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Correlations Among Grain Mold Severity, Seed Weight, and Germination Rate of Sorghum Association Panel Lines Inoculated With *Alternaria alternata*, *Fusarium thapsinum*, and *Curvularia lunata*

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Abstract
The sorghum association panel was evaluated for grain mold severity, seed weight, and germination rate. The 377 accessions were inoculated with *Alternaria alternata* alone, a mixture of *A. alternata*, *Fusarium thapsinum*, and *Curvularia lunata*, and untreated water-sprayed control during 2010, 2013-2015 growing seasons at the Texas AgriLife Research Farm, Burleson County, Texas. Each accession was evaluated at least twice. Across accessions, Spearman's rank correlation was performed for non-parametric correlation analysis for grain mold severity, seed weight, and germination rate. There were significant negative correlations between grain mold severity with seed weight and germination rate for the individual treatment and when combined. A significant positive correlation between seed weight and germination rate was observed. The results indicated that higher grain mold severity reduces both sorghum seed weight and germination rate whether deliberately inoculated with fungal pathogens or naturally infected. It can be argued that correlations from this study were more robust due to the large number of accessions from all major sorghum races used and may represent the true association among the three parameters for this pathosystem. Thus, the use of grain mold resistant lines, resulting in sound seeds and higher germination rates is recommended.
Keywords: Sorghum accession; Fungal species; Disease resistance; Seed quality.

1. Introduction
Sorghum (*Sorghum bicolor* (L.) Moench) production continues to increase and gain in importance as a food commodity, and more recently as a potential source of biofuel [1-3]. As production expands, there is a renewed effort to minimize the impact of biotic stress such as grain mold, a disease complex associated with diverse genera of fungi, including *Fusarium thapsinum, Curvularia lunata, Alternaria alternata, F. semitectum, Phoma sorghina*, and *Colletotrichum sublineola* [4-8]. Depending on the fungi/fungus involved in the disease development, symptoms of grain mold on sorghum may appear as different discoloration of the kernels, small seed size, and presence of fungal fruiting bodies [4, 8, 9]. In addition to yield losses which can reach 100% on susceptible cultivars under conducive environmental conditions [8, 10], seed quality can be significantly compromised and is compounded by the fact that many of the fungal genera, in particular *Fusarium* spp. associated with sorghum grain mold are mycotoxigenic either in the field or in stored grains, further reducing the value of the crop for human and animal consumption [11-16]. Thus, this study was undertaken to determine the correlations among grain mold severity, seed weight, and germination rates of hundreds of sorghum accessions inoculated individually or in combination with grain mold fungi.

2. Materials and Methods
Field trial: A total of 377 accessions maintained by the USDA-ARS, Plant Genetic Resources Conservation Unit, Griffin, Georgia were selected for the study. These accessions are part of the sorghum association panel (SAP) compiled by Casa, *et al.* [17]. This SAP was evaluated for grain mold response by inoculation with *A. alternata* alone, mixture of *A. alternata*, *F. thapsinum*, and *C. lunata*, and untreated control during 2010, 2013-2015 growing seasons in a randomized complete block design at the AgriLife Research Farm, Burleson County, TX. Each accession was replicated thrice and evaluated at least in two growing seasons. Fertilizer application and other agronomic managements were followed as recommended.

Screening for grain mold: The inoculum preparation and inoculation method were as previously described by Prom, *et al.* [18]. The fungi *A. alternata*, *F. thapsinum*, and *C. lunata* were grown separately in Petri plates containing half-strength potato dextrose agar and incubated at 25˚C for 10 days. Spore suspensions were harvested by flooding the plates with sterilized water and filtered through layers of sterile cheesecloth into separate beakers and diluted with sterile water to final concentrations of 1 x 10^6 for *F. thapsinum* and 2 x 10^4 conidia/ml for *A. alternata* and *C. lunata*, respectively. Three sorghum panicles per accession/replicate/treatment were arbitrarily tagged at 50% bloom then inoculated using a hand-held spray bottle at different dates the during the growing season in June and July for the experiments. The untreated control panicles were sprayed with sterile distilled water. To promote disease development, treated and untreated control panicles were covered with paper bags for 24 h and thereafter, misted twice with sterile water a day (once in the morning and afternoon) for 7 consecutive days.

