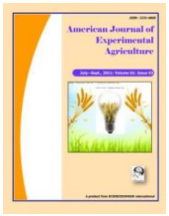

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Mycoflora Analysis of Hybrid Sorghum Grain Collected from Different Locations in South Texas

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Authors' contributions

This work was carried out in collaboration between all authors. Author LKP prom conceived and designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Authors RP, ZJ and GR performed the mycoflora analysis. Authors TI and CM assisted in the collection of samples and reviewed the experimental design and all drafts of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Mycoflora characterization of sorghum grain collected from different locations in Texas during the 2008 and 2009 growing seasons revealed *Alternaria* spp. as the dominant genus. At the beginning of grain development to soft dough stage, *Alternaria* spp. accounted for 66% of the recovered fungal species in 2008 and 55.8% in 2009. At grain maturity in 2009, *Alternaria* spp. represented 66.8% of the recovered fungal species across locations, followed by *Bipolaris* spp. (12.8%), *C.*

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lunata (6.8%) and other *Fusarium* spp. (6.8%). Other fungal taxa recovered from sorghum grain included *F. semitectum*, *F. thapsinum*, *Aspergillus* spp. *Phoma* spp. *Colletotrichum* spp. and *Rhizopus* spp. In conclusion, *Alternaria* spp. was the most dominant fungal genus recovered from sorghum grain collected from different locations in southern Texas. However, there is little or no information on the reaction of sorghum hybrids in the region against *Alternaria* spp.

Keywords: Sorghum grain mold; Sorghum bicolor; fungal pathogens; mycoflora analysis.

1. INTRODUCTION

One of the major obstacles to sorghum [*Sorghum bicolor* (L.) Moench.] productivity and seed quality is grain mold, a complex fungal disease. The disease is most prevalent and severe in areas with moist conditions later in the growing season [1,2]. Numerous fungal taxa, including *Fusarium thapsinum* Klittick, Leslie, Nelson et al. Manasas; *Fusarium semitectum* Berk. & Ravenel; *Curvularia lunata* (Wakk.) Boedijn; *Colletotrichum graminicola* (Ces.) G. W. Wilson; *Alternaria alternata* (Fr.:Fr.) Keissl and *Phoma sorghina* (Sacc.) Boerema, Dorenbosch & Van Kesteren have been shown to be involved with the disease complex [1,3,4]. Generally, *F. thapsinum*, *F. nygamy* and *C. lunata* are considered the most common grain molding fungi [1,3,5]. Pathogen infection can occur during flowering to grain harvest [3,6]. Grain mold symptoms include small or deteriorated seed, with discoloration ranging from pink to reddish-pink or black to grayish-black [3,6]. The establishment of excellent plant stand in the field requires high quality seed that produce vigorous seedlings. Seeds infected with grain mold fungi exhibit poor germination and seedling vigor [6-10]. Diseased grain may also not be suitable for food or feed since some of the infecting fungi have the capacity to produce mycotoxins [11,12].

In this study, we evaluated the frequency and diversity of fungal genera and species infecting or contaminating sorghum grain collected from several counties in southern Texas.

2. MATERIALS AND METHODS

Hybrid sorghum seeds were collected from a number of commercial fields in south Texas during the 2008 and 2009 growing seasons. In 2009, samples were collected when plants were at the beginning of grain development to soft dough stage (June 4) and at maturity (July 10). The sampled fields were located in Bee, Goliad, Hidalgo, Kleberg, Nueces, Refugio and Willacy Counties. In each County, four fields were sampled using a W-shaped pattern. Five to 10 panicles were collected arbitrarily from each location. The panicles from each location were threshed and grains bulked. The mycoflora analysis used in this study was previously described by Prom [10]. One hundred and fifty randomly selected seeds from each location were placed in vials and soaked in tap water and then immersed in 10% Clorox bleach (NaOCl) for 1 minute, rinsed three times in distilled water and dried under a laminar flow hood. The seeds were plated on half-strength potato dextrose agar and incubated at 25ºC with a 12 hours photoperiod for 7 days. Identification of fungal genera and species was based on the conidia, conidiophores, colony morphology and color and, in the case of *Fusarium* spp. the descriptions provided by Booth [13] and Nelson et al. [14].

PROC GLIMMIX (SAS version 9.3, SAS Institute, Cary, NC) was used to determine the main effect of fungal species using the beta distribution as the conditional distribution and a logit link function. To include extreme values (0, 1) into the analysis, the constant 0.001 was added to the lowest value and subtracted from the highest before performing the analysis. Least-squares means for the fungal species were compared using the Tukey-Kramer *P*-value (0.05) adjustment for multiple comparisons.

