Characterization and management of major fungal diseases and mycotoxin contamination of grain sorghum in the mid-Atlantic U.S.

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ABSTRACT

Industry demand for local sources of grain for animal feed has increased sorghum production in the mid-Atlantic region of the U.S. Sorghum anthracnose (causal agent *Colletotrichum sublineola*) and the grain mold complex, which includes mycotoxin-producing *Fusarium* spp., limit the yield and quality of grain sorghum in humid climates worldwide. A majority of U.S. grain sorghum production is in arid regions, and management strategies have not been developed for the mid-Atlantic U.S. where warm, wet conditions favor disease. The specific objectives of this research were to: (1) determine the effectiveness of fungicides and their application timing for the management of sorghum foliar anthracnose, (2) compare five grain sorghum hybrids for their susceptibility to foliar anthracnose, grain mold and mycotoxin contamination under field conditions, (3) integrate host resistance and fungicide application to manage anthracnose and grain mold, and (4) identify *Fusarium* spp. associated with grain mold and mycotoxin contamination of sorghum in the mid-Atlantic U.S. For Objective 1, it was determined that a single application of pyraclostrobin-containing fungicide no later than flowering reduced anthrancose, protected yield and maximized farm income. Objective 2 focused on sorghum hybrid selection as a disease management tactic, and it was determined that hybrids with high yield potential and moderate disease resistance should be selected for mid-Atlantic sorghum production in order to maximize grain yield and quality while minimizing the need for fungicide inputs. Objective 3 focused on integrated management and demonstrated that under moderate disease pressure, a high-yielding susceptible hybrid required a single application

of pyraclostrobin-based fungicide to minimize fungal diseases and maintain acceptable yields, whereas under high disease pressure it was necessary to integrate hybrid resistance and judicous applications of fungicides. The aim of Objective 4 was to characterize potential causal agents of mycotoxin contamination in mid-Atlantic sorghum, and thirteen phylogenetically distinct *Fusarium* species (*F. lacertarum, F. graminearum. F. armeniacum, F. proliferatum, F. fujikuroi, F. verticillioides, F. thapsinum* and several in *Fusarium incarnatum-equiseti* species complex) were found to be associated with grain mold and fumonisin and/or deoxynivalenol contamination of sorghum grain. This work has provided insights into the impacts of fungal diseases on grain sorghum yield and quality in the mid-Atlantic and has aided in development of best management practices for the region.

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GENERAL AUDIENCE ABSTRACT

Sorghum is grown in tropics, sub-tropics and semi-arid region worldwide for food, feed, forage and fuel. Sorghum acreage in the mid-Atlantic is increasing due the demand for locally grown grain by poultry and swine industries. During the growing season, warm and humid conditions are common in the southeastern and mid-Atlantic states favoring fungal diseases development that reduce the grain yield and quality. Anthracnose and grain mold, which includes toxic mycotoxin-producing *Fusarium* species, are the two major constraints in sorghum production in the region. However, management alternatives have not been developed. The main goal of this research was to develop management strategies to protect yield and maximize farm profitability by controlling anthracnose and grain mold of sorghum using chemicals and/or host resistance. The specific objectives were to: (1) determine the effectiveness of fungicides and their application timing for the management of sorghum foliar anthracnose, (2) compare grain sorghum hybrids for their susceptibility to foliar anthracnose, grain mold and mycotoxin contamination under field conditions, (3) assess the value of integrating host resistance and judicious use of fungicides to manage sorghum anthracnose and grain mold, and (4) identify *Fusarium* spp. associated with grain mold and mycotoxin contamination of sorghum in the mid-Atlantic U.S. Results from this research indicate that a single application of pyraclostrobin-containing fungicides no later than flowering reduces anthrancose, protects yield, and increases farm income. Sorghum hybrids varied in susceptibility to anthrancnose and grain mold, and planting a moderately resistant hybrid and applying a fungicide under high disease risk

conditions provided the greatest return on investment. Both fumonisin and deoxynivalenol were frequently detected from sorghum grain, and mycotoxin contamination was associated with 13 different *Fusarium* species from three distinct species complexes. Based on the results of this work, best management practices for minimizing sorghum disease losses were developed for the mid-Atlantic region.

