



Plant Pathology Journal

ISSN 1812-5387

science
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Seed Mycoflora for Grain Mold from Natural Infection in Sorghum Germplasm Grown at Isabela, Puerto Rico and their Association with Kernel Weight and Germination

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Abstract: Seed mycoflora, kernel weight and seed germination were evaluated for 20 sorghum germplasm lines naturally infected with grain mold during the 2002 and 2003 growing seasons in Isabela, Puerto Rico. Mycoflora analysis of the kernels revealed *Fusarium semitectum* as the most frequently recovered fungal species. *Fusarium verticillioides*, *F. thapsinum*, other *Fusarium* species, *Curvularia lunata*, *Bipolaris* spp., *Alternaria* spp., *Colletotrichum graminicola* and several unknown fungal species were also isolated from mold infected grain. *Bipolaris* spp., *Alternaria* spp. and *C. graminicola* were isolated at the lowest frequencies from infected grain. Variation was observed in the frequency of fungal species isolated between years and between germplasm lines. Seed germination was positively correlated with kernel weight and negatively correlated with the incidence of *F. semitectum*, *C. lunata* and *Bipolaris* spp. Non-significant negative associations between germination with *Fusarium* spp. and between kernel weight with *F. semitectum*, *F. thapsinum* and *C. lunata* were also observed. Significant negative associations occurred between the incidence of *F. semitectum* with the frequency of *C. lunata* and *F. thapsinum*. The incidence of grain mold did not reduce seed germination for several sorghum lines, suggesting potential sources of tolerance for sorghum improvement.

Key words: *Sorghum bicolor*, grain mold fungi, Sureño, Dorado, germplasm

INTRODUCTION

Grain mold is considered the most important sorghum disease and is a major constraint to sorghum production and profitability, especially in areas where the crop matures during warm and moist weather conditions^[1-4]. Fungal species in over 40 genera have been associated with grain mold in sorghum^[4]. Grain mold fungal species commonly isolated from sorghum include *Fusarium thapsinum* Klittick, Leslie, Nelson *et al.*, Manasas, *Curvularia lunata* (Wakk.) Boedijn, *Fusarium semitectum* Berk. And Ravenel, *Colletotrichum graminicola* (Ces.) G.W. Wilson, *Alternaria alternata* (Fr.: Fr.) Keissl, *Phoma sorghina* (Sacc.) Boerema, Dorenbosch and Van Kesteren and *Cladosporium* spp.^[2,4,5-7]. *Fusarium thapsinum* is considered the most prevalent grain mold fungal species infecting sorghum^[2].

Grain mold infection may occur any time after anthesis up to grain harvest^[4,6,8,9]. Disease symptoms include small and deteriorated seed with seed discoloration ranging from pink to reddish-pink or black

to grayish-black^[4,8,10]. Grain mold affects both grain yield and quality. Yield losses of 30 to 100% have been observed on susceptible sorghum lines under severe disease pressure^[4].

Management strategies aimed at minimizing the impact of grain mold involve planting of sorghum cultivars that mature during periods of dry weather or the use of cultivars with colored grain high in tannins that limit mold infection^[4]. The use of chemical fungicides is not economically practical in controlling the disease in production fields. Although, chemical seed dressings are typically used to enhance germination and seedling vigor of grain mold infected seed^[4,11,12]. The development of genetically resistant sorghum lines to grain mold is the most practical method of reducing the impact of the disease.

Extensive research has been conducted on the reaction of various sorghum cultivars to grain mold in India and the United States^[6,13-15]. In Puerto Rico, there is little information on seed mycoflora for grain mold and their association with germination and seed weight of

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Table 1: Country of origin and phenotypic characteristics of the 20 sorghum germplasm lines included in the grain mold evaluation conducted in Isabela, Puerto Rico during the 2002 and 2003 growing seasons