3. Data Collection

At maturity, treated and nontreated control panicles were hand harvested and threshed. The hand threshed kernels from each treatment/replicate were assessed for grain mold severity using a 1-5 scale, where 1 = no mold observed on the seeds; 2 = 1 to 9 %, 3 = 10 to 24%, 4 = 25 to 49% and 5 = 50% or more of the seeds molded [19, 20]. Seed weight was based on weight in grams of 100 randomly selected seeds from each accession/replication/treatment. Germination rate was based on the number of seeds that germinated after 7 d from 300 seeds (100seeds/replication) per experiment placed in Anchor germination paper. The data for grain mold severity, seed weight, and percent germination rate were recorded in a laboratory note book.

3.1. Statistical Analysis

Spearman's rank correlation was performed with JMP Pro 14 for non-parametric correlation analysis for grain mold severity, seed weight, and germination rate data across accessions.

Fig-1. Scatter plots showing correlations between grain mold (GM) infection score, seed weight (SW), and germination rate (GR) (Top left= *A. alternate* alone inoculation, top right= a mixture of *A. alternata*, *F. thapsinum*, and *C. lunata* inoculation, bottom left= untreated control inoculation, bottom right= sum of all three data)

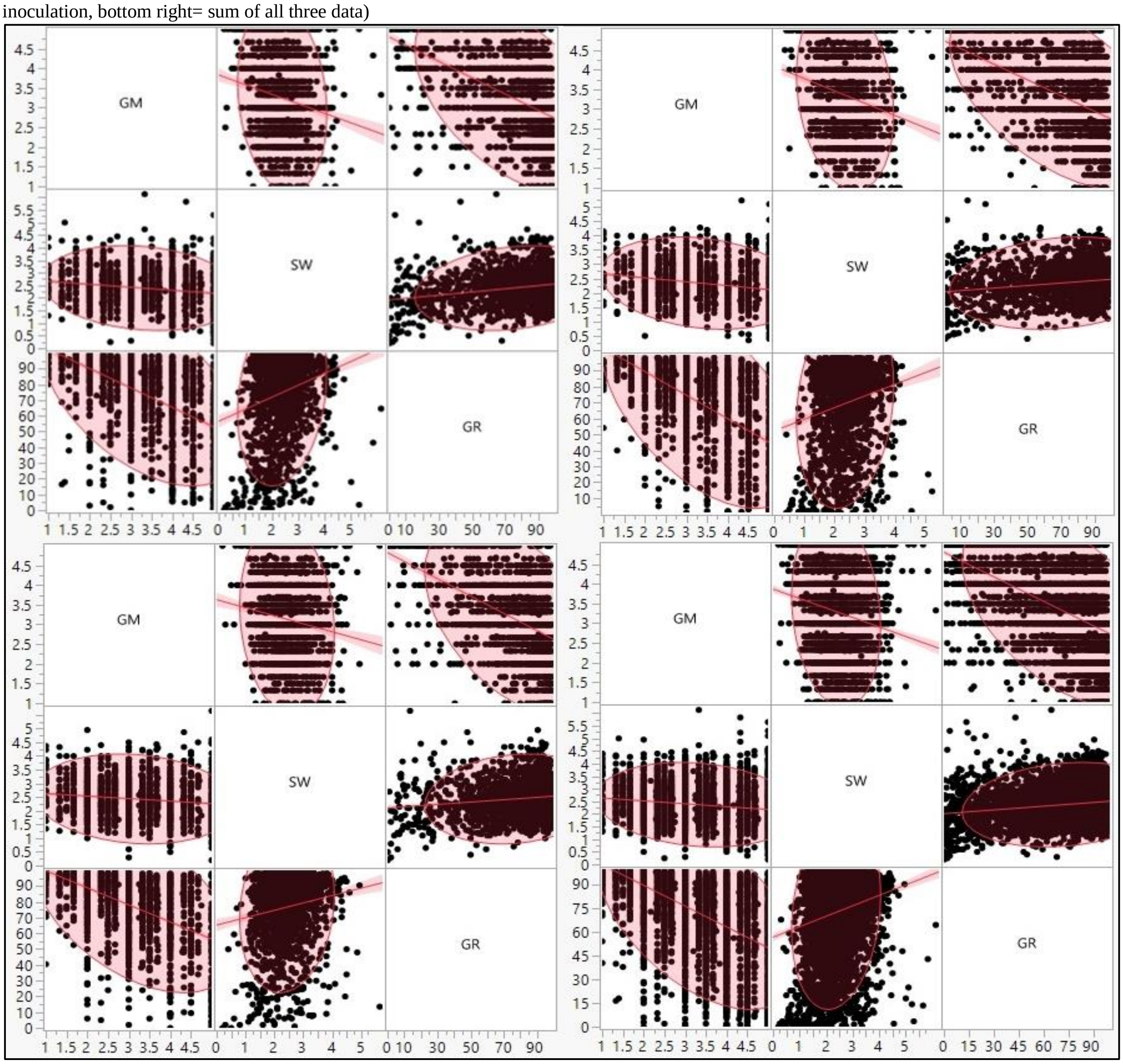

Table-1. Correlations between grain mold infection score, seed weight, and germination rate