3. RESULTS AND DISCUSSION

In both years, the main effect of fungal species was highly significant (*P<0.0001*). In 2008, *Alternaria* spp. (66%) were the most frequently isolated fungal genus across locations followed by *Bipolaris* spp. (17%) and other *Fusarium* spp. (11.3%) (Table 1). *Curvularia lunata* accounted for 2.4%, *F. semitectum* 1.2% and *F. thapsinum* 0.5% of the recovered fungal species. *Aspergillus* spp. and *Colletotrichum* spp. were also isolated but with much lower frequency. Castor and Frederiksen [15] also reported that *Alternaria* spp. were the most frequently isolated fungal species in naturally infected sorghum grains collected in Texas followed by

F. semitectum, *C. lunata* and *C. protuberate*. Similarly, Prom [10] showed that *Alternaria* spp. and *F. semitectum* were the most frequently recovered fungal species from naturally-infected seeds of sorghum grown in Burleson County in central Texas. In Isabela, Puerto Rico, *F. semitectum* was the most frequently isolated fungal species from seeds naturally infected with grain mold, followed by other *Fusarium* spp. *C. lunata* and *Bipolaris* spp. *Alternaria* spp. and *Colletotrchum graminicola* were also isolated, but at lower frequencies [16]. Sorghum grain collected from Santa Fe Province in Argentina had *Fusarium* spp. (*F. moniliforme*, *F. subglutinans*, *F. chlamydosporum* and *F. semitectum*), *A. alternata*, *C. lunata* and *Bipolaris cynodontis*as the most frequently recovered fungal species [11]. Mycoflora analysis of sorghum grain collected from different locations in West and Central Africa revealed *Phoma* spp. and *Fusarium* spp. as the dominant fungal taxa followed by *Curvularia* spp. [17].

In 2009, sorghum grains were collected from the same fields at the beginning of grain development to soft dough stage and also at maturity (Tables 2 and 3). Mycoflora analysis of sorghum grain conducted at beginning of grain development to soft dough stage across locations revealed *Alternaria* spp. with a mean frequency of 55.8% as the dominant genus, followed by non-colonized or clean seed, 22.5%, and *Bipolaris* spp. 14.3%. *C. lunata* and other *Fusarium* spp. accounted for 3.3% and 2.3%, respectively (Table 2). Other fungal genera and species, *F. thapsinum*, *F. semitectum*, *Aspergillus* spp. and *Phoma* spp. were isolated at lower frequencies.

At harvest, *Alternaria* spp. accounted for 66.8% of the recovered fungal genera and species across locations, followed by *Bipolaris* spp. 12.8%, *C. lunata* 6.8% and other *Fusarium* spp. 6.8% (Table 3). Other fungal genera isolated in low frequencies included *Rhizopus* spp. and *Phoma* spp.

Table 1. Frequency of recovery (%) of various fungal genera and species isolated from sorghum grain collected from fifteen locations in southern Texas, 2008[1] fungal species

County	Alt[2]	Bipo	Coll	Cur	FT	FS	Fus	Unkn	Asper
Bee	60	12	2	10	0	0	8	6	2
Bee	74	14	0	0	0	0	10	0	0
Refugio	64	20	0	8	0	0	8	0	0
Refugio	78	16	0	0	0	0	6	0	0
San Patricio	60	28	0	0	0	2	6	4	0
San Patricio	42	24	0	0	0	0	34	0	0
Nueces	64	18	0	0	0	4	8	6	0
Nueces	84	4	0	0	4	0	6	2	0
Kleberg	82	4	0	2	0	0	12	0	0
Kleberg	38	48	0	0	0	4	8	2	0
Willacy	80	20	0	0	0	0	4	0	0
Willacy	74	4	0	0	0	0	14	6	0
Goliad	90	2	0	6	0	0	20	2	0
Hidalgo	46	24	0	0	0	0	22	0	0
Hidalgo	60	16	0	10	4	8	4	0	0
Overall[3] mean	66a	17b	0.1c	2.4c	0.5c	1.2c	11.3b	1.7c	0.1c

[1]Sorghum grains were collected at harvest. one hundred and fifty seeds from each location were analyzed.
[2]Alt=Alternaria species, bipo=Bipolaris species, coll=Colletotrichum species, cur=C. lunata, FT=Fusarium thapsinum, FS=Fusarium semitectum, Fus=other Fusarium species, unkn=unknown, asper=Aspergillus species, Ph=Phoma species, Cln=clean seed (not colonized).
[3]Means within a row followed by the same letter(s) are not significantly different at the 5% probably level based on tukey-kramertest for multiple comparisons

Table 2. Frequency of recovery (%) of fungal genera and species isolated from sorghum grain collected from several locations in southern texas, 2009[1]fungal species

