oxygen availability is usually never a limiting factor and the microorganisms involved, either individually or collectively, are able to grow over a wide range of temperatures (Mc Gree, 1986). In this study, the moisture added by the sprinkler irrigation at CW96 and CW97 modify the order of predominant molding species in comparison to the experiments without sprinkler irrigation at the same location (CD96 and CD97). At CW96 and CW97 the predominant fungal specie was *F. verticillioides* while at CD96 the most abundant specie was *F. semitectum* and at CD97 the predominant species was *Alternaria* spp. (Table 1).

Grain mold infection should be seen as a community of different fungal populations interacting among them. In the present study, *C. lunata* was negatively correlated with *Alternaria* spp. and *Dreschlera* sp. and no relation was found between *C. lunata* with *Phoma sorghina* (Table 2). On the contrary, in the literature a positive relation has been reported between *C. lunata* with *Dreschlera* and *C. lunata* with *Alternaria* (Denis and Girard, 1980) and negative relation of *C. lunata* with *Phoma sorghina* (Singh and Agarwal, 1986). In this case, the significant correlations between different sorghum grain molding fungi over locations should not be seen as fungal interactions, but rather as the influence of environmental conditions.

Environmental requirements for spore production, fungal infection and fungal growth in sorghum grains is different among molding fungi, i.e., *Alternaria* spp produce abundant spores during wet conditions and at temperatures higher than 13°C. In contrast, *F. verticillioides* is adapted to warmer conditions (Humpherson-Jones and Maude, 1982). The inoculum abundance of the different fungal species at the experimental site plays a big role on grain mold. When airborne spores of *Fusarium*, *Curvularia* and *Alternaria* species were monitored, it was found that the concentration of spores of the different mold fungi in the air varied between season and within the season. Besides, the frequency of *Fusarium* and *Alternaria* spores in the two years study differed while that of *Curvularia* was similar in both years (Bandyopadhyay *et al.*, 1991).

F. verticillioides was negatively related with *Alternaria* sp. and positively related with *C. lunata*. In

Table 1: Fungi isolations (%) from sorghum kernels produced under 8 environments*

Environment	Moisture condition	Soil type	<i>Fusarium verticillioides</i>	<i>Fusarium semitectum</i>	<i>Alternaria</i> sp.	<i>Dreschlera</i> sp.	<i>Aspergillus</i> sp.	<i>Phoma</i> sp.	<i>Curvularia lunata</i>	Unknown	<i>Penicillium</i> sp.	<i>Nigrospora</i> spp.
BE95	Dryland	Weesa-tche-Clareville	62.6		15.8			0.8	21.0			
CW95	Spinkler irrigation	Miller	77.3		8.6	4.8	0.2	0.8	10.5			
CW96	Spinkler irrigation	Miller	43.3		33.1	2.7	0.8	0.2	2.80			
CD96	Dryland	Miller		56	43.1	0.9						
CW97	Spinkler irrigation	Miller	48.5		43.8	3.0	0.2	1.2	2.60	0.2		
CD97	Dryland	Miller	37.6		52.2	3.0		0.4	6.60	0.2		
BE97	Dryland	Weesa-tche-Clareville	61.4		22.6	5.4		5.0	5.00	0.4		
CC97	Dryland	Victoria Clay	35.2		35.2	5.4	3.8	0.6	18.8		0.2	0.4
mean			45.8	7	31.8	3.2	0.6	1.1	8.40	0.1	0.03	0.06

*All values are means derived from assays of 600 seed per location

Table 2: Correlation coefficients for fungi association based on the total isolations per environment

