Journal of Agriculture and Crops
ISSN(e): 2412-6381, ISSN(p): 2413-886X
Vol. 7, Issue. 1, pp: 14-19, 2021
URL: https://arpgweb.com/journal/journal/14
DOI: https://doi.org/10.32861/jac.71.14.19

Academic Research Publishing Group

Original Research **Open Access**

Sorghum Seed Fungal Community and Their Association with Grain Mold Severity, Seed Weight, and Germination Rate

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Article History
Received: 10 October, 2020
Revised: 15 November, 2020
Accepted: 27 November, 2020
Published: 1 December, 2020

Abstract

Grain mold, considered the most important disease of sorghum, is associated with several fungal genera. The disease reduces both yield and quality. In this study, over 300 sorghum seed samples collected from Texas, Florida, and Georgia were evaluated for grain mold severity, seed weight, germination rate, and seed fungal community. Grain mold severity of the seed samples, except for those collected from Cameron, Texas, were rated 3 or higher, indicating that these sorghum lines were moderately susceptible under naturally-infected field conditions during the 2016 and 2017 growing seasons. Seed weight across surveyed locations ranged from 1.1 g to 4.0g for samples collected in Texas during the same period. Percent germination rates for samples collected in Texas ranged from 59.6% to 86.7%. Sorghum samples collected from Florida and Georgia exhibited moderately susceptible response to grain mold infection. Mean seed weight was 1.9 g for samples collected from Florida, while in Georgia, mean seed weight was 2.3 g. Germination rate was low for samples collected from Florida and Georgia. Mycological analysis of sorghum seed samples collected from farmers' fields in Central and South Texas during the 2016 and 2017 growing seasons showed *Alternaria* species as the most frequently isolated fungal genus, accounting for 40% and 42 % in 2016 and 2017, followed by *Fusarium incarnatum*, *F. acuminatum*, *F. equiseti*, & *F. semitectum* Complex. In Florida and Georgia, *Fusarium incarnatum*, *F. acuminatum*, *F. equiseti*, & *F. semitectum* Complex was the most frequently recovered fungal species, accounting for 77% and 72% of the total. genera/species isolated from seed samples. Other fungal species, including *Curvularia lunata*, *Bipolaris* sp., *Colletotrichum sublineola*, *F. verticillioides*, *Penicillium* sp., *Aspergillus flavus*, *F. thapsinum*, *F. oxysporum*, *F. sporotrichioides*, *F. graminearum*, *F. proliferatum*, and *Aspergillus niger* were also isolated from sorghum seeds in various frequencies. In conclusion, the presence of large number of fungal genera associated with grain deterioration and their effect on other traits, makes management of this disease complex challenging. To identify grain mold resistant sources in a region, using the most dominant species in that region to screen the sorghum germplasm is recommended.

Keywords: *Sorghum bicolor*; Grain mold; Fungi; Mycoflora; Sorghum seed; Fungi.

1. Introduction

Sorghum is considered a vital cereal crop that provides the calorie intake needs of millions of people, especially in the drier tropics [1]. The production and profitability of the crop are hampered by both abiotic and biotic stresses. Among the biotic constraints on sorghum production is grain mold, a complex fungal disease considered to be the most important worldwide. Sorghum grain mold is associated with fungi in several genera, including *Fusarium thapsinum* Klittick, Leslie, Nelson *et al.*, Manasas; *Fusarium semitectum* Berk. & Ravenel; *Curvularia lunata* (Wakk.) Boedijn; *Colletotrichum sublineola* Henn ex Sacc & Trotter; *Alternaria alternata* (Fr.: Fr.) Keissl.; and *Phoma sorghina* (Sacc.) Boerema, Dorenbosch, & Van Kesteren [2-4]. Some of the fungal genera, in particular *Fusarium* species, are mycotoxigenic either during the grain development or post- harvest during storage [5-9]. Severity of this pathosystem is most pronounced in areas where wet conditions occur later in the growing season and if mature grains are not harvested on time [3, 10]. Manifestation of the disease may range from seed discoloration to smaller seed size [4, 11, 12]. Moreover, losses in grain yield on highly susceptible sorghum lines can reach 100 % [13]. *Fusarium thapsinum*, *F. nygamy* and *C. lunata* are considered the most important contributors to grain molding