County	Alt[2]	Bipo	Coll	Cur	FT	FS	Fus	Unkn	Asper	Ph	Cln
Bee	24	2	0	16	0	0	2	4	0	10	42
Refugio	68	20	0	4	0	0	8	0	0	0	0
Hidalgo	88	10	0	0	0	0	0	0	0	0	0
Hidalgo	74	0	2	2	2	2	4	4	4	4	20
San patricio	60	12	0	0	0	0	2	0	0	0	26
Nueces	6	2	0	0	0	0	0	0	0	0	92
Willacy	46	50	0	4	0	0	0	0	0	0	0
Kleberg	80	18	0	0	0	0	2	0	0	0	0
overall[3]mean	55.8a	14.3b	0.3b	3.3b	0.3b	0.3b	2.3b	1.0b	0.5b	1.8b	22.5b

[1]Sorghum grains were collected at the beginning of kernel development to soft dough stage, June 4. One hundred and fifty seeds from each location were analyzed. [2]Alt=Alternaria species, bipo=Bipolaris species, coll=Colletotrichum species, cur=C. Lunata, FT=Fusarium thapsinum, FS=Fusarium semitectum, Fus=other Fusarium species, Unkn=unknown, Asper=Aspergillus species, Ph=Phoma species, Cln=clean seed (not contaminated). [3]Means within a row followed by the same letter(s) are not significantly different at the 5% probably level based on tukey-kramertest for multiple comparisons

Table 3. Frequency of recovery (%) of various fungal genera and species isolated from sorghum grain collected from several locations in texas, 2009[1] Fungal species

County	Alt	Bipo	Cur	FS	Fus	Unkn	Ph	Cln	Rhz
Bee	56	6	2	0	4	4	4	12	12
Refugio	58	18	20	2	2	0	0	0	0
Hidalgo	72	8	6	8	6	0	0	0	0
Hidalgo	62	22	6	2	8	0	0	0	0
San patricio	62	16	6	4	14	0	0	0	0
Nueces	78	10	0	6	6	0	0	0	0
Willacy	66	16	6	4	8	0	0	0	0
Kleberg	80	6	8	0	6	0	0	0	0
Overall[3]mean	66.8a	12.8b	6.8b	3.3b	6.8b	0.5b	0.5b	1.5b	1.5b

[1]Sorghum grains were collected at maturity, July 10. one hundred and fifty seeds from each location were analyzed.[2]Alt=Alternaria species, bipo=Bipolaris species, Cur=C. Lunata, FS=Fusarium semitectum, Fus=other Fusarium species, Unkn=unknown, Ph=Phoma species, Cln=clean seed (not contaminated), Rhz=Rhizopus species.[3]Means within a row followed by the same letter(s) are not significantly different at the 5% probably level based on tukey-kramer test for multiple comparisons

The recovery of *Alternaria* spp. in Bee, Nueces and Willacy counties, *C. lunata* in six of the seven counties*, F. semitectum* in Hidalgo and Nueces counties and other *Fusarium* spp. in five of the seven counties increased at harvest, whereas the percentage of non-colonized or clean seeds and, to a lesser degree, *Bipolaris* spp. and *Phoma* spp. decreased. Generally, this indicates that some fungal species that are present early on the developing grain tended to be the dominant species in the grain mycoflora later in the season. The frequency of isolation of different fungal species from sorghum grain collected in a number of locations in South Korea showed *A. alternata*, *A. flavus*, *C. lunata*, *F. moniliforme* and *Phoma* sp. as the dominant species [18], while, *F. thapsinum*, *F. nygamy* and *C. lunata* are the most commonly-isolated grain mold fungi at other locations [1,3,5]. However, this study and others have shown, *Alternaria* spp. *F. semitectum* and other fungal species are usually the dominant species associated with naturally- infected grain [10,11,15,16,18]. Table 4 shows the weather data for the 2008 and 2009 growing seasons. In 2008, mean temperature for the months of June and July ranged from 28ºC to 38ºC and mean precipitation ranged from 1.6 mm to 6.4 mm across locations, while in 2009, mean temperature for the same months ranged from 32ºC to 37ºC and mean precipitation ranged from 0.03 mm to 0.94 mm The lack of rain and high temperatures during the 2008 and 2009 growing seasons were not conducive for grain mold development in the regions where the sorghum grain was collected.