DEDICATION

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CHAPTER 1: Literature Review

Introduction

Sorghum [*Sorghum bicolor* (L.)] is a crop with multiple uses that is cultivated for grain (food and feed), forage, silage, and sugar. It is a staple food to more than 5 million people and is grown mostly in warm regions of more than 100 countries (Marley et al. 2005). In terms of produciton, sorghum ranks third among cereal grains in the United States (www.grains.org) and is the fifth most important grain crop grown in the world after maize, wheat, rice, and barley. In the U.S., South America, and Australia, sorghum is mainly used for livestock feed in the poultry, beef and pork industries (Vanderlip 1993). More recently, sorghum has appeared in the gluten free food products in the U.S. (www.sorghumgrowers.com). It is also viewed as a promising source of ethanol (Cuevas et al. 2014b). In African countries, it is used in the production of fermented and unfermented beverages (Marley et al. 2005). One of the major advantages of sorghum is its ability to survive in relatively harsher climatic conditions and on marginal lands (Thakur and Mathur 2000). The climatic conditions of southern U.S. provide favorable conditions for sorghum cultivation. The U.S. is a top exporter of grain sorghum, exporting almost 30% of the global trade (www.grains.org). Interest in growing sorghum is increasing in mid-Atlantic mainly due to the demand for locally sourced animal feed. More recently, changes in precipitation patterns and fluctuations in daily temperature are reported due to climate change (Alexander et al. 2006). Since sorghum is drought tolerant and can grow well on marginal lands, it might be a preferred crop to corn and soybean for the region in the future.

There are a number of factors that hinder the production of such an important crop including biotic factors namely diseases and insect-pests. Among many different diseases

caused by viruses, bacteria and fungi, the two major diseases of sorghum in the mid-Atlantic U.S. that impact both grain yield and quality are sorghum anthracnose and sorghum grain mold.

Sorghum Anthracnose

Sorghum anthracnose, a disease caused by a fungus (*Colletotrichum sublineolum* P. Henn in Kabát and Bubk [formerly *C. graminicola* (Ces.) G. W. Wilson]) is one of the most destructive diseases capable of infecting all the aerial parts of the plant including leaves, leaf sheaths, stalk, panicle, and seed (Thakur and Mathur 2000). In addition, it degrades both the quantity and quality of grain and stover (Prom et al. 2012b; Tesso et al. 2012). It is a polycyclic disease and infection is enhanced by rain splash and contact of leaves with infested soil (Ngugi et al. 2000; Thakur and Mathur 2000). This fungus shows hemibiotrophic interaction with the host (Crouch and Beirn 2009; Wharton et al. 2001). The penetration of the host by the fungus occurs within 42 hours of conidia coming in contact with the plant surface, which is followed by a short biotrophic phase of about 24 h (Wharton et al. 2001). The necrotrophic phase begins after 66 h of penetration and rapidly degrades the host tissue (Wharton et al. 2001). Anthracnose is prevalent in regions of high rainfall and severity is high during extended periods of cloudy, warm and humid weather (Thakur and Mathur 2000). Expansion of the lesion and formation of acervuli in the lesion is favored by low light conditions coupled with warmer temperatures close to 30^{o}C (Crouch and Beirn 2009). The development of anthracnose was reported to be slow during the early growth stages while it was rapid after flowering through the grain filling stage (Li and TeBeest 2009; Ngugi et al. 2000). Foliar anthracnose is considered the most common type of anthracnose, which interferes with the photosynthate accumulation by covering the leaf surface with anthracnose lesions. Yield losses as high as 50% have been reported in susceptible cultivars under severe infection (Thakur and Mathur 2000). Symptoms of anthracnose can occur

at any stage of plant development but usually appear at flag leaf emmergence (30-40 days after seedling emergence) or later (Thakur and Mathur 2000; Vanderlip 1993). Typical foliar symptoms appear as small, circular, elliptical, or elongated spots that have gray to straw-colored centers with margins having tan, orange, or red to blackish purple color. However, leaf symptoms can vary due to differences in the pathogen genotype, host reaction or the physiological stage of the host during infection and environment (Tesso et al. 2012). Under favorable conditions, the number or size of spots can increase and coalesce covering the majority of the leaf area. At the center of the spots, fruiting bodies of the fungi, acervuli, develop which are small, circular, black dots with a small, black, hair-like structure protruding from it called setae. Within the acervuli, conidia are excreted in a mucilaginous matrix containing glycoprotein that serves as an anti-desiccant allowing conidia to survive under adverse conditions (Leite and Nicholson 1992). Water is an important component in the infection process of this pathogen that reduces the concentration of the strong germination inhibitor (mycosporine-alanine) in the mucilaginous matrix and allows conidia to germinate (Leite and Nicholson 1992) when splashed to a susceptible host tissue. In severe cases, premature senescence and severe defoliation occur (Thakur and Mathur 2000). Symptoms and signs in stalk, midrib, leaf sheath, panicle and seed are similar to those observed in leaf. Yield losses of 2-15% can result from panicle anthracnose, however, up to 30-50% can occur following severe epidemics (Thakur and Mathur 2000). Panicles or heads from the infected plants are usually small, lightweight and grains ripen prematurely (Thakur and Mathur 2000). Infected heads do not develop normally resulting in partial sterility, however severe infection results in completely discolored grains in the head (Thakur and Mathur 2000).