Line ¹	Country	Race ²	FD ³	Seed Color	Pericarp Color	Mesocarp	Testa ⁴	Endosperm Texture ⁵
Dorado	El Salvador	C	66	white	white	thin	A	P
Sureño	Honduras	C	69	white	white	thin	A	P
PI 533830	Sudan	C	53	brown	red	thick	P	P
PI 533900	Japan	C	63	yellow	yellow	thin	A	P
PI 533951	Nigeria	C	55	red	red	thick	P	P
PI 533967	Uganda	C	60	white	white	thin	A	P
PI 533993	Sudan	C	59	white	white	thin	A	P
PI 534010	India	D	51	white	white	thin	A	P
PI 534013	India	D	53	brown	red	thick	P	S
PI 534034	India	D	69	white	white	thin	A	P
PI 534056	Nigeria	C	64	brown	red	thick	P	AS
PI 534112	Uganda	C	64	brown	red	thick	P	P
PI 534141	Kenya	C	60	white	white	thin	A	P
PI 534162	China	D	59	white	white	thin	A	P
PI 576328	Swaziland	C	50	white	white	thick	P	AS
PI 576339	Zimbabwe	K	56	white	white	thick	A	P
PI 576344	Ethiopia	DB	59	brown	red	thin	P	AS
PI 576353	Sudan	C	45	white	white	thick	P	P
PI 576373	Japan	CB	59	white	white	thick	P	P
PI 576374	Tanzania	C	61	brown	red	thick	P	P

¹Plant introduction number for the sorghum accessions (USDA-ARS National Genetic Resources Program, 2005, <http://www.ars-grin.gov/>); Sureño (PI 561472) and Dorado (Grif 642).

²Race: Sorghum race classification Harlan and de Wet^[23]; C = caudatum, D = durra, K = kafir, DB = durra-bicolor, CB = caudatum-bicolor.

³FD: Flowering date was averaged over multiple growing seasons and refers to the number of days after planting when 50% of the panicles were at 50% anthesis.

⁴Testa: A = absence of a pigmented testa layer; P = presence of a pigmented testa. ⁵Endosperm Texture: P = partly corneous; AS = almost starchy; S = completely starchy

naturally infected sorghum cultivars. *Fusarium moniliforme* (Sheldon), *C. lunata*, *Alternaria* spp., *F. semitectum* and *F. roseum* var. *longipipes* Ellis and Everhart were isolated by Hepperly *et al.*^[16] from mold infected sorghum seed produced in Mayaguez, Puerto Rico. Since the occurrence of grain mold fungal species can vary between locations^[4], information is needed on the prevalence of the various grain mold fungal species occurring in Isabela, Puerto Rico prior to evaluating sorghum germplasm to identify resistant sources.

The environmental conditions in Isabela, Puerto Rico are favorable for the development of grain mold and the location is ideal for the evaluation of the sorghum collection maintained by the US National Plant Germplasm System to identify sources of resistance to grain mold. Thus, an evaluation was conducted during the 2002 and 2003 growing seasons to determine the occurrence and frequency of grain mold fungal species from naturally mold infected sorghum seed. Kernel weight and seed germination were measured to determine the effect grain mold had on seed quality in order to effectively screen for resistance. Because the host plant response to grain mold can vary between fungal species, the frequency of each species determined from seed mycoflora analysis was used to evaluate associations between kernel weight, seed germination and the incidence of the various fungal species.

MATERIALS AND METHODS

‘Sureño’ (PI 561472), ‘Dorado’ (Grif 642) and 18 sorghum germplasm lines were selected for the evaluation of grain mold in Isabela, Puerto Rico. Sureño is moderately resistant to grain mold and Dorado is susceptible^[5,15,17]. The 18 sorghum germplasm lines included in the grain mold evaluation were selected to represent genetically diverse sorghum germplasm for the evaluation of host plant response to infection (Table 1). Selected lines had a similar flowering date to Sureño and Dorado and varied for kernel characteristics such as seed and pericarp color, presence or absence of a pigmented testa, mesocarp thickness and endosperm texture.

The grain mold evaluation was conducted at the USDA-ARS Tropical Agriculture Research Station in Isabela, Puerto Rico during the 2002 and 2003 growing seasons. The experiments were planted on 12 September 2002 and 25 September 2003 in a Randomized Complete Block Design with three replications. The planting dates correspond to the wet growing season when climatic conditions are more favorable for disease development. The 20 sorghum germplasm lines were planted in single rows 1.8 m in length with 0.9 m row spacing. Experiments were fertilized at a rate of 560 kg ha⁻¹ (15-5-10 NPK) during planting with a second application of fertilizer conducted approximately 30 days after planting. Fields

