

Original Research Article

Mycoflora analysis of hybrid sorghum grain collected from different locations in South Texas

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. lunata* (6.8%) and *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.

Key words: *Sorghum* grain mold, *Sorghum bicolor*, fungal pathogens, mycoflora analysis.

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* (Wak.) 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. nygam* 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 species infecting or contaminating sorghum grain collected from several counties in southern Texas.

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. Five to 10 panicles were collected 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 25°C with a 12-hour photoperiod for 7 days. Identification of fungal 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].

RESULTS AND DISCUSSION

In 2008, *Alternaria* spp. (66%) were the most frequently isolated fungal species 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 less 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. protuberata*. 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 Sabela, Puerto Rico, *F. semitectum* was the most frequently isolated fungal species from seeds naturally infected with grain mold, followed by other

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80 *Fusarium* spp., *C. lunata* and *Bipolaris* spp. *Alternaria* spp. and *Colletotrichum graminicola* were
81 also isolated, but at lower frequencies [16]. Sorghum grain collected from Santa Fe Province in
82 Argentina had *Fusarium* spp. (*F. moniliforme*, *F. subglutinans*, *F. chlamydosporum*, and *F.*
83 *semitectum*), *A. alternata*, *C. lunata*, and *Bipolaris cynodontis* as the most frequently recovered
84 fungal species [11]. Mycoflora analysis of sorghum grain collected from different locations in
85 West and Central Africa revealed *Phoma* spp. and *Fusarium* spp. as the dominant fungal taxa
86 followed by *Curvularia* spp. [17].

87 Table1.Frequencyofrecovery (%)ofvariousfungalspeciesisolated fromsorghumgraincollected fromfifteenlocationsinsouthern
88 Texas,2008¹

County	Alt ²	Fungal species							
		Bipo	Coll	Cur	FT	FS	Fus	Unkn	Asper
Bee60	12	2	10	0	0	8	6	2	
Bee74	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
SanPatricio	60	28	0	0	0	2	6	4	0
SanPatricio	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
Kleberg82	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

Comment [H2]: otherFusariumspp

107	Hidalgo	46	24	0	0	0	0	22	0	0
108	Hidalgo	60	16	0	10	4	8	4	0	0
109										
110	Overall									
111	mean66	17	0.1	2.4	0.5	1.2	11.3	1.7	0.1	

112

113 ¹*Sorghum* grains were collected at harvest. One hundred and fifty seeds from each location were analyzed.

114 ²Alt=*Alternaria* species, Bipo=*Bipolaris* species, Coll=*Colletotrichum* species, Cur=*C. lunata*, FT=*Fusarium thapsinum*,

115 FS=*Fusarium semitectum*, Fus=*Fusarium* species, Unkn=unknown, Asper=*Aspergillus* species, Ph=*Phoma* species, Cln=clean seed

116 (not colonized).

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117
118 In 2009, sorghum grains were collected from the same fields at the beginning of grain development
119 to soft dough stage, and also at maturity (Tables 2 and 3). Mycoflora analysis of sorghum grain
120 conducted at beginning of grain development to soft dough stage across locations
121 revealed *Alternaria* spp. with a mean frequency of 55.8% as the dominant species, followed by non-
122 colonized or clean seed, 22.5%, and *Bipolaris* spp., 14.3%.
123 *C. lunata* and other *Fusarium* spp. accounted for 3.3% and 2.3%, respectively (Table 2).
124 Other fungal species, *F. thapsinum*, *F. semitectum*, *Aspergillus* spp. and *Phoma* spp. were isolated at
lower frequencies.