Primary sources of inoculum for sorghum anthracnose include microsclerotia, mycelia, infected seeds and weed hosts. As mycelia, *C. sublineola* can survive on crop residues and on soil surfaces for up to 18 months but not in buried residue. Microsclerotia, produced in the stalks of susceptible sorghum hybrids, can survive on soil surfaces or in buried residue for longer periods of time (Tesso et al. 2012; Thakur and Mathur 2000). Seed transmission is also important, and Cardwell et al. (1989) showed that 23% of seedlings emerging from infected seeds were found to exhibit both poor germinaiton and anthracnose symptoms. Two common weed hosts, Johnson grass (*Sorghum halepense*) and centipede grass (*Eremochloa ophiuroides*), can help the pathogen survive in the absence of a sorghum host (Crouch and Beirn 2009). Conidia produced by the pathogen ensure the secondary spread, and *C. sublineola* produces two types of conidia: falcate conidia mostly in solid media and small oval conidia in liquid media (Souza-Paccola et al. 2003). Souza-Paccola et al. (2003) found both types of conidia to be pathogenic, developing typical anthracnose symptoms on sorghum plants. The authors further suggested considering the role of oval conidia in the studies that involve virulence testing and development of disease, particularly in the strains that do not produce falcate conidia.

In the past, *C. graminicola* was considered the causal agent of anthracnose on cereals that included corn and sorghum. However, rDNA sequence identified *C. sublineola* as a separate species and was confirmed as the species causing anthracnose in sorghum (Sherriff et al. 1995). Several studies have also confirmed *C. sublineola* is a heterogenous species with diverse pathotypes (Costa et al. 2011; Moore et al. 2010b; Rosewich et al. 1998). Within *C. sublineola*, differentiation of pathotypes has previously been characterized based on the conidial morphology and culture characteristics, but this is not sufficient for characterizing genetic diversity as the environmental conditions would have a high influence on the morphological trait

(Prom et al. 2012b). One explanation for the presence of highly diverse pathotypes, despite the absence of sexual reproduction in nature, is through parasex which was confirmed by the occurrence of heterokaryosis (Souza-Paccola et al. 2003). Another study in Brazil using mutants resistant to chlorate and unable to use nitrate (Nit mutants) suggested that the genetic instability in the strains of *C. sublineola* could be due to transposable elements activity, despite the same genetic background compared to the original strains (Favaro et al. 2007). Using different sets of host differentials, more than 40 different pathotypes have been reported from different parts of the world (Thakur et al. 2007). However, based on DNA comparisons, the diversity was found to be less than expected (Neya and Le Normand 1998; Prom et al. 2012b; Rosewich et al. 1998; Tesso et al. 2012). The study by Prom et al. (2012) found a high genetic similarity between the isolates from Texas, Arkansas, Georgia and Puerto Rico even though the polymorphism was high among the isolates. Furthermore, they did not find any correlation between genetic distances of the isolates with the geographical distances that they were collected from, and all the isolates were of the same mating type, suggesting long distance dispersal of the pathogen. Due to the diversity in *C. sublineola* pathotypes, differences in levels of virulence as well as host genotype specificity have been found (Prom et al. 2012b; Were and Ochuodho 2012). For instance, Were and Ochuodho (2012) found isolates from stems and panicles more virulent than isolates from leaves when inoculated in 20 different host genotypes. At a location, one pathotype can dominate the others in the population as noticed by Casela et al. (2001) when the same amount of inoculum was used to inoculate. Hence, identification of many pathotypes with differences in the level of virulence and host genotype specificity suggests a gene-for-gene interaction of *C. sublineola* with sorghum, with numerous potential *Avr* genes present (Costa et al. 2011; Prom et al. 2012b). However, several studies have suggested

involvement of more than one gene in the resistance response (Biruma et al. 2012; Burrell et al. 2015) . Also, the variability of *C. sublineola* has complicated the use of host resistance in managing sorghum anthracnose.