***A. alternate* inoculation**	**Correlation**	**p-value**
SW-GM	-0.1751	<0.0001
GR-GM	-0.4974	<0.0001
GR-SW	0.1668	<0.0001
A mixture inoculation		
SW-GM	-0.2043	<0.0001
GR-GM	-0.5397	<0.0001
GR-SW	0.1393	<0.0001
Mock inoculation		
SW-GM	-0.1440	<0.0001
GR-GM	-0.4643	<0.0001
GR-SW	0.1003	<0.0001
Sum of all treatments		
SW-GM	-0.1704	<0.0001
GR-GM	-0.5159	<0.0001
GR-SW	0.1452	<0.0001

4. Results and Discussion

Grain mold is one of the most destructive diseases of sorghum. The complexity of grain mold makes management strategies a challenging endeavor because of several factors such as environmental conditions, fungal pathogen(s) involved, maturity group of the cultivar/line, shape of the panicle, limitation in visual scoring of kernels, and genotype x isolate interaction [8, 18, 19, 21, 22]. In this study, SAP was evaluated to determine the relationships among grain mold severity, seed weight, and germination rate when inoculated with grain mold fungi either individually or in combination. Figure 1 shows scatter plots of the correlations between grain mold infection score, seed weight, and germination rate. There were significant negative correlations between grain mold severity and seed weight, grain mold severity and germination rate for the individual treatments and when combined, indicating that higher the disease severity due to inoculation with *A. alternata* alone, mixture of *A. alternata*, *F. thapsinum*, and *C. lunata*, or untreated control, the lower the seed weight or germination rate (Table 1). The use of high- quality seed is essential for seedling vigor and field establishment which translates to higher yields. Seed infected grain mold fungi are usually small in size and deteriorate faster in storage and loses viability. Prom and Erpelding [23] also noted a significant negative correlation between grain mold severity and germination rate due to inoculation with *F. thapsinum*. Similarly, Garud, *et al.* [24] noted that germination was significantly reduced in the presence of *Fusarium* spp. Sorghum and foxtail seedling emergence rate was reduced when inoculated separately with individual grain mold fungi *A. alternata*, *C. lunata*, *F. moniliforme*, and *F. solani*. [25]. A significant positive correlation between seed weight and germination rate was observed (Table 1), indicating that the larger/heavier seeds, the higher germination rate. Heavier tobacco seeds were found to germinate earlier and at higher rates than light weight seeds [26]. McKersie, *et al.* [27], noted that larger seed sizes of bird's-foot trefoil improved field establishment. Similarly, Saeed and Shaukat [28] observed that large *Senna occidentalis* seeds had a higher germination rate, longer seedling roots and shoots than small seeds. Mao, *et al.* [29], noted that large seed had higher 1000-seed weight and soluble sugar concentration and exhibited higher germination index, vigor index, and seedling biomass than small seeds. In silver sagebrush, Hou and Romo [30] recorded an increased germination rate with increased seed weight up to a certain level. The higher germination rate of larger seeds is the result of larger food reserve compared to smaller seeds [31]. Amylase that enhances the hydrolysis of starch in germinating seeds has been shown to be higher during the germination of large seeds due to a higher amount of storage protein and carbohydrate [31]. However, Adebisi, *et al.* [32] noted that a soybean variety M-351 had medium 100-seed weight (11.7 g) but exhibited a higher germination rate than two other varieties with higher 100-seed weight. Our study has shown that higher disease severity reduces both sorghum seed weight and germination rate. The correlation coefficients (r) for the grain mold severity and germination was moderate ranging from -0.46 to -0.54 and lower for grain mold severity and seed weight (Table 1). Across the treatments, correlation coefficients for the seed weight and germination rate were low. In conclusion, whereas in most studies on correlations between the three parameters of grain mold resistance are usually done with small number of sorghum accessions/lines, in this study hundreds of accessions from all major races of the crop were used to determine the association among grain mold severity, seed weight, and germination. The correlations from this work were more robust and may represent the true association among the three parameters for this pathosystem.

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