fungi [3, 4, 14]. However, the frequency and recovery of the grain molding fungi vary from location to location, and in some sorghum production areas, the frequency of isolation of these fungal species is either low or non-existent [15-18]. Thus, this study reports the grain mold severity, seed weight, germination rate, and seed fungal community of sorghum samples collected from Texas, Georgia, and Florida.

2. Materials and Methods

A total of 165 and 109 hybrid sorghum seed samples were collected from farmers' fields in Central and South Texas during the 2016 and 2017 growing seasons, respectively. These samples were obtained from 6 counties in 2016 and 9 counties in 2017, using a X-shaped pattern. Additionally, 19 samples from the state of Florida and 10 samples from Georgia were collected in 2017. In each sorghum field, three panicles were arbitrarily collected using a diagonal pattern. Panicles were threshed and the seed samples were put in separate paper bags, carried to the laboratory and stored at 7°C until mycological analysis.

Grain mold severity was assessed on the naturally-infected threshed seeds. Severity was based on a scale of 1 to 5 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 [10, 19].

The protocol for determining sorghum seed mycoflora and percentage germination rates was previously described by Prom, *et al.* [20] and Prom [21]. Briefly, seed mycoflora was determined for 50 surfaced-disinfected seeds per sample. Seeds placed in vials were put in a small beaker containing 10% NaCl for 1 min., then rinsed three times with sterilized water and dried under a laminar flow hood. Ten seeds were plated on each Petri dish containing half-strength potato dextrose agar and incubated at 25±2°C for 5-7 days. Identification of fungal species under microscope was based on the conidia, conidiophores, colony morphology, and color, according to descriptions provided by Booth [22], Nelson, *et al.* [23], and Barnett and Hunter [24]. The FIESC (i.e., *Fusarium incarnatum, F. acuminatum, F. equiseti, & F. semitectum* Complex) was identified based on TEF-1-alpha primers and checking on the NCBI database for confirmation.

2.1. Data Collection

Seed weight in grams was based on weight of 100 randomly selected seeds per sample. Germination rates were obtained by placing 100 randomly selected seeds on Anchor seed germination paper (Anchor Paper CO, St. Paul, MN) and evaluating the number of seeds that germinated in 7 days.

2.2. Statistical Analysis

Data for the mycoflora, grain mold severity, seed weight, percent germination rates were analyzed using the command PROC MEANS and PROC GLM (SAS Institute, SAS version 9.4, Cary, NC). Differences in means for grain mold severity, seed weight, and percent germination rates among counties were determined at the 5% probability level based on pairwise comparisons of least-square means with *t*-tests. Grain mold severity, seed weight, percent germination rates of the samples collected from Florida and Georgia were analyzed using the command PROC UNIVARIATE.

3. Results

3.1. Mycoflora Analysis

Mycological analysis was performed on 165 and 109 sorghum seed samples collected from farmers' fields in Central and South Texas during the 2016 and 2017 growing seasons. *Alternaria* species was the most frequently isolated fungal genus from the samples, accounting for 40% and 42 % in 2016 and 2017, respectively (Fig. 1). In 2016, *Alternaria* spp., followed by *Fusarium incarnatum, F. acuminatum, F. equiseti, & F. semitectum* Complex (FIESC) (27.8%) and *C. lunata* (13.7%) were the most recovered species. Other fungal genera and species recovered from seed samples included *Bipolaris* sp., *C. sublineola, F. verticillioides, Penicillium* sp., and *Aspergillus flavus*. *Fusarium thapsinum, F. oxysporum, F. sporotrichioides,* and *A. niger* were also isolated but in trace amounts. During the 2017 survey, FIESC (32.9%) also was the second most frequently recovered fungal species, followed by *Bipolaris* sp. (13.2%), other unidentified fungal species (4.7%) and *C. lunata* (4.6%). Other fungi isolated from seed samples in trace amounts were *F. graminearum, F. thapsinum, F. sporotrichioides, F. verticillioides, Aspergillus* sp., and *A. niger.* However, *Colletotrichum sublineola, F. proliferatum, Penicillium* sp., *Aspergillus flavus*, and *F. oxysporum* were not detected in sorghum seeds collected in 2017. In both years, trace amounts of bacteria were detected.