Table 2. Frequency of recovery (%) of fungal species isolated from sorghum grain collected from several locations in southern Texas, 2009¹

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	0	8	0	0	0	0
Hidalgo	88	10	0	0	0	0	0	0	0	0	0	0
Hidalgo	74	0	2	2	2	2	2	4	4	4	4	20
San Patricio	60	12	0	0	0	0	0	2	0	0	0	26
Nueces	6	2	0	0	0	0	0	0	0	0	0	92
Willacy	46	50	0	4	0	0	0	0	0	0	0	0
Kleberg	80	18	0	0	0	0	0	2	0	0	0	0
Overall												
Mean	55.8	14.3	0.3	3.3	0.3	0.3	0.3	2.3	1.0	0.5	1.8	22.5

144 ¹*Sorghum* grains were collected at the beginning of kernel development to soft dough stage, June 4. One hundred and fifty seeds
145 from each location were analyzed.

146 ²Alt=*Alternaria* species, Bipo=*Bipolaris* species, Coll=*Colletotrichum* species, Cur=*C. lunata*, FT=*Fusarium thapsinum*, FS=*Fusarium*
147 *semitectum*, Fus=*Fusarium* species, Unkn=unknown, Asper=*Aspergillus* species, Ph=*Phoma* species, Cln=clean seed (not
148 contaminated).

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

Table 3. Frequency of recovery (%) of various fungal species isolated from sorghum grain collected from several locations in Texas, 2009¹

Fungal species										
County	Alt	BipoCur	FS	FusUnkn	PhClnRh	Rhz				
Bee	56	6	2	0	4	4	4	12	12	
Refugio	58	18	20	2	2	0	0	0	0	0
Hidalgo	72	8	6	8	6	0	0	0	0	0
Hidalgo	62	22	6	2	8	0	0	0	0	0
San Patricio	62	16	6	4	14	0	0	0	0	0
Nueces	78	10	0	6	6	0	0	0	0	0
Willacy	66	16	6	4	8	0	0	0	0	0
Kleberg	80	6	8	0	6	0	0	0	0	0
Overall										
Mean	66.8	12.8	6.8	3.3	6.8	0.5	0.5	1.5	1.5	

¹Sorghum grains were collected at maturity, July 10. One hundred and fifty seeds from each location were analyzed.

174 ²Alt=Alternaria species, Bipo=Bipolaris species, Cur=C. lunata, FS=Fusarium semitectum, Fus=Fusarium species, Unkn=unknown,
175 Ph=Phoma species, Cln=clean seed (not contaminated), Rhz=Rhizopus species.

176 The recovery of Alternaria spp. in Bee, Nueces, and Willacy counties, C. lunata in six of these seven counties, F. semitectum in Hidalgo and Nueces
177 counties, and other Fusarium spp. in five of the seven counties increased significantly at harvest, whereas the percentage of non-colonized or
178 clean seeds and, to a lesser degree, Bipolaris spp. and Phoma spp. decreased. Generally, this indicates that some fungal species that are
179 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
180 species from sorghum grain collected in a number of locations in South Korea showed A. alternata, A. flavus, C. lunata, F.
181 moniliforme, and Phoma spp. as the dominant species [18], while, F. thapsinum, F. nygamya and C. lunata are the most commonly isolated grain
182 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
183 the dominant species associated with naturally-infected grain [10, 11, 15, 16, 18]. Data obtained from two weather stations (Nueces and
184 Hidalgo Counties) in 2008 noted temperature for the months of June and July ranged from 20.6°C to 37.8°C and precipitation ranged from 5.1 mm
185 to 78.7 mm, while in 2009, temperature for the same months ranged from 18.9°C to 39.4°C and precipitation ranged from 2.5 mm to 20.3 mm. The
186 lack of rain and high temperatures during the 2008 and 2009 growing seasons were not conducive for grain mold development in the regions
187 where the sorghum grain was collected.

188 189 CONCLUSION

190
191 In conclusion, Alternaria spp. was the most dominant fungal genus recovered from sorghum grain collected from different locations in
192 southern Texas in 2008 and 2009. However, there is little or no information on the reaction of sorghum germplasm against this fungal species.

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