	<i>Fusarium verticillioides</i>	<i>Alternaria</i> sp.	<i>Dreschlera</i> sp.	<i>Aspergillus</i> sp.	<i>Phoma sorghina</i>	<i>C. lunata</i>	Unknown
<i>Alternaria</i>	-0.77***						
<i>Dreschlera</i>	-0.22	0.41					
<i>Aspergillus</i>	-0.05	-0.07	0.32				
<i>P. sorghina</i>	0.41	-0.26	0.24	-0.19			
<i>C. lunata</i>	0.51*	-0.55*	-0.53*	-0.35	-0.06		
Unknown	0.28	-0.23	0.24	0.45	0.15	-0.18	
<i>Penicillium</i>	-0.21	0.27	0.18	0.45	-0.23	0.06	-0.6

*, **, *** Significant at 0.05, 0.01 and 0.001 probability levels, respectively

contrast, some reports state that *F. verticillioides*, is antagonistic to *C. lunata* (Singh and Agarwal, 1986; Denis and Girard, 1980). No reports about the relationship between *F. verticillioides* and *Alternaria* in sorghum kernel exist. These relations among molding fungi may be attributed to competition for substrate, production of antagonistic substances, or environmental conditions that differentially influence sorghum kernel infection by these fungi. Although, a better understanding about grain mold fungal interaction should be sought at a sample level or preferably, on an individual kernel basis i.e., different frequency of fungal molding species was recovering depending of the culture media used as a substrate (Ali *et al.*, 1991).

The influence of molding fungi on germination: Pearson product moment correlation coefficients between seed germination and molding fungi frequency are shown on Table 3. In the present study, a positive and significant correlation was found between germination and *F. verticillioides* and germination and *C. lunata*. On the contrary, there are some papers that report a negative correlation between germination and *F. verticillioides* (Denis and Girard, 1980; Mathur *et al.*, 1975). There are two possible explanations to these results. First, the *F. verticillioides* strains present during the current study

may not have reached the embryo at the time of seed harvest. Second, mycotoxins that inhibit germination may be absent in the *F. verticillioides* strains present in the Texas sorghum samples. Although *F. verticillioides* has been related to many mycotoxins like fusaric acid, fusarins, fusariocins, moniliform, T-2 toxin and zearalenone, only some of these mycotoxins (like zearalenone) have been associated with the inhibition of seed germination. Variability on zearalenone production for different *F. verticillioides* strains has been reported (Brodnik, 1975). In the present study, the zearalenone content of the *F. verticillioides* strains was not determined. However, Seitz *et al.* (1983) reported that no zearalenone was detected in sorghum grain samples harvested at College Station, TX. More biochemical studies are needed to determine if the *F. verticillioides* strains present in Texas sorghum area lack of zearalenone or other metabolite toxic to seed embryo.

In the present study, although not significant ($p>0.05$), a negative trend was observed between the frequency of *Alternaria* spp. *Dreschlera* spp. and *Aspergillus* spp. and germination (Table 3). A possible explanation for the low significance of this negative relationship in our study may be that initial kernel infection by *F. verticillioides* may serve as an important deterrent to subsequent kernel invasion by other seed

Table 3: Pearson correlation coefficients between seed germination and fungi incidence

Fungi specie	Correlation coefficient
<i>F. verticillioides</i>	0.59**
<i>C. lunata</i>	0.61***
<i>Alternaria</i> spp.	-0.25
<i>Dreschlera</i> spp.	-0.23
<i>Aspergillus</i> sp.	-0.45
Unknown	-0.23
<i>Phoma sorghina</i>	0.12
<i>Penicillium</i> spp.	0.05

, * Significant at 0.01 and 0.001 probability levels, respectively

Table 4: Average values of seed germination, Diversity index and scoring rates and correlation coefficients among these variables

Scoring rates	Seed germination	Diversity index
1	75.1	1.95
2	74.5	1.94
3	47.9	1.88
4	36.9	1.87
5	24.1	1.85

Spearman correlations:

Scoring rate vs Seed germination	-0.64***
Diversity index vs Seed germination	0.09*
Scoring rate vs Diversity index	-0.03

*, *** Significant at 0.05 and 0.001 probability levels, respectively

infecting fungi and may have a “protective effect” against infection by other pathogens that kill the embryo (Rheeder *et al.*, 1990).