In Florida and Georgia, FIESC was the most frequently recovered fungal species, accounting for 77% and 72.4% of the total genera/species isolated from seed samples (Fig. 2). In both states, *C. lunata* (7.2% and 8.4%) was the second most isolated species. Other fungal species isolated were *F. oxysporum, Alternaria* sp., and *Bipolaris* sp. *F. thapsinum, F. verticillioides, F. sporotrichioides,* and *A. niger.* Trace amounts of bacteria were also isolated on seed samples from Florida and Georgia, while fungi recovered on 6.1% of the seed samples from Florida and 3.4% from Georgia were not classified.

Figure-1. CS= *Colletotrichum sublineola*; CL=*Curvularia lunata*; FIESC=*Fusarium incarnatum, F. acuminatum, F. equiseti, and F. semitectum* Complex; FO=*F. oxysporum*; FP=*F. proliferatum*; FG=*F. graminearum*; FV=*F. verticillioides*; FT=*F. thapsinum*; FSPOR= *F. sporotrichioides*; FSP=*Fusarium* species; ASPN=*Aspergillus niger*; ASPFL=*A. flavus;* ASPSP=*Aspergillus* species; ALT=*Alternaria* species; BIP=*Bipolaris* species; PEN=*Penicillium* species; and OTHER=unknown fungal species i.e., not identified