Numerous studies have been conducted with the aim of identifying resistance genes in sorghum that can be deployed in commercial cultivars (Cuevas et al. 2014a; Marley et al. 2005; Moore et al. 2009; Prom et al. 2012b; Thakur and Mathur 2000). A number of putative resistance genes (dominant as well as recessive) and loci conferring resistance to specific *C. sublineola* genotypes have been identified (Biruma et al. 2012; Costa et al. 2011; Erpelding and Prom 2004; Mehta et al. 2005; Murali Mohan et al. 2010; Perumal et al. 2009; Ramasamy et al. 2008). In addition, sorghum genotypes with dilatory resistance, characterized by slow development of the disease, have also been documented (Buiate et al. 2010; Casela et al. 2001b). However, the host resistance approach has not succeeded due to the diversity in the pathogen population that can adapt rapidly to the changes in the resistant sorghum lines deployed. In addition, lines/ hybrids with disease resistance in one geographical area may be susceptible in another area (Ali and Warren 1987) and the breakdown of resistance in a very short period of time under different environmental conditions has also been reported (Wharton et al. 2001). A resistance gene (*Cg1*) contributed by a sorghum genotype 'SC748-5' on chromosome 5 was found to be effective against sorghum anthracnose across five different environmental conditions tested (Mehta et al. 2005; Perumal et al. 2009). However, a recent study suggested that anthracnose resistance in 'SC748-5' is not controlled by a single gene but by several resistance genes in a linkage block (Burrell et al. 2015). Four additional AFLP markers that co-segregated with the *Cg1* have also been identified (Perumal et al. 2009). An AFLP marker, *Xtxa6227,* mapped within 1.8cM of the resistance locus while another

polymorphic simple sequence repeat (SSR) marker, *Xtxp549*, identified by sequencing the BAC clones, mapped within 3.6cM of the resistance locus (Perumal et al. 2009). In another study, eight loci associated with disease resistance were identified by association analysis, using mini core collection of sorghum landraces at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) (Upadhyaya et al. 2013b). However, no information on the introgression of these genes and/or loci into an elite sorghum cultivar is available. Therefore, to identify resistant sorghum genotypes for a location, Prom et al. (2012) suggested screening of sorghum lines with a mix of local *C. sublineola* isolates in the target environment as a practical way of coping with this problem. Limited successes in utilizing host resistance and the absence of a standard set of differentials have hindered the widespread use of resistance genes for anthracnose management (Prom et al. 2012b; Thakur and Mathur 2000). Nonetheless, exotic sorghum germplasms are continuously being evaluated to identify new sources of resistance that are stable across environments and more than 160 sorghum accessions from different parts of the world have been identified as potential sources of resistance to anthracnose (Cuevas et al. 2014d; Erpelding and Prom 2004, 2006; Pande et al. 1994; Prom et al. 2011; Tesso et al. 2012).

Apart from host resistance, some additional research has been done in an attempt to manage sorghum anthracnose. In 2009, Moore et al. evaluated the effect of previous cropping history on grain sorghum yields and on severity of sorghum anthracnose. They found that planting of sorghum a year after maize, soybeans or rice significantly reduced sorghum anthracnose severity on most of the hybrids used in the study compared to sorghum followed by sorghum. In addition, a number of different cultural practices such as field sanitation, elimination of weed hosts, clean planting, adjusting planting date, use of healthy seeds or fungicide-treated seeds, soil solarization, and use of bio-controls are also recommended as

measures to manage anthracnose in problematic fields (Marley et al. 2005; Moore et al. 2009; Singh 2008; Thakur and Mathur 2000).

Ngugi et al. (2000) found that delayed planting reduced time for disease onset probably due to increased latent period of pathogen, which when combined with the use of resistant cultivars resulted in the lower disease level. Similar observations were made by Gwary et al. (2008) in Nigeria where delayed planting (14th July) had significantly lower disease severity compared to the early planting (30th June). However, early planting resulted in significantly higher yield compared to the later planting despite the disease pressure.