Table 4. Weather data for the 2008 and 2009[1]

County	2008		2009	
	MaxT (°C)[2]	Prec (mm)[3]	MaxT (°C)	Prec (mm)
Bee	34.4	2.03	37.0	0.64
Goliad	34.8	2.7	36.5	0.64
Hidalgo	34.1	6.4	36.4	0.38
Kleberg	28.0	1.8	32.0	0.03
Nueces	33.1	3.8	35.6	0.05
Refugio	32.6	1.6	33.7	0.04
San Patricio	33.4	2.8	36.0	0.94
Willacy	34.1	5.2	36.6	0.25

[1]*Weather data for juneand July (plant development stages from flowering to maturity).*
[2]*MaxT (°C) =mean maximum temperature in celsius.*
[3]*Prec (mm)=mean precipitation in millimeters*

4. CONCLUSION

In conclusion, *Alternaria* spp. was the most dominant fungal genus recovered from sorghum grain collected from different locations in southern Texas in 2008 and 2009. However, there is little or no information on the reaction of sorghum germplasm against this fungal species.

DISCLAIMER

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendations or endorsement by the U.S. Department of Agriculture

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bandyopadhyay R, Chandrashekar A. Biology and management of sorghum grain mold in: Proceedings of Consultative Group Meeting on Technical and Institutional Options for Sorghum Grain Mold Management. 18-19 May. Chandrashekar A, Bandyopadhyay R, Hall AJ, eds. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India; 2000.
2. Garud TB, Sawant LV, Ismail S. Development of grain mold in sorghum-pigeonpea intercropping systems. Int. Sorghum Millets Newsl. 2000b;41:55.
3. Singh SD, Bandyopadhyay R. Grain mold. Pages 38-40 in: Compendium of Sorghum Diseases. Frederiksen RA, Odvody GN, eds. The American Phytopathological Society. St. Paul, MN, USA; 2000.
4. Esele JP, Frederiksen RA, Miller FR. Importance of plant colour and modifier genes in grain mould resistance in sorghum. J. E. Afr. Agric. For. 1995;61:31-37.
5. Forbes GA, Bandyopadhyay R, Garcia G. A review of sorghum grain mold. Pages 253-264 in: Sorghum and Millets Diseases: A Second World Review. De Milliano WAJ, Frederiksen RA, Bengston GD, eds. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India; 1992.
6. Castor LL. Grain mold histopathology, damage assessment and resistance screening within *Sorghum bicolor* (L.) Moench lines. Ph. D. Dissertation Texas A. & M. Univ. College Station, TX; 1981.
7. Hepperly PR, Feliciano C, Sotomayor A. Chemical control of seedborne fungi of sorghum and their association with seed quality and germination in Puerto Rico. Plant Dis. 1982;66:902-904.
8. Garud TB, Ismail S, Shinde BM. Effect of two mold-causing fungi on germination of sorghum seed. Int. Sorghum Millets Newsl. 2000a;41:54.
9. Girish AG, Deepti S, Rao VP, Thakur RP. Detection of seedborne grain mold fungi in sorghum and their control with fungicidal seed treatment. Int. Sorghum Millets Newsl. 2004;45:31-33.
10. Prom LK. The effects of *Fusarium thapsinum* and *Curvularia lunata* and their combination on sorghum germination and seed mycoflora. Journal of New Seed. 2004;6:39-49.

11. Saubois A, Piontelli Laforet E, Nepote MC, Wagner ML. Mycological evaluation of a sorghum grain of Argentina, with emphasis on the characterization of *Fusarium* species. Food Microbiol. 1999;16:435-445.
12. Isakeit T, Prom LK, Wheeler M, Puckhaber LS, Liu J. Mycotoxigenic potential of ten Fusarium species grown on sorghum and in vitro. Plant Pathol. J. 2008;7:183-186.
13. Booth C. The Genus *Fusarium*. Commonwealth Mycological Institute. Surrey, England; 1971.
14. Nelson PE, Toussoun TA, Marasas WFO. Fusarium species: An Illustrated Manual for Identification. Pennsylvania State University Press, University Park, PA; 1983.
15. Castor LL, Frederiksen RA. Fusarium head blight occurrence and effects on sorghum yield and grain characteristics in Texas. Plant Dis. 1980;64:1017-1019.
16. Erpelding JE, Prom LK. Seed mycoflora for grain mold from natural infection in sorghum germplasm grown at Isabela, Puerto Rico and their association with kernel weight and germination. Plant Pathol. J. 2006;5:106-112.
17. Ratnadass A, Marley PS, Hamada MA, Ajaya O, Cissé B, Assamoi F, Atokple IDK, Beyo J, Cissé O, Dakouo D, Diakite M, Dossou-Yovo S, Le Diambo B, Vopeyande MB, Sissoko I, Tenkouano A. Sorghum head-bugs and grain molds in West and Central Africa: I. Host plant resistance and bug-mold interactions on sorghum grains. Crop Prot. 2003;22:837-851.
18. Yago JI, Roh JH, Bae S, Yoon YN, Kim HJ, Nam M. The effect of seed-borne mycoflora from sorghum and foxtail millet seeds on germination and disease transmission. Mycobiol. 2011;39:206-218.

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