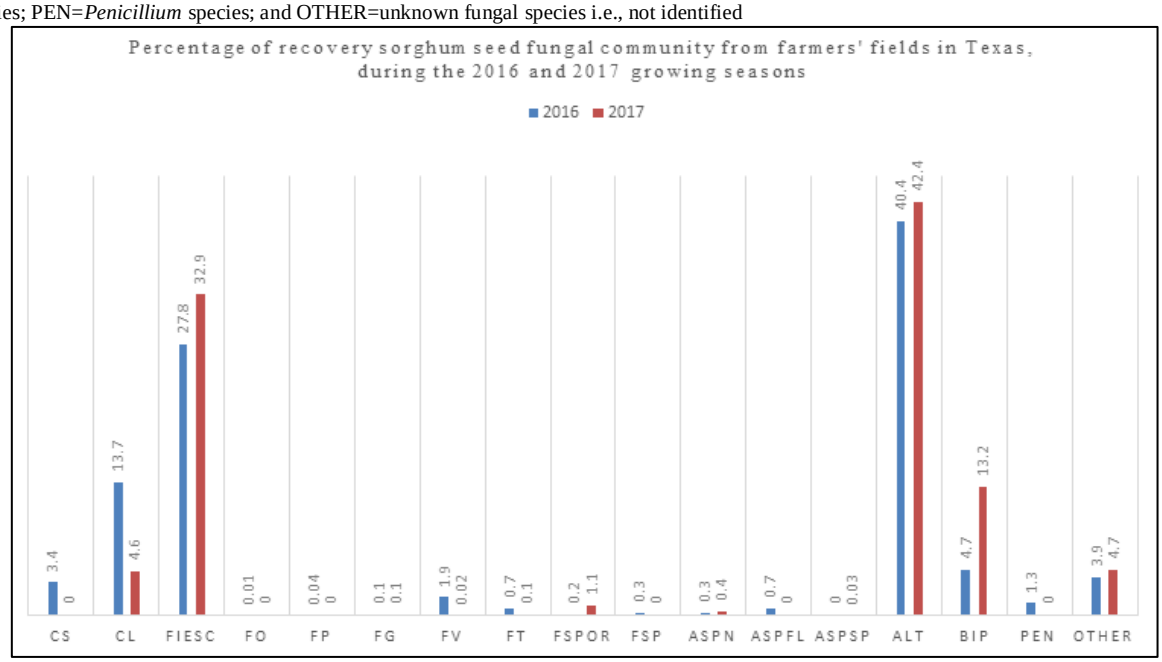
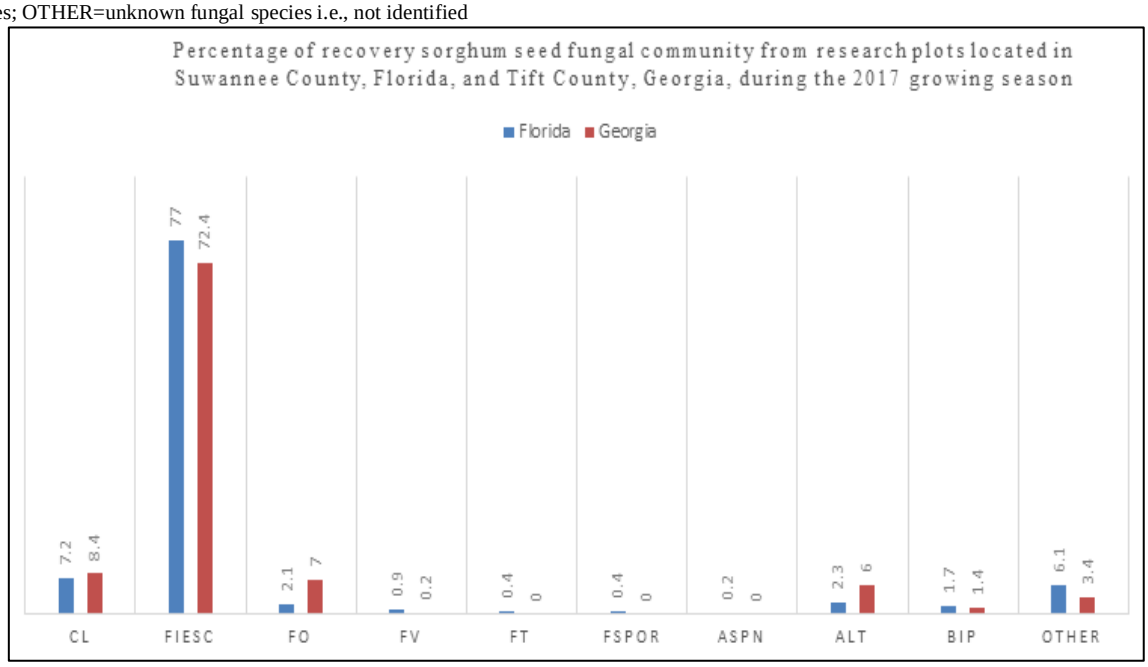


Figure-2. CL=*Curvularia lunata*; FIESC=*Fusarium incarnatum, F. acuminatum, F. equiseti, and F. semitectum Complex*; FO=*F. oxysporum*; FV=*F. verticillioides*; FT=*F. thapsinum*; FSPOR= *F. sporotrichioides*; ASPN=*Aspergillus niger*; ALT=*Alternaria* species; BIP=*Bipolaris* species; OTHER=unknown fungal species i.e., not identified