were irrigated after planting for stand establishment and as necessary during the growing season. There was no supplemental overhead irrigation conducted after floral initiation. For the 2002 evaluation, Furadan (carbofuran) insecticide (FMC Corporation, Philadelphia, PA) was applied at a rate of 0.2 L ha⁻¹ to prevent seed loss from fire ants. To reduce fire ant damage in 2003, Lorsban 15G (O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate) granular insecticide (Dow AgroSciences, Indianapolis, IN) was applied at a rate of 8 kg ha⁻¹ during planting. Weed control was conducted using mechanical tillage and hand hoeing. Prior to anthesis, panicles were bagged to prevent seed loss from birds. Bagged panicles were hand harvested approximately 100 days after planting, dried at 50°C and threshed using a single plant thresher (Almaco Single Plant and Head Thresher, Allan Machine Company, Nevada, IA).

Seed mycoflora, germination rate and 100-kernel weight were determined for the 20 sorghum germplasm lines using a seed sample from each replication. Seed mycoflora analysis and germination evaluation have been described by Prom *et al.*^[15] and Prom^[10]. Briefly, germination rate was based on the number of seeds that germinated after seven days from 400 seeds planted in flats containing Metro Mix 200 potting medium (Scotts-Sierra Horticultural Products Company, Maryland, OH). For seed mycoflora analysis, 50 seeds from each replication were surface disinfected and allowed to dry in a laminar flow hood. Seeds were transferred to half-strength potato dextrose agar media and incubated at 25°C with a 12 h photoperiod for seven days. Fungal species were identified based on conidium, conidiophore and colony morphology and color. Confirmation of *Fusarium* spp. was conducted at the Fusarium Research Center, Pennsylvania State University, College Park, PA based on fungal characteristics of reference cultures. Kernel weight was based on weight in grams of 100 randomly selected seeds from each replication.

Data for the 100-kernel weight and percent seed germination rate were analyzed using PROC ANOVA (SAS version 8.1, SAS Institute, Cary, NC). Since the data showed homogeneity of error variances, data for the two years were combined for analysis. Mean comparisons were conducted using Tukey's studentized range test at the 5% probability level. Pearson correlation coefficient was calculated for incidence of fungal species, 100-kernel weight and percent seed germination from the 2002 and 2003 data.

RESULTS

Fusarium semitectum was the most frequently isolated fungal species from naturally grain mold infected sorghum kernels obtained from Isabela, Puerto Rico in 2002 and 2003 (Table 2 and 3). During the 2002 growing season, *F. semitectum* accounted for 59.8% of the total fungal species isolated from kernels and for 38.4% of those isolated in 2003. Other *Fusarium* spp. accounted for 19.7% of the fungal species isolated in 2002. *Curvularia lunata* (13.4%), *F. thapsinum* (6.4%) and *Bipolaris* spp. (0.5%) were also isolated from mold infected grain in 2002. In 2003, 17.8% of the kernels were infected with *F. verticillioides*, 16.1% were infected with *F. thapsinum*, 12.4% were infected by other *Fusarium* spp. and 7.3% were infected with *C. lunata*. *Alternaria* spp., *Bipolaris* spp. and *C. graminicola* were also isolated in 2003, but at frequencies lower than 1%. Fungal species isolated from 2.9% of the infected sorghum grain in 2003 could not be classified and 3.2% of the kernels were free of infection.

Kernel weight and seed germination were significantly ($p = 0.0001$) affected by sorghum genotype (Table 4). Kernel weight averaged 1.9 g for the 20 sorghum germplasm lines with five of the sorghum lines (PI 533900, PI 534010, PI 534162, PI 576339 and PI 576374) having similar kernel weights as compared to Sureño. The

Table 2: Frequency of the various fungal species isolated from Sureño, Dorado and 18 sorghum lines naturally infected with grain mold at Isabela, Puerto Rico in 2002¹

