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 10⁶ for *F. thapsinum* and 2 x 10⁴ 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 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 non-treated 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 (100 seeds/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.

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**Fig-1.** Scatter plots showing correlations between grain mold (GM) infection score, seed weight (SW), and germination rate (GR) (Top left= *A. alternata* 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)
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 1000 -seed weight (11.7 g) but exhibited a higher germination rate than two other varieties with higher 1000-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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| Table 1. Correlations between grain mold infection score, seed weight, and germination rate |
|---------------------------------------------|
| Grain mold fungi | Correlation | p-value  |
| A. alternata inoculation | -0.1751 | <0.0001 |
| GR-GM | -0.4974 | <0.0001 |
| GR-SW | 0.1668 | <0.0001 |
| A mixture inoculation | -0.2043 | <0.0001 |
| GR-GM | -0.5397 | <0.0001 |
| GR-SW | 0.1393 | <0.0001 |
| Mock inoculation | -0.1440 | <0.0001 |
| GR-GM | -0.4643 | <0.0001 |
| GR-SW | 0.1003 | <0.0001 |
| Sum of all treatments | -0.1704 | <0.0001 |
| GR-GM | -0.5159 | <0.0001 |
| GR-SW | 0.1452 | <0.0001 |
References

[1] Frederiksen, R. A. and Odvody, G. N., 2000. *Compendium of sorghum diseases*. St. Paul, MN, USA: The American Phytopathological Society. p. 78.

[2] Gonzalez, R., Phillips, R., Saloni, D., Jameel, H., Abt, R., Pirraglia, A., and Wright, J., 2011. "Biomass to energy in the Southern United States: Supply chain and delivered cost." *BioResources*, vol. 6, pp. 2954-2976.

[3] Kumar, A. A., Reddy, B. V. S., Sharma, H. C., Hash, C. T., Rao, P. S., Ramaiah, B., and Reddy, P. S., 2011. "Recent advances in sorghum genetic enhancement research at ICRISAT." *Amer. J. Plant Sci.*, vol. 2, pp. 589-600.

[4] Williams, R. J. and Rao, K. N., 1981. "A review of sorghum grain moulds." *J. Pest Manage.*, vol. 27, pp. 200-211.

[5] Bandyopadhyay, R. and Chandrashekar, A., 2000. "Biolog and management of sorghum grain mold." In *Proceedings of Consultative Group Meeting on Technical and Institutional Options for Sorghum Grain Mold Management*, ICRISAT, Patancheru-502 324, India, 18-19 May. pp. 2-2.

[6] Esele, J. P., Frederiksen, R. A., and Miller, F. R., 1995. "Importance of plant colour and modifier genes in grain mould resistance in sorghum." *J. E. Afr. Agric. For.*, vol. 61, pp. 31-37.

[7] Melake-Berhan, A., Butler, L., Ejeta, G., and Menkir, A., 1996. "Grain mold resistance and polyphenol accumulation in sorghum." *J. Afric. Food, Chem.*, vol. 44, pp. 2428-2434.

[8] Singh, S. D. and Bandyopadhyay, R., 2000. *Mold In: Compendium of sorghum diseases*. St. Paul, MN, USA: The American Phytopathological Society. pp. 38-40.

[9] Rao, V. T., Reddy, P. S., Thakur, R. P., and Reddy, B. V. S., 2013. "Physical kernel properties associated with grain mold resistance in sorghum Bicolor (L.) Moench." *Intern. J. Plant Breed. Genet.*, vol. 7, pp. 176-181.

[10] Navi, S. S., Bandyopadhyay, R., Reddy, R. K., Thakur, R. P., and Yang, X. B., 2005. "Effects of wetness duration and grain development stages on sorghum grain mold infection." *Plant Dis.*, vol. 86, pp. 872-878.

[11] Funnell-Harris, D. L., Prom, L. K., Sattler, S. E., and Pedersen, J. F., 2013. "Response of near-isogenic sorghum lines, differing at the P locus for plant colour, to grain mould and head smut fungi." *Ann. Appl. Biol.*, vol. 163, pp. 91-101.

[12] Isakeit, T., Prom, L. K., Wheeler, M., Puckhaber, L., and Liu, J., 2008b. "Mycotoxigenic potential of ten Fusarium species grown on sorghum and in vitro." *Plant Pathol.*, vol. 7, pp. 183-186.

[13] Leslie, J. F., Zeller, K. A., Lamprecht, S. C., Rheeder, J. P., and Marasas, W. F., 2005. "Toxicity, pathogenicity, and genetic differentiation of five species of fusarium from sorghum and millet." *Phytopathol*, vol. 95, pp. 275-83.

[14] Little, C. R., Perumal, R., Tesso, T., Prom, L. K., and Magill, C. W., 2012. "Sorghum pathology and biotechnology: A fungal disease perspective: Part I. Grain mold, head smut, and ergot." *Eur J. Plant Sci. Biotechnol.*, vol. 6, pp. 10-30.