3.2. Disease Assessment, Seed Weight, Germination Rate

The main effect of Texas county for grain mold severity (*P*=0.0107) was highly significant and significant for seed weight (*P*=0.0313) and germination rate (*P*=0.0376) in 2016. In 2017, grain mold severity and seed weight were highly significant (*P*=0.0001) and significant for percent seed germination rate (*P*=0.0318), indicating differences in response among the counties for the measured traits (Table 1). In 2016, Willacy and Burleson Counties recorded the lowest mean grain mold severities 3.0 and 3.1, respectively. Hidalgo County recorded the highest mean grain mold severity of 3.8 (Table 1). Mean seed weight was highest on samples collected from farmers' fields in Nueces County (4.0 g) and this was significantly higher than the level recorded from Willacy County. Seed samples from Willacy County exhibited the highest germination rate (82.3%), followed by samples from Burleson and Kleberg Counties. In 2017, mean grain mold severity of samples from Cameron County (2.4) was significantly lower than mean levels obtained from the rest of the counties surveyed (Table 1). Similarly, samples from Cameron County recorded the highest mean seed weight (3.2 g); whereas, samples from Jim Wells County exhibited the lowest (2.2 g). Mean percent seed germination rate was highest on samples collected from Jim Wells County (86.7%) and this amount was significantly higher than samples collected from Hidalgo County (59.6%) which was the lowest rate among the counties surveyed in 2017.

Table-1. Mean grain mold severity, seed weight, and germination rate of sorghum samples collected from farmers' fields location in a number of Counties in Central and South Texas, during the 2016 and 2017 growing seasons

County	2016			2017		
	GM[1]	Seed WT[2]	Germ[3]	GM	Seed WT	Germ
Burleson	3.1b[4]	3.6ab	78.0a	3.6bc	2.8ab	76.9ab
Cameron	-[5]	-	-	2.4d	3.2a	78.8a
Hidalgo	3.8a	2.2bc	72.3ab	3.4bc	2.4cd	59.6b
Jim Wells	-	-	-	3.2c	2.2d	86.7a
Kleberg	3.6ab	2.2abc	76.2ab	3.4bc	2.8ab	79.8a
Nueces	3.3b	4.0a	71.4b	3.2c	2.8ab	77.5a
Refugio	3.7ab	2.2abc	63.7b	4.7a	2.8ab	76.9ab
San Patricio	-	-	-	3.4bc	3.1ab	78.5a
Willacy	3.0b	1.1c	82.3a	4.0ab	2.6.bcd	74.7ab

[1]GM=grain mold severity based on a scale of 1 to 5 where, 1 = no mold observed on the panicle; 2 = 1 to 9 %, 3 = 10 to 24%, 4 = 25 to 49% and 5 = 50% or more of the panicle molded [10, 19]. Grain mold severity was assessed on naturally-infected threshed seeds.
[2]Seed WT=seed weight (100 seeds in grams). Main effect of seed weight for 2016 was non-significant.
[3]Germ=percent germination rates based on the number seeds that germinated after one week on a blotter paper.
[4]Means within a column with the same letter(s) are not significantly different at the 5% probability level based on pairwise comparisons of least-square means with *t*-tests.
[5]Counties not surveyed in 2016.

Table 2 shows the descriptive statistics of the data collected from samples obtained in Florida and Georgia. Mean grain mold severity of 3.4 was recorded for the seed samples collected from both locations. The percent seed germination rate was low, 30.7% and 38% from Florida and Georgia, respectively. While mean seed weight was 1.9 g for samples collected from Florida, and 2.3 g for those from Georgia.

Table-2. Mean grain mold severity, seed weight, and germination rate of sorghum samples collected from Florida and Georgia in 2017 growing seasons

	Florida			Georgia		
	N	Mean	SD	N	Mean	SD
GM[1]	19	3.4	0.68	10	3.4	0.70
Seedwt[2]	19	1.9	0.64	10	2.3	0.50
Germ[3]	19	30.7	26.2	10	38.8	20.6

[1]GM=grain mold severity based on a scale of 1 to 5 where, 1 = no mold observed on the panicle; 2 = 1 to 9 %, 3 = 10 to 24%, 4 = 25 to 49% and 5 = 50% or more of the panicle molded [10, 19]. Grain mold severity was assessed on naturally-infected threshed seeds.
[2]Seed WT=seed weight (100 seeds in grams). Main effect of seed weight for 2016 was non-significant.
[3]Germ=percent germination rates based on the number seeds that germinated after one week on a blotter paper.