Line	Fungal species ²				
	FT	CL	FS	BI	F spp.
Dorado	3.3	12.7	74.7	0.0	9.3
Sureño	2.0	12.0	18.7	0.0	65.3
PI 533830	10.7	10.0	73.3	0.0	6.0
PI 533900	9.3	15.3	34.0	0.0	41.3
PI 533951	10.7	8.0	74.7	0.0	9.3
PI 533967	7.3	39.3	50.7	0.2	2.0
PI 533993	1.3	9.3	81.3	2.0	6.7
PI 534010	1.3	8.7	56.0	3.3	30.7
PI 534013	7.3	14.0	52.7	1.3	25.3
PI 534034	6.0	6.6	54.0	0.0	34.0
PI 534056	6.0	4.7	72.0	1.3	10.0
PI 534112	18.0	9.3	43.3	0.0	29.3
PI 534141	7.3	10.7	68.0	0.0	14.0
PI 534162	8.7	7.3	49.3	0.0	34.7
PI 576328	6.0	17.3	72.7	0.0	4.0
PI 576339	3.3	6.7	67.3	0.0	21.3
PI 576344	2.7	34.0	40.0	1.3	22.7
PI 576353	1.3	26.7	69.3	0.0	2.7
PI 576373	6.7	14.7	69.3	0.7	8.7
PI 576374	8.0	0.0	75.3	0.0	17.3

¹Frequency of recovery (%) of the various fungal species was based on assays of 150 seed per line plated on half-strength potato dextrose agar medium with 50 seed evaluated from each replication. ²Fungal species: FT = *Fusarium thapsinum*; CL = *Curvularia lunata*; FS=*Fusarium semitectum*; BI = *Bipolaris* spp.; F spp. = other *Fusarium* spp.

Table 3: Frequency of the various fungal species isolated from Sureño, Dorado and 18 sorghum lines naturally infected with grain mold at Isabela, Puerto Rico, 2003¹

Line	Fungal species ²									
	FT	CL	FS	BI	F spp.	ALT	CG	FV	NC	Other
Dorado	17.3	6.0	40.0	0.0	9.3	2.7	0.0	18.0	2.7	3.3
Sureño	6.0	5.3	22.0	3.3	9.3	1.3	0.0	20.0	28.7	3.3
PI 533830	12.01	0.7	42.0	1.3	6.7	2.0	0.0	25.3	0.0	0.0
PI 533900	12.7	1.3	28.0	6.2	2.0	0.0	0.0	26.0	7.3	3.3
PI 533951	11.3	6.0	50.0	0.7	12.7	0.7	0.0	12.0	4.0	2.7
PI 533967	16.7	20.7	34.0	0.0	12.0	0.7	0.0	10.7	2.0	5.3
PI 533993	15.3	8.0	39.3	0.0	31.0	0.7	0.0	10.0	0.0	2.7
PI 534010	20.7	1.3	20.0	0.0	26.0	0.0	0.0	30.0	4.7	2.7
PI 534013	18.0	5.3	45.3	0.0	13.3	1.3	0.0	9.3	0.0	7.3
PI 534034	16.0	0.7	32.7	0.0	13.3	0.7	0.0	31.3	5.3	0.0
PI 534056	5.3	13.3	42.0	0.7	6.0	0.7	0.7	28.7	2.7	3.3
PI 534112	16.7	0.0	46.7	0.0	11.3	0.0	0.0	20.0	4.7	0.7
PI 534141	36.7	5.3	26.7	2.0	11.3	2.7	0.0	11.3	0.0	4.0
PI 534162	28.0	5.3	22.0	2.0	16.0	2.0	0.0	14.7	0.0	6.7
PI 576328	7.3	10.0	47.3	0.7	13.3	1.3	0.0	14.0	0.7	5.3
PI 576339	9.3	4.0	52.7	0.0	14.0	0.0	0.0	20.0	0.0	0.0
PI 576344	2.0	29.3	30.7	1.3	14.7	2.7	0.0	14.0	0.0	5.3
PI 576353	13.3	8.7	54.7	0.0	11.3	0.0	0.0	10.7	0.0	1.3
PI 576373	21.3	2.7	52.7	0.0	8.7	0.0	0.0	13.3	1.3	0.0
PI 576374	35.3	2.0	39.3	0.0	6.0	0.0	0.0	16.7	0.7	0.01

¹Frequency of recovery (%) of the various fungal species was based on assays of 150 seeds per line plated on half-strength potato dextrose agar medium with 50 seed evaluated from each replication.