[15] Oliveira, R. C., Davenport, K. W., Hovde, B., Silva, D., Chain, P. S. G., Correa, B., and Rodrigues, D. F., 2017. "Draft genome sequence of sorghum grain mold fungus Epicoccum sorghinum producer of tenuazonic acid." *Genome Announc.*, vol. 5, pp. e01495-16. Available: [https://doi.org/10.1128/genomeA.01495-16](https://doi.org/10.1128/genomeA.01495-16)

[16] Sashidha, R. B., Ramakrishna, Y., and Bhat, R. V., 1992. "Moulds and mycotoxins in sorghum stored in traditional containers in India." *J. Stored Products Res.*, vol. 28, pp. 257-260.

[17] Casa, A. M., Pressoir, G., Brown, P. J., Mitchell, S. E., Rooney, W. L., Tuinstra, M. R., Franks, C. D., and Kresovich, S., 2008. "Community resources and strategies for association mapping in sorghum." *Crop Sci.*, vol. 48, pp. 30-40.

[18] Prom, L. K., Waniska, R. D., Kollo, A. I., and Rooney, W. L., 2003. "Response of eight sorghum cultivars inoculated with Fusarium trichophilus, Curvularia lunata and a mixture of the two fungi." *Crop Prot.*, vol. 22, pp. 623-628.

[19] Thakur, R. P., Rao, V. P., Reddy, B. V. S., and Reddy, S. P., 2007. "Grain mold." In *Screening Techniques for Sorghum Diseases* (R.P. Thakur, B.V.S. Reddy, K. Mathur, eds.). ICRISAT, Patancheru-502 324, India, Bull. 76. p. 76.

[20] Isakeit, T., Collins, S. D., Rooney, W. L., and Prom, L. K., 2008a. "Reaction of sorghum hybrids to anthracnose, grain mold and grain weathering in Burleson County, Texas." *Plant Dis. Manage. Rep.*, vol. 2, p. FC003.

[21] Kumar, A. A., Reddy, B. V. S., Thakur, R. P., and Ramaiah, B., 2008. "Improved sorghum hybrids with grain mold resistance." *J. SAT Agric. Res.*, vol. 6, pp. 1-4.

[22] Mpofu, L. T. and McLaren, N. W., 2014. "Ergosterol concentration and variability in genotype-by-pathogen interaction for grain mold resistance in sorghum." *Planta*, vol. 240, pp. 239-250.

[23] Prom, L. K. and Erpelding, J. E., 2013. "Evaluation of sorghum accessions from Ethiopia and Mali against Fusarium trichophilus." *J. Trop. Agric.*, vol. 51, pp. 92-97.

[24] Garud, T. B., Ismail, S., and Shinde, B. M., 2000. "Effect of two mold-causing fungi on germination of sorghum seed." *Intern. Sorghum and Millet Newl.*, vol. 41, p. 54.

[25] Yago, J. I., Roh, J. H., Bae, S. D., Yoon, Y. N., Kim, H. J., and Nam, M. H., 2011. "The effect of seedborne mycoflora from sorghum and foxtail millet seeds on germination and diseases transmission." *Mycobiol.*, vol. 39, pp. 206-218.
Kasperbauer, M. J. and Sutton, T. S., 1977. "Influence of seed weight on germination, growth, and development of tobacco." *Agron. J.*, vol. 69, pp. 1000-1002.

McKersie, B. D., Tomes, D. T., and Yamamoto, S., 1981. "Effect of seed size on germination, seedling vigor, electrolyte leakage, and establishment of Bird’s- Foot Trefoil [Lotus corniculatus (L.)]." *Canadian J. Plant Sci.*, vol. 61, pp. 337-343.

Saeed, S. and Shaukat, S. S., 2000. "Effect of seed size on germination, emergence, growth, and seedling survival of Senna occidentalis Link." *Pakistan J. Biol. Sci.*, vol. 3, pp. 292-295.

Mao, P., Guo, L., Gao, Y., Qi, L., and Cao, B., 2019. "Effects of seed size and sand burial on germination and early growth of seedlings of coastal Pinus thunbergii in the Northern Shandong Peninsula, China." *Forests*, vol. 10, p. 281.

Hou, J. and Romo, J. T., 1998. "Seed weight and germination time affect growth of two shrubs." *J. Range Manage.*, vol. 51, pp. 699-703.

Santi, M. M., Chakraborty, D., and Gupta, K., 2008. "Seed size variation: influence on germination and subsequent seedling performance in Hyptis suaveolens Lamiaceae." *Res. J. Seed Sci.*, vol. 1, pp. 26-33.

Adebisi, M. A., Kehinde, T. O., Salau, A. W., Okesola, L. A., Porbeni, J. B. O., Esuruoso, A. O., and Oyekale, K. O., 2013. "Influence of different seed size fractions on seed germination, seedling emergence and seed yield characters in tropical soybean [Glycine max (L.) Merrill]." *Intern. J. Agric. Res.*, vol. 8, pp. 26-33.