4. Discussion

Globally, grain mold is considered the most important sorghum disease [18]. The fact that large number of fungal genera are associated with this pathosystem makes management challenging [3, 4, 11, 18, 21]. In this study, sorghum samples collected from Texas, Georgia, and Florida were evaluated for disease severity, seed weight, germination rate, and seed fungal community. Grain mold severity of the seed samples, except for those collected from Cameron, Texas were rated 3 or higher, indicating that the sorghum lines being grown were moderately susceptible under naturally-infected field conditions. Over the years, grain mold resistance studies conducted either under naturally-infected fields or inoculated with individual fungal species or in combination have yielded sorghum lines with high levels of resistance [10, 25-29]. Seed weight across surveyed locations ranged from 1.1 g to 4.0g for samples collected in Willacy and Nueces Counties, Texas, while germination rate ranged from 59.6% to 86.7%. The mean seed weight and germination rate of samples from Texas were higher than those from Florida and Georgia. Seed weight and germination rate are factors to consider when evaluating sorghum germplasm response to grain mold. Mycological analysis of sorghum seed samples collected from farmers' fields in Central and South Texas, showed *Alternaria* species as the most frequently isolated fungal genus. Similarly, Prom, *et al.* [18], noted that *Alternaria* spp. was the dominant fungal species isolated from sorghum seeds across several counties in South Texas. Also, *Alternaria* spp. and *F. semitectum* were the most frequently recovered fungi on naturally infected sorghum seeds collected from Burleson County, Texas [21]. Turgay and Ünal [30], also found that *Alternaria alternata* was the most frequently recovered fungal species on sorghum seeds collected from different locations in Turkey. Also, Naqvi, *et al.* [31], isolated *Alternaria* spp. on all sorghum seed samples collected from 14 locations in Eritrea, North East Africa. Unlike the Texas samples in this study, *F. incarnatum, F. acuminatum, F. equiseti, & F. semitectum* Complex (FIESC) was the most frequently recovered fungal species from Florida and Georgia seeds. Erpelding and Prom [32], also noted that *F. semitectum* was the dominant fungal species infecting or contaminating sorghum seeds collected from Isabela, Puerto Rico, during the 2002 and 2003 growing seasons. In the present study other fungal species, including *C. lunata*, *Bipolaris* sp., *C. sublineola*, *F. verticillioides*, *Penicillium* sp., *Aspergillus flavus*, *F.thapsinum*, *F. oxysporum*, *F. sporotrichioides*, and *A. niger* were also isolated. Fungal community of sorghum seeds collected from Northwestern Sierra Leone, West Africa, revealed *Phoma sorghina* as the most frequently isolated fungus, followed by *F. verticillioides*, and *Bipolaris bicolor*, but *C. lunata* was among the least recovered fungal species [16]. However, sorghum samples collected from six regions in Egypt, showed *Aspergillus*

niger, followed by *A. flavus*, *Alternaria* spp., and *Fusarium* spp. as the most frequently isolated fungi infecting or contaminating the seed [17].

Overall, similar fungal communities on sorghum seeds collected from Texas, Florida, and Georgia were noted in the study. The climatic classification of the collection sites in these states is similar according to Köppen Classification Cfa i.e., warm temperate, fully humid, and hot summer [33, 34].

5. Conclusion

In conclusion, the presence of a large number of fungi associated with grain deterioration and their effect on other traits makes management of this disease complex very challenging. Future research in grain mold studies would require planting sorghum lines in multiple geographic locations to identify stable resistant lines. However, in specific regions, exposing the lines to a mixture of the predominant fungal species will be more practical and beneficial in identifying the most stable grain mold resistant sources. Recommendation is when screening sorghum germplasm for resistance to grain mold in a particular region, one should use the dominant fungal species either contaminating or infecting sorghum grain in that region.

Acknowledgement

This research (CRIS # 3091-22000-034-00D) was supported by the U. S. Department of Agricultural Research Service.

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