²Fungal species: FT = *Fusarium thapsinum*; CL = *Curvularia lunata*; FS = *Fusarium semitectum*; BI = *Bipolaris* spp.; F spp. = other *Fusarium* spp.; ALT = *Alternaria* spp.; CG = *Colletotrichum graminicola*; FV = *Fusarium verticillioides*; NC = non-contaminated seed and Other = other fungal spp. not classified

Table 4: Kernel weight and percent seed germination observed for Sureño, Dorado and 18 sorghum lines naturally infected with grain mold in Isabela, Puerto Rico during 2002 and 2003¹

Line	Kernel weight ²	Germination ³
Dorado	2.32ab ⁴	72.8a-e
Sureño	2.37a	69.8a-f
PI 533830	1.75d-h	70.2a-f
PI 533900	2.25a-c	77.5a-d
PI 533951	1.89b-f	68.8a-f
PI 533967	1.81c-g	47.0fg
PI 533993	1.46f-h	49.0e-g
PI 534010	2.17a-d	54.8d-g
PI 534013	1.30h	63.2c-g
PI 534034	1.69e-h	72.2a-f
PI 534056	1.88b-f	93.2a
PI 534112	1.47f-h	84.7a-c
PI 534141	1.77d	75.2a-d
PI 534162	2.43a	74.2a-e
PI 576328	1.75d-h	67.0b-f
PI 576339	2.59a	89.8ab
PI 576344	1.54f-h	77.3a-d
PI 576353	1.37gh	38.7g
PI 576373	1.82c-g	63.3c-g
PI 576374	2.13a-e	88.0a-c

¹Error variances were homogenous between years and data was combined to represent an average for the two years.

²Kernel weight = mean weight in grams of 100 randomly selected seed from each replication.

³Germination = mean seed germination percentage after seven days with 400 seed evaluated for each replication.

⁴Means within a column followed by the same letter (s) are not significantly different at the 5% probability level. Mean comparison was conducted using Tukey's studentized range test

average seed germination rate for the 20 sorghum lines was 69.8%. Ten of the sorghum lines had a higher germination rate than Sureño.

A highly significant positive relationship between kernel weight and seed germination ($r = 0.41$; $p = 0.0011$) was observed in 2003, but not in 2002 ($r = 0.24$; $p = 0.0603$). There were significant negative correlations between seed germination and the incidence of *C. lunata* ($r = -0.27$; $p = 0.0380$), *F. semitectum* ($r = -0.28$; $p = 0.0328$) and *Bipolaris* spp. ($r = -0.27$; $p = 0.0394$) in 2002. Non-significant negative associations between kernel weight and the incidence *C. lunata*, *F. semitectum* and *F. thapsinum* were also observed. In 2003, non-significant negative correlations were observed between seed germination and the incidence of *F. semitectum*, *Bipolaris* spp. and other *Fusarium* spp. The incidence of *F. semitectum* exhibited a significant negative association with the frequency of *C. lunata* ($r = -0.33$; $p = 0.0096$) and other *Fusarium* spp. ($r = -0.78$; $p = 0.0001$) isolated in 2002 and with the incidence of *F. thapsinum* ($r = -0.42$; $p = 0.0007$) and *F. verticillioides* ($r = -0.28$; $p = 0.0275$) in 2003. A non-significant negative associate was observed in 2002 for the frequency of *F. semitectum* with the occurrence of *F. thapsinum*. In 2003, the incidence of *F. thapsinum* showed a significant negative association with the occurrence of *C. lunata* ($r = -0.26$; $p = 0.0413$).

DISCUSSION

Seed mycoflora, kernel weight and seed germination are important factors in determining the reaction of sorghum germplasm to grain mold^[2,4-7]. Mycoflora analysis

of kernels naturally infected with grain mold showed that *F. semitectum* was the most frequently recovered fungal species for the 20 sorghum germplasm lines evaluated in Isabela, Puerto Rico during 2002 and 2003. *Fusarium verticillioides*, *F. thapsinum*, other *Fusarium* species, *C. lunata*, *Bipolaris* spp., *Alternaria* spp., *C. graminicola* and several unknown fungal species were also isolated at various frequencies from mold infected kernels. *Bipolaris* spp., *Alternaria* spp. and *C. graminicola* were isolated at the lowest frequencies and the majority of the sorghum lines were not infected by these species, suggesting that these fungal species may not be aggressive in causing grain mold on sorghum grown in Isabela, Puerto Rico. Hepperly *et al.*^[16] identified *F. moniliforme*, *C. lunata* and *Alternaria* spp. as the most frequently recovered fungal species from naturally grain mold infected sorghum seed produced in Mayaguez, Puerto Rico. *Fusarium moniliforme* has been shown to be comprised of a number of *Fusarium* spp, including *F. thapsinum*^[18]. In South America, *Fusarium* spp., *Curvularia* spp., *Alternaria* spp., *Cladosporium* spp., *C. graminicola*, *Bipolaris* spp. and *Drechslera* spp. have been isolated from mold infected sorghum seed^[19,20]. *Curvularia lunata* and *F. verticillioides* were the dominant species isolated in India from naturally grain mold infected sorghum seed^[12]. *Alternaria* spp., *F. semitectum* and *C. lunata* were shown to be the most prevalent grain mold species occurring in Texas from naturally infected sorghum seed^[10]. This would suggest that *F. semitectum*, *F. thapsinum* and *C. lunata* would be the most important fungal species for evaluating sorghum germplasm for resistance to grain mold in Isabela, Puerto Rico.

