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Frequency of Isolation of Four Fungal Species Colonizing Sorghum Grain Collected from Six Lines in an Anthracnose-Infected Field

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Abstract

Sorghum seed mycoflora analysis from six lines grown in an anthracnose infected field was conducted in 2020. Seed samples were collected in July and August, and the frequency of isolation of four grain mold fungi was recorded. In July, seeds collected from SC748 exhibited the highest isolation of *Alternaria alternata* (40%) while 38% of seeds collected from SC1103-654 and 32% from SC1103-590 also were infected with the pathogen. In August, *A. alternata* was recovered in 34% of seeds from SC748 and SC265-375. Seeds obtained from RTx430 exhibited the lowest isolation of *A. alternata* in July and August. In July, *Fusarium semitectum* was most frequently isolated from BTx623 seeds, followed by SC1103-590 and SC1103-654. Higher recovery of *F. semitectum* was observed in August, with seeds collected from SC748 exhibiting 55%. In both collection periods, *Colletotrichum sublineola* was most frequently isolated from anthracnose susceptible lines RTx430 and BTx623 while the resistant SC748 had zero infected seeds. The highest frequency of isolation of *Curvularia lunata* was recorded from sorghum seeds collected from SC265-375 (20%), followed by SC1103-590 (6%) and 4% from SC748 seeds. This study showed that fungal species once present on the seeds are likely to persist during the growing season at various concentrations. And *C. sublineola*, causal agent of sorghum anthracnose seemed to be present on/in seeds of susceptible lines, while absent in seeds of the resistant line.

Keywords: Sorghum; Seed mycoflora; *Alternaria alternata*; *Colletotrichum sublineola*; *Curvularia lunata*; *Fusarium semitectum*.

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1. Introduction

Globally, sorghum ranks fifth among cereals and supplies the daily caloric needs for millions of people, especially in the drier tropics [1-3]. Despite its importance, the crop is hampered by several biotic stresses which limit its productivity and profitability [2, 4, 5]. Among the biotic constraints, grain mold ranks at the top of these maladies, causing yield losses of up to 100% [5, 6]. Also, the grain quality is impacted because some of the fungi (i.e., *Fusarium thapsinum*, *F. semitectum*, and other *Fusarium* spp.) associated with the disease produce mycotoxins either in the field or under poor storage conditions [7-10]. Other fungi species associated with grain mold complex include *Alternaria alternata*, *Phoma sorghina, Curvularia lunata, Colletotrichum sublineola, and Bipolaris* spp. [4, 5]. Due to the large number of fungal genera associated with the disease, management can be challenging.

Thus, this communication explored the frequency of isolation of four common grain mold fungi in a field infected with anthracnose at the Texas AgriLife Research Farm, Burleson County, Texas.

2. Materials and Method

Sorghum panicles from six lines RTx430, BTx623, SC748, SC265-375, SC1103-590, and SC1103-654 were collected from an anthracnose-infected field at the Texas A&M AgriLife Farm, Burleson County, Texas, during the 2020 growing season. Two panicles were collected from each line in July and again in August from the same row. 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.

The protocol for determining sorghum seed mycoflora analysis was previously described [11, 12]. Briefly, for each sorghum line and collection date, seed mycoflora was determined for 50 surface-disinfected seeds by placing them in vials and put in a small beaker containing 10% NaCl for 1 min. The vials were then rinsed three times with sterilized water and dried under a laminar flow hood. Analysis was conducted on 150 seeds (50 seeds/vial) per line per collection date. For each sorghum line and sample collection date, seeds were placed on five Petri dishes (10 seeds per Petri dish) containing half-strength potato dextrose agar and incubated at 25±2°C for 5-7 d. Fungal species were identified under microscope based on the conidia, conidiophores, colony morphology, and color, according to descriptions provided by Booth [13], Nelson, *et al.* [14], and Barnett and Hunter [15].