Eleven different fungicides were tested in Brazil (tebuconazole, benomyl, mancozeb, triforine, fenarimol, prochloraz, imibenconazole, azoxystrobin, chlorothalonil, captan and carbendazim), and these were applied three times starting at the moderate incidence of foliar anthracnose at 10 days interval (Pinto 2003). The results showed that fungicides prochloraz, carbendazim, benomyl and azoxystrobin were most efficient in the controlling leaf anthracnose. Gwary et al. (2008) also tested seed treatment fungicides, Apron plus 50DS (metalaxyl+carboxin) and Super homai 70% WP (thiram+thiophanate methyl), in three local sorghum varieties and found that both the fungicides were effective in reducing leaf anthracnose severity up to 70 days after sowing. An integration of host resistance with application of fungicides, particularly combination of foliar application and seed treatment gave an effective control of anthracnose and increased grain yield in all the varieties under study (Marley 2004). In this study, seed treatment with fungicides (metalaxyl + carboxin + furathiocarb) alone did not control the disease while foliar fungicide (3 sprays of benomyl at weekly intervals) alone or in combination with seed treatment was effective and provided better yields compared to the control. However, additional chemistries have been recently labelled for sorghum, and these

newer chemistries have not been tested for their efficacies in controlling sorghum anthracnose. In a preliminary study in 2013 by Mehl and coworkers (Dr. Mehl, personal communication), a single application of the fungicide Headline (pyraclostrobin) at first flower stage reduced anthracnose severity by 15% compared to other registered fungicides and also resulted in reduced peduncle anthracnose. Based on the experiments in 2014, a single early flowering application resulted in the highest yield, however, the later application (14-days after 50% heading) resulted in higher test weight (Dr. Mehl, personal communication). In another study, Resende et al. (2013) found that application of silicon 30 days before sowing seeds can suppress the anthracnose progress. In the study, silicon as calcium silicate used at the rate of 6 tons per ha, with or without fungicide (Opera) reduced the anthracnose by 44% and 37%, respectively. They further suggest using silicon could be beneficial in reducing the number of fungicide applications which in turn reduces production cost and chances of developing fungicide resistance in the pathogen. Since the use of host resistance alone has not proven durable, integration of host resistance with the judicial use of fungicides is the best approach for increasing the yield and also managing the pathogen population below the economic threshold level.

Grain Mold

Grain mold, another major constraint in sorghum production, is a disease complex and more than 40 different genera of fungi have been reported to be involved (Singh and Bandyopadhyay 2000). Species of *Fusarium, Curvularia, Alternaria, Poma* and *Colletotrichum sublineola* are considered of major concern (Singh and Bandyopadhyay 2000), but the fungi associated with grain mold varies geographically. For instance, a study in India found *Fusarium moliniforme, F. pallidoroseum, Curvularia lunata, Cladosporium oxysporium, Bipolaris*

australiensis and *Poma sorghina* associated with grain mold (Bandyopadhyay et al. 1991a) while a study in South Africa found *Fusarium thapsinum, F. graminearum, Curvularia lunata, Alternaria alternata* and *Poma sorghina* to be associated (Mpofu and McLaren 2014). Yield losses of 30-100% have been reported in sorghum depending on the cultivar, time of flowering, maturity, and soil type. Among different fungal species associated with grain mold in sorghum, species of *Fusarium, Aspergillus* and *Penicillium* are of major concern because they produce different types of mycotoxins (Apeh et al. 2016; Chala et al. 2014; Kange et al. 2015; Nagaraja et al. 2016; Osman et al. 2017; Oueslati et al. 2014). Contamination of grain sorghum with mycotoxins produced by *Aspergillus* spp. and *Penicillium* spp. are of major concern in countries in Africa, South America and Asia (Apeh et al. 2016; Ayalew et al. 2006; Chala et al. 2014; González et al. 1997; Hussaini et al. 2009; Kange et al. 2015; Osman et al. 2017; Silva et al. 2000; Yassin et al. 2010) while this problem is minimal in North America where *Aspergillus* spp. and *Penicillium* spp. and associated mycotoxins are seldom detected from grain sorghum (Bluhm and Faske 2015; Hagler et al. 1987; Prom et al. 2015). However, *Fusarium* spp. and their associated mycotoxins are of major concern in sorghum production regions worldwide and species within *Fusarium* also vary by geographical location. Seven *Fusarium* species (*F. thapsinum, F. proliferatum F. andiyazi, F. sacchari, F. verticillioides, F. graminearum* and members of the *F. incarnatum equiseti* species complex) were isolated from grain sorghum in India (Apeh et al. 2016; Divakara et al. 2014; Sharma et al. 2011), five species (*F. moniliforme, F. semitectum, F. graminearum, F. equiseti, F. solani,* and *F. avenaceum*) in Egypt (Osman et al. 2017), four species (*F. nygamai, F. semitectum, F. thapsinum* and *F. verticillioides*) in Saudi Arabia (Yassin et al. 2010), five species (*F. oxysporum, F. semitectum, F. solani, F. verticillioides,* and *F. equiseti*) in Nigeria (Hussaini et

