

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* (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 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 hours 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. 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

80 *Fusarium* spp., *C. lunata* and *Bipolaris* spp. *Alternaria* spp. and *Colletotrchum 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 Table 1. Frequency of recovery (%) of various fungal species isolated from sorghum grain collected from fifteen locations in southern
 88 Texas, 2008¹

	Fungal species									
	County	Alt ²	Bipo	Coll	Cur	FT	FS	Fus	Unkn	Asper
94	Bee	60	12	2	10	0	0	8	6	2
95	Bee	74	14	0	0	0	0	10	0	0
96	Refugio	64	20	0	8	0	0	8	0	0
97	Refugio	78	16	0	0	0	0	6	0	0
98	San Patricio	60	28	0	0	0	2	6	4	0
99	San Patricio	42	24	0	0	0	0	34	0	0
100	Nueces	64	18	0	0	0	4	8	6	0
101	Nueces	84	4	0	0	4	0	6	2	0
102	Kleberg	82	4	0	2	0	0	12	0	0
103	Kleberg	38	48	0	0	0	4	8	2	0
104	Willacy	80	20	0	0	0	0	4	0	0
105	Willacy	74	4	0	0	0	0	14	6	0
106	Goliad	90	2	0	6	0	0	20	2	0

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	mean	66	17	0.1	2.4	0.5	1.2	11.3	1.7	0.1

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

114 ²*Alt=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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118 In 2009, sorghum grains were collected from the same fields at the beginning of grain
119 development to soft dough stage, and also at maturity (Tables 2 and 3). Mycoflora analysis of
120 sorghum grain 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
122 non-colonized or clean seed, 22.5%, and *Bipolaris* spp., 14.3%. *C. lunata* and other *Fusarium*
123 spp. accounted for 3.3% and 2.3%, respectively (Table 2). Other fungal species, *F. thapsinum*,
124 *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	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											
Mean	55.8	14.3	0.3	3.3	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 ²*Alt*=*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
153 fungal species isolated in low frequencies included *Rhizopus* spp. and *Phoma* spp.

154 Table 3. Frequency of recovery (%) of various fungal species isolated from sorghum grain collected from several locations in Texas,
 155 2009¹

156

157 Fungal species

158

159 County	Alt	Bipo	Cur	FS	Fus	Unkn	Ph	Cln	Rhz
161 Bee	56	6	2	0	4	4	4	12	12
162 Refugio	58	18	20	2	2	0	0	0	0
163 Hidalgo	72	8	6	8	6	0	0	0	0
164 Hidalgo	62	22	6	2	8	0	0	0	0
165 San Patricio	62	16	6	4	14	0	0	0	0
166 Nueces	78	10	0	6	6	0	0	0	0
167 Willacy	66	16	6	4	8	0	0	0	0
168 Kleberg	80	6	8	0	6	0	0	0	0
169									
170 Overall									
171 Mean	66.8	12.8	6.8	3.3	6.8	0.5	0.5	1.5	1.5
172									

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

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

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 significantly 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]. Data obtained from two weather stations (Nueces and Hidalgo Counties) in 2008 noted temperature for the months of June and July ranged from 20.6C to 37.8C and precipitation ranged from 5.1 mm to 78.7 mm, while in 2009, temperature for the same months ranged from 18.9C to 39.4C and precipitation ranged from 2.5 mm to 20.3 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.

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.

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