3. Results

In this study, recovery of four fungal species that are associated with grain mold and weathering, *A. alternata*, *C. lunata*, *F. semitectum*, and *C. sublineola* were collected from seeds of six sorghum lines in July and August.

In July, *A. alternata* was isolated from 40% of the seeds collected from SC748, followed by 38% from SC1103-654, and 32% from SC1103-590 (Fig. 1A). In August, *A. alternata* was recovered in 34% of seeds from SC748 and SC265-375. Seeds obtained from RTx430 exhibited the lowest isolation of *A. alternata* overall. The highest frequency of isolation for *C. lunata* was 20% in sorghum seeds collected from SC265-375 in July (Fig. 1B). Both RTx430 and BTx623 exhibited lower levels of seed contamination or infection with *C. lunata* in July and August. *Curvularia lunata* was not isolated from SC748 and SC1103-654 seeds in August. The next highest frequency during July was 6% (SC1103-590), followed by 4% from SC748 seeds.

In July, *F. semitectum* was most frequently isolated from BTx623 (37%) seeds, followed by SC1103-590 and SC1103-654 each with 34% infestation (Fig. 1C). Higher recovery of *F. semitectum* was observed in August, with seeds collected from SC748 and SC265-375 exhibiting 55% and 50%, respectively. Seeds for this mycoflora study were collected in a field infected by *C. sublineola*, the causal agent of sorghum anthracnose. In July and August collection periods, *C. sublineola* was most frequently isolated from RTx430 seeds, 66% and 60%, respectively (Fig. 1D). Seeds from BTx623 exhibited the second highest recovery of the anthracnose pathogen with 36% in July and 46% in August. Seeds collected from SC748 recorded zero recovery of *C. sublineola*. In August, no anthracnose pathogen was recovered from SC265-375 and SC1103-654.

4. Discussion

Globally, grain mold is considered as one of the most important sorghum diseases [4, 16-18]. This disease is associated with many genera of fungi; however, the frequency of isolation of these fungi from sorghum seeds varies from location to location and from year to year [10, 12, 19]. In this work, the recovery of four fungal species *A. alternata*, *C. lunata*, *F. semitectum*, and *C. sublineola* associated with sorghum grain mold and weathering are discussed. In both collection periods, ***A. alternata*** was most frequently isolated from SC748. *Alternaria alternata* was the most common fungal species isolated from sorghum seeds collected from different locations in Turkey [20]. Also, Prom, *et al.* [12] noted that *Alternaria* spp. was the dominant fungal species recovered from sorghum seeds in South Texas. Fungal flora from sorghum seed collected from Eritrea, Northeast Africa, showed that *Alternaria* spp. was detected on all samples [21]. *Curvularia lunata* is considered one of the most important grain molding fungus [5]; however, studies have shown that *C. lunata* had been isolated in low frequency in seeds collected from different sorghum growing regions [10, 12, 19]. This report showed that *C. lunata* was most frequently isolated from SC265-375 sorghum seeds collected in July (Fig. 1B). While *F. semitectum* was most frequently isolated from BTx623 (Fig. 1C). Anthracnose is the most important foliar disease of sorghum, and losses in yield on susceptible lines can reach 50% when panicles are infected [22-24]. These sorghum lines RTx430 and BTx623 are found to be highly susceptible to anthracnose [25]; as a result, exhibited the highest percentage of *C. sublineola* recovery from seeds. Whereas *C. sublineola* was absent in seeds collected from SC748 (Fig. 1D), a line which is resistant to the disease [25].

Weather has been shown to influence sorghum diseases such as anthracnose which tends to increase during wet conditions [26]. In this study, the total rainfall in July was higher than in August; however other weather parameters were similar (Table 1). In July, frequency of isolation of the anthracnose pathogen was high in SC265-375 sorghum seeds but absent in August (Fig. 1D). Other factors may also play a role in the recovery of different fungal species from seeds. Prom [11] noted negative associations between *F. semitectum* and *C. lunata* and between *Alternaria* spp. and *F. thapsinum.*

Fig-1. Frequency of recovery (A) *A. alternata*, (B) *C. lunata*, (C) *F. semitectum*, and (D) *C. sublineola* contaminating or infecting sorghum seeds of six sorghum lines collected in July and August in a field infected with anthracnose.