al. 2009), two species (*F. proliferatum* and *F. graminearum*) in South Africa (Tarekegn et al. 2006), three species (*F. proliferatum, F. graminearum* and *F. thapsinum*) in Ethiopia (Tarekegn et al. 2004), fourteen species (*F. moniliforme, F. semitectum, F. proliferatum, F. graminearum, F. equiseti* in higher frequency and *F. sporotrichioides, F. avenaceum, F. heterosporum, F. sambucinum, F. subglutinans, F. oxysporum, F. solani, F. napiforme* and *F. chlamydosporum* in lower frequency) in Argentina (González et al. 1997; Saubois et al. 1999) and at least ten species (*F. thapsinum, F. proliferatum, F. verticillioides, F. semitectum, F. incarnatum equiseti* species complex, *Fusarium fujikuroi* species complex, *F. chlamydosporum, F. andiyazi, F. nygamai,* and *F. pseudonygamai*) in the United States (Bluhm and Faske 2015; Leslie et al. 2005; Prom et al. 2015). This indicates that species in the *F. fujikuroi* species complex are consistently present in the grain mold complex throughout the world. A majority of these *Fusarium* species found in grain mold are also associated with stalk rot in different sorghum producing regions (Kelly et al. 2017; Petrovic et al. 2009; Tesso et al. 2010).

The source of inoculum for grain mold can be soil-borne fungi or plant debris on soil surfaces, additional crop hosts as grain mold fungi are non-host specific, or fungal spores that are naturally available in the air over sorghum fields that can initiate disease epidemics under favorable environmental conditions (Bandyopadhyay et al. 1991a; Ratnadass et al. 2003). Members of the *Fusarium fujikuroi* species complex that can be the source of inoculum for grain mold were found in soil (Funnell-Harris and Pedersen 2011). Similarly, *F. thapsinum, F. verticilloides, F. proliferatum,* and *F. andiyazi* in *F. fujikuroi* species complex and *F. graminearum, F. subglutinans* and several members of *F. incarnatum-equiseti* species complex were found in the air samples above sorghum fields (Funnell-Harris and Pedersen 2011).

The development of grain mold signs and symptoms depends on the fungi involved, and time and severity of infection, and these can include include grains with pink, orange, gray, white, or black discoloration. Rao et al. (2012) broadly categorized grain mold into pre-physiological grain deterioration and grain weathering. Pre-physiological grain deterioration is caused by a small number of fungal species that interact pathologically and/or saprophytically with the developing grain interfering with grain filling, yield reduction, grain quality and grain weight (Rao et al. 2012). The fungi in this category are the primary players in causing the disease including the mycotoxin producers. Grain weathering is a post physiological maturity problem and the fungi involved colonize the exposed parts of the grain saprophytically causing grain discoloration and tissue damage when the weather is wet. One example of grain weathering fungi are *Aspergillus* spp. which also produce mycotoxins and further deteriorate the grain quality after harvest and in storage. Grain contaminated with mycotoxins can be rejected by the processing industries because of its toxicity to humans and animals. However, timely harvest and grain drying can prevent grain weathering fungi (Rao et al. 2012).

The time for grain mold infection is from flowering/anthesis to maturity with milk and soft dough stage being the most suscpetible (Funnell-Harris and Pedersen 2011; Melake-Berhan et al. 1996; Navi et al. 2005; Tarekegn et al. 2004). The major routes of infection by grain mold fungi are suggested to be through the florets and developing grains (Navi et al. 2005). In a review, Chandrashekar and Satyanarayana (2006) mentioned the pericarp as the first site of infection after which the endosperm is infected. They further elucidated that *Curvularia* spp. and *Fusarium* spp. spread from the hylar region on the pericarp surface to the endosperm while *Phoma* were restricted to the pericarp. Furthermore, insect infestation (e.g.: sorghum head-bugs) also allows for easy penetration of *Fusarium* spp. and other grain mold fungi into the developing