Associations between grain mold rating, seed germination and fungal diversity: A Spearman rank-order correlation addressed the relationship between rank scores on diversity index, seed germination and grain mold rating for the 8 environments. The observed correlation between scoring rate and seed germination was found to be statistically significant ($r_s = -0.64$, $p < 0.001$), suggesting that as grain mold infection increase, so does seed embryo mortality (Table 4). i.e., seed germination decreased 35% when grain mold infection increased from rate 2 to rate 3.

The same correlation test compared the mean of seed germination and the molding fungi diversity index (Table 4). This test was found to be statistically significant ($r_s = 0.09$, $p < 0.05$), suggesting that as fungal species diversity increases so does seed germination. The increase of seed germination may be the indirect effect of an increase in fungal diversity. That is, as fungi diversity increases there is greater competition for limited substrate between fungi that kill the embryo and those that do not affect the embryo. A Spearman correlation test to compare the ranks of fungi diversity index with grain mold rating scores was performed. This test found not significant differences between these variables ($r_s = -0.003$, $p > 0.05$), indicating that fungi diversity index does not increase as grain molding infection does. However, a slight trend to decrease the fungi diversity as the grain mold infection increases was observed.

Relative role of molding fungi within each grain rating:

Rate 1: Frequency of 5 molding fungi was measured on a set of RIL with the seed bright and free from mold damage (Table 5). A PC analysis indicated that the first PC has an eigenvalue of 2.11 and explains 96% of the total variation in the data set this suggests that PC1 represents the equivalent of 2.1 individual variables. *C. lunata* and *Dreschlera* have the largest weights. Therefore, the first PC probably represents a latent interaction of these two fungal species. That is, kernel samples with less *Curvularia* frequency and more *Dreschlera* frequency had lower values for PC1.

Rate 2: Seed slightly discolored from moderate resistant RIL to grain mold was analyzed for molding fungi presence (Table 5). The PC analysis indicates that the first two PC's of the fungi data account for 94% of the total variance among fungi frequency mean (64 and 29% of the variance, respectively). Consequently, sample variation is summarized very well by two principal components and a reduction in the data from 8 environments on 4 fungi species to 8 environments on 2 principal components is reasonable. The first PC represents an interaction of three fungi species, almost contributing at the same level; *Fusarium* and *Curvularia* with positive and *Alternaria* with negative weights. The second factor is mainly accounting for *Dreschlera*, which was not very well represented by the first PC. This result is common because the smaller factors frequently represent instances where the process focuses on the variance of an individual variable that had relatively little variance on earlier factors.

Rate 3: Considerable discolored seed from moderately susceptible RIL was classified in this molding rate. Table 5 shows the factor pattern and summary of a principal component analysis of the grain molding fungi frequency by the rate 3. Three of the 5 PC 's have eigenvalues greater than 1.0 and are retained in the analysis. Together they account for 84% of the variance of the original variables with the 16% unexplained being largely due to random variation. The first PC reports high positive weights for *Dreschlera* and *Fusarium* and high negative weights for *Alternaria*. That is, kernel samples with less frequency of *Dreschlera* and *Fusarium* and greater frequency of *Alternaria* had lower values for PC1. The second PC has a positive weight for *Phoma* frequency and a negative weight for *Aspergillus*. It accounts for 21% of the variation almost half of the variation explained by the first PC (44%). The third factor is mainly accounting for *Dreschlera*, which was not very well represented by either of the first two factors. That is, in the scoring rate of 3, the interaction between *Dreschlera*, *Fusarium* and *Alternaria* explains most of the variation. However, the interaction between *Phoma* and *Aspergillus* is also important.