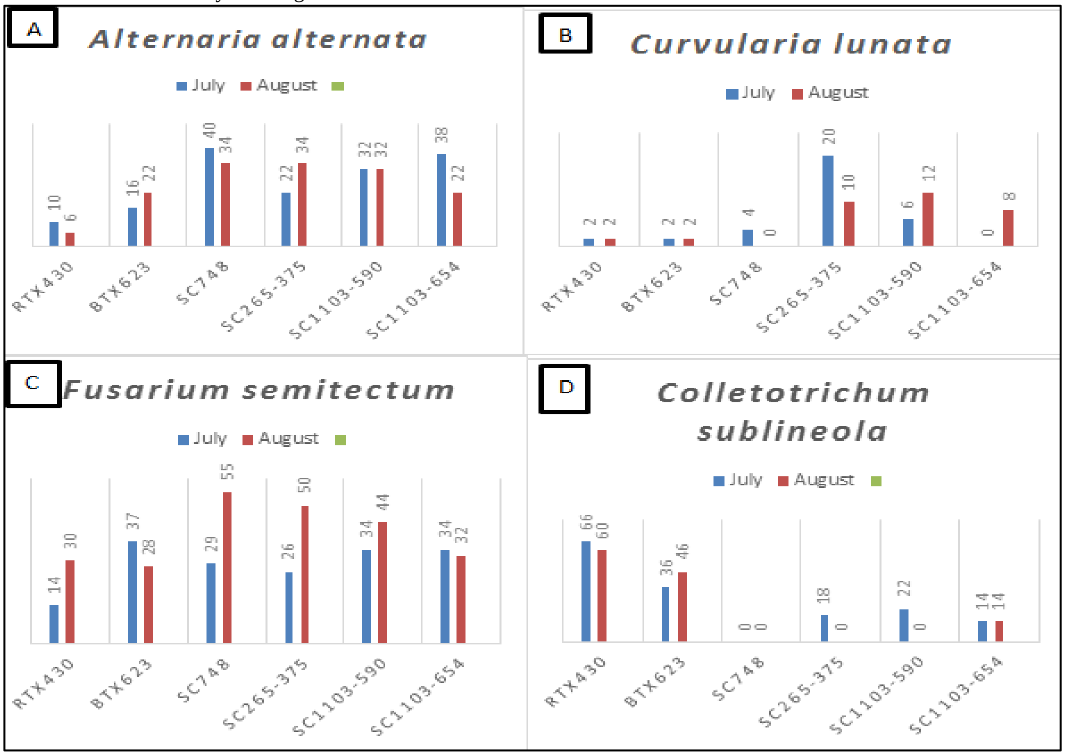

Table-1. Weather data for July and August 2020[1]

Month	Total rainfall	Mean Temp	Avg High	Avg Low
July	2.74	86.7	96.3	77.0
August	0.58	86.5	97.8	75.3

Data accessed November 25, 2022. https://www.weather.gov/hgx/climate_cll_normals_summary#2020. Total rainfall (inches) and temperature (Fahrenheit).

5. Conclusion

This study showed that these fungal species *A. alternata*, *C. lunata*, *F. semitectum*, and *C. sublineola*, once present, are likely to persist during the growing season at various infestation frequencies. In case of *C. sublineola*, causal agent of sorghum anthracnose seemed to be present on/in seeds of susceptible lines, while absent in seeds of the resistant line, SC748. One can conclude that spores on anthracnose-infected sorghum leaves in the field are continuously splashed during windy rains onto other plants and panicles during the growing season and persist on seeds of those lines that are susceptible.

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