The mycoflora analysis also indicated that the occurrence and frequency of the different grain mold fungal species on sorghum seed will vary between growing seasons and for the sorghum genotypes. In 2002, *C. lunata* was recovered at a higher frequency than in 2003. In contrast, *F. thapsinum* was more frequently recovered from infected seed in 2003 as compared to 2002. Even though *F. semitectum* was the most frequently observed species in both years, this species was recovered at a greater frequency in 2002 than in 2003. The diversity of fungal species isolated from infected grain also varied between growing seasons. A greater number of fungal species were identified in 2003 as compared to 2002. *Alternaria* spp. and *C. graminicola* were isolated at a low frequency in 2003 and it is possible that in 2002 the frequency of these two species was below the detection threshold. In contrast, *F. verticillioides* was isolated at a high frequency in 2003, which would suggest

other factors were involved in the occurrence of this species.

The negative effect of grain mold on seed viability was observed in this study. Seed germination was significantly reduced with an increase in the incidence of *F. semitectum*, *C. lunata* and *Bipolaris* spp. Hepperly *et al.*^[16] observed significant negative correlations between seed germination with the incidence of *C. lunata* and *F. moniliforme* for mold infected sorghum seed from Mayaguez, Puerto Rico. Castor^[8] and Garud *et al.*^[3] noted significant negative correlations between germination and *Fusarium* spp. Prom^[10] and Shinde *et al.*^[21] observed significant negative correlations between seed germination with the incidence of *F. thapsinum*, *F. moniliforme* and *C. lunata* isolated from sorghum panicles inoculated with grain mold. This association between grain mold incidence and seed germination would suggest that germination rate could be effectively used in the evaluation of sorghum germplasm. Grain mold can also influence seed development resulting in a significant reduction in size seed due to the premature development of the black layer^[4], but the lack of significant associations between the incidence of the various fungal species with kernel weight may have resulted from infection occurring at later stages of seed development. This would suggest that kernel weight may provide limited information on grain mold susceptibility from natural infection.

Several of the sorghum germplasm lines evaluated may provide sources of tolerance to grain mold for sorghum improvement. Seed viability was not significantly reduced by grain mold for the sorghum lines PI 534056, PI 534112, PI 576339 and PI 576374. With the exception of PI 576339, these lines are characterized as having brown seed with a red pericarp and a pigmented testa. Esole *et al.*^[22] suggested that these seed characteristics conferred resistance to grain mold. Four of the germplasm lines with seed germination rates greater than Sureño were white seeded and lacked a pigmented testa, suggesting other factors may provide tolerance to grain mold or that grain mold did not negatively influence seed germination for these lines.

Although, *F. thapsinum* and *C. lunata* are considered the most common grain molding fungi^[4] and were isolated in this study, they are generally not the dominant species isolated from naturally infected sorghum seed. *Fusarium semitectum* and other grain mold fungi have been shown to be negatively correlated with these two species. This may explain why *F. semitectum* was the most frequently recovered fungal species from this grain mold evaluation. In addition, the lower

incidence of *F. semitectum* in 2003 may have been a contributing factor to the greater diversity of fungal species observed on infected seed as compared to 2002. This would also suggest that other grain mold fungal species may interfere when screening sorghum lines for resistance to *F. thapsinum* and *C. lunata* under natural conditions. However, panicle inoculation could be effectively used to evaluate host plant resistance to specific fungi species not frequently observed under natural infection or to determine the resistant response of the host plant to specific grain mold fungal species.

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