Table 5: Eigenvectors from principal component analysis of molding fungi species on food type sorghum kernels

Variable	Grain mold rate											
	Rate 1			Rate 2			Rate 3			Rate 4		
	PC1	PC1	PC2	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
<i>F. verticillioides</i>	0.08	0.567	0.33	0.44	0.04	-0.43	0.505	-0.130	0.399	0.570	0.396	-0.10
<i>Alternaria</i> spp.	-0.28	-0.55	-0.40	-0.50	0.13	0.22	-0.500	0.317	-0.190	-0.600	0.051	-0.10
<i>Dreschlera</i> sp.	-0.63	-0.32	0.76	0.49	0.16	0.55	0.181	0.581	0.097	0.070	0.528	0.46
<i>Aspergillus</i> sp.				0.35	-0.60	0.45	-0.220	0.296	0.838	-0.100	-0.020	0.72
<i>P. sorghina</i>	0.36			0.25	0.78	0.17	0.644	0.231	-0.190	0.220	-0.050	-0.40
<i>C. lunata</i>	0.62	0.526	-0.40	0.34	-0.10	-0.48	-0.050	-0.630	0.232	0.480	-0.280	0.30
Unknown												
Eigen values	2.11	2.57	1.17	2.62	1.27	1.11	3.080	1.870	0.940	2.180	1.530	1.24
% of the variance	96.00	64.00	29.00	44.00	21.00	19.00	48.000	29.000	15.000	31.000	22.000	18.00
Cum. % of the variance	96.00	64.00	94.00	44.00	65.00	84.00	48.000	76.000	91.000	31.000	53.000	71.00

Rate 4: In this rate, seed with extensive discoloration and deterioration from susceptible RIL were analyzed. The first PC explains 48% of total sample variance. The first three PC's collectively, explain 91% of the total sample variance, given the component coefficient (Table 5). The first PC appears to be essentially an interaction of *F. verticillioides*, *Phoma* and *Alternaria*. The two former fungi with positive weights and *Alternaria* with a negative weight. The second PC appears to contrast *C. lunata* with weighted *Dreschlera* frequency. The third factor is mainly accounting for *Aspergillus* spp frequency. The first two PC's have an eigenvalue of 3.08 and 1.87, respectively. This suggests that PC1 and PC2 represent the equivalent of 3 and 2 individual variables. In this scoring rate, two interactions seem to be very important, first the interaction between *Phoma*, *Fusarium* and *Alternaria* and second, the interaction between *Curvularia* and *Dreschlera*.

Rate 5: In the scoring rate 5, seed essentially dead with embryo and endosperm deteriorated was collected from very susceptible RIL. The PC analysis indicated that the first PC has an eigenvalue of 2.8 and explains 31% of the total variation in the data set (Table 5). PC2 accounts for an additional 22% of the variation and has an eigenvalue of 1.53. PC3, with an eigenvalue of 1.24, explains 18% of the variation, while PC4 accounts for 14%, with an eigenvalue of 0.99. The total amount of the variation explained by the first four principal components was 85%. The first PC may be viewed as an interaction of *Fusarium*, *Curvularia* and *Alternaria*. The two former fungi with positive weights and *Alternaria* with a negative weight. The second PC appears to be essentially a weighted average of unknown fungi, *Fusarium* and *Dreschlera*. The third PC appears to contrast *Phoma* with *Dreschlera* and *Aspergillus*.

Competition *in vivo* and *in vitro* among sorghum grain mold fungi has been reported (Singh and Agarwal, 1986). Principal component analysis allows the estimation of fungal interactions that may not be detected by univariate statistical analyses. In addition, it seems that as scoring rate increases more PC's are needed to explain the variation. As grain molding rates increase, the interaction among fungi species becomes more complex. Some of these interactions among sorghum grain molding fungi may be competition, mutualism, comensalism, parasitism, etc. Knowledge about fungal flora in different grain mold rating scores allows the identification of the most predominant fungi species, the most important interaction among those species and may help to enhance selection processes for resistant sorghum varieties. Besides, some of the grain molding fungi have been related to mycotoxin production and seed viability, understanding the fungal flora at the different rating scores allows for the prediction of the level of mycotoxins and seed health.

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