grain and worsens the condition either by opening up avenues for the fungi though feeding or suppressing host defenses (Bluhm and Faske 2015; Ratnadass et al. 2003; Sharma et al. 2000). Variation in the infection frequency among fungi indicates that individual fungi in grain mold might have different windows for maximum infection during the grain development stages (Navi et al. 2005). Similar conclusions can be drawn from the study by Mpofu and McLaren (2014) where they did not find any correlation among the fungi infecting grain with an exception for *Alternaria alternata* and *F. thapsinum*. Another study found that inoculation of sorghum cultivars with a mixture of fungi (*Fusarium thapsinum* and *Curvularia lunata*) did not result in significantly higher level of grain mold severity when compared to individual fungal treatments (Prom et al. 2003). The same study resulted in re-isolation of *C. lunata* in higher frequency than *F. thapsinum,* and *C. lunata* had the most negative effect on germination of the grains. A significant amount of fungal biomass was accumulated for *C. lunata* and *P. sorghina* across all the sorghum genotypes used in a study indicating that they caused significant damage compared to the other fungi (Mpofu and McLaren 2014). *A. alternata* was deduced to be a superficial fungus without contributing any significant damage as it was grouped with control in a dendogram analysis, while Little et al. (2012) identified *Fusarium* spp., *Curvularia* spp. and *Alternaria* spp. as major pathogens in their study. Mpofu and McLaren (2014) also found that *F. graminearum* caused very little grain mold infection and *F. thapsinum* showed significant genotype specificity. Hence, environmental conditions at a location might have also played a role in determining the pathogenicity of a particular fungal species. Studies from India found that host genetics had greater influence in grain mold occurring before physiological maturity while environment had greater influence in grain mold occurring after the physiological maturity (Ambekar et al. 2011; Audilakshmi et al. 2011). This indicates that evaluation of

sorghum germplasm for grain mold resistance should be done at or before physiological maturity. In addition, pathogens causing grain mold vary from location to location and at a particular location some pathogens might be predominant compared to the others (Little et al. 2012). This can make the deployment of pathogen-specific resistance genes ineffective across diverse regions (Little et al. 2012) but two loci linked to grain mold resistance have been identified with one non-host resistance gene *Rxo1*, originally discovered from corn (Upadhyaya et al. 2013a). Similarly, five quantitative trait loci for grain mold resistance have been mapped on chromosomes 4, 6, 7, 9 and 10 (Klein et al. 2001). Besides these, a number of evaluations of sorghum genotypes from different parts of the world, that included accessions and cultivars, have led to the identification of sorghum genotypes that can be a good source of resistance to tackle this disease (Cuevas et al. 2016; Cuevas et al. 2014b; Prom and Erpelding 2009; Prom et al. 2011; Prom et al. 2014; Sharma et al. 2010). However, none of them have been integrated into the elite germplasm for use by the growers (Ambekar et al. 2011).

Presence of certain biochemical compounds in grains is thought to be an indicator of mold resistance. Ergosterol content in the grain has been deployed as criteria for selecting resistance in sorghum germplasm. Hybrids with high ergosterol and white colored grain were susceptible to grain mold compared to low ergosterol containing and red colored hybrids (Rao et al. 2012). Grain mold resistance were also observed in sorghum germplasm with harder grain, higher levels of seed phenols and darker glumes (Audilakshmi et al. 1999). Sorghum hybrids with higher content of proanthocyanidins (Pas), 3 – deoxyanthocyanidins (3-DAs) and flavan-4-ols were resistant to biotic and abiotic stresses, including grain mold (Dicko et al. 2005). However, flavon-4-ols content in the grains was not suggested to be a good indicator for identifying resistant hybrids because white grained hybrids had negligible amounts of flavan-4-

ols compared to that of red grained hybrids and were resistant to grain mold (Audilakshmi et al. 1999; Rao et al. 2012). Sorghum seed antifungal proteins (AFPs) are also known to inhibit spore germination of several grain mold fungi including some of the mycotoxin producers (Seetharaman et al. 1997). AFPs increase during grain development but are reported to leach out of immature grains upon imbibition (Seetharaman et al. 1996) which makes it susceptible to mold infection when relative humidity (RH) or precipitation are high after anthesis. A report from Arkansas showed that *Aspergillus* isolation and aflatoxin contamination was rare in grain sorghum even though they were isolated from corn every year (Bluhm and Faske 2015). A similar observation was made in a study in North Carolina where aflatoxin levels were very low in sorghum grains even though deoxynivalenol and zearalenone levels were high (Hagler et al. 1987). This could be due to the low molecular weight proteins present in sorghum seeds that inhibit spore germination and growth of aflatoxin producing *Aspergillus* species (Gosh and Ulaganathan 1996).

Integration of host resistance and late planting in July was beneficial in reducing grain mold severity in Nigeria, however, grain yield was higher in early planting of resistant hybrids which could be due to early access to soil nutrients and early vigor development leading to disease tolerance (Marley 2004). Similar observation was made in Mexico where late planting in July reduced grain mold severity which also reduced grain yield compared to early planting (Montes-Belmont et al. 2003). Environmental factors inevitably have influence in the disease development but little work has been done regarding epidemiology of grain mold, probably due to the large number of pathogens involved that are highly variable across sorghum production regions. High RH during early plant developmental stages and between the end of flowering and harvest was strongly correlated with mold incidence (Ratnadass et al. 2003). The grain mold

increased notably when RH exceeded 95% by providing favorable conditions for infection or by enhancing sporulation. A wetness duration of 16 h was reported as optimal for infection at physiological maturity but required at least 72 h wetness for infection at flowering (Bandyopadhyay et al. 2000). In a study using five cultivars in South Africa, averages of minimum temperature, total rainfall and frequency of rainfall during post-flowering periods were found to be good predictors of grain mold compared to others (Tarekegn et al. 2006). With the increase in averages of minimum temperature and precipitation, the grain mold incidence also increased. In an another study, a wetness duration of 40 h was found to be optimal for most of the grain mold fungi (Navi et al. 2005). However, a study from Mexico (Montes-Belmont et al. 2003) found that the increase in grain mold incidence was predicted by mean temperature but not by mean relative humidity. In the same study, approximately 97% of the grains from field samples also had *Fusarium thapsinum* infestation. Based on the above mentioned studies, the isolation of the fungi causing grain mold depends on the presence and predominance of fungal species and environmental conditions at a particular location. In the mid-Atlantic region of the U.S., the fungi causing grain mold are not characterized. This demands some studies to identify fungal species causing grain mold with particular emphasis on the mycotoxin producers.

Mycotoxins

Mycotoxins are chemically diverse and harmful secondary metabolites that spoil about twenty five percent of the food globally (Waliyar et al. 2008). In the U.S. alone, over $1.5 billion in crop losses is estimated to occur annually due to mycotoxin contamination (Waliyar et al. 2008). Mycotoxin contamination can occur at pre-harvest during crop development and postharvest during storage, processing, transportation, or marketing. More than 400 mycotoxins are known today, however, aflatoxins, fumonisins, ochratoxins, zearalenone, and trichothecenes

are widely investigated because of their frequent occurrence and adverse health effects on humans and animals (Ediage et al. 2015). Only certain strains of some fungal species causing grain mold produce mycotoxins (Waliyar et al. 2008), and some of them are also involved in pathogenesis and antagonism (Mulè et al. 2005). Trichothecenes, zearalenone, fumonisin, aflatoxins, and alternaria toxins are some important mycotoxins produced by grain mold fungi, but fumonisin and aflatoxin are the two major problems in sorghum (Chala et al. 2014; Osman et al. 2017; Silva et al. 2000; Waliyar et al. 2008) even though other mycotoxins such as deoxynivalenol (DON) and zearalenon (ZEA) have been frequently detected (Chala et al. 2014; Hagler et al. 1987). Most of the mycotoxin studies have focused on the occurrence and toxicology of single mycotoxins and regulations also do not consider the combined effects of mycotoxins (Smith et al. 2016). However, combinations of mycotoxins are commonly present in food and feed, and co-occurrence of mycotoxins and their interaction is of major concern at present since there is very limited toxicological information on their simultaneous exposure effects (Smith et al. 2016; Waliyar et al. 2008).

Compared to corn, mycotoxin contamination severity is low in sorghum due to the hard seed coat (Waliyar et al. 2008). Conditions that favor grain mold, insect infestation, delayed harvesting and improper drying and storage are important factors contributing to mycotoxin production (Waliyar et al. 2008). Mycotoxin can enter human food chain directly through the consumption of contaminated cereals or indirectly through animal products (e.g. milk) when animals are fed with mycotoxin contaminated grains. In the U.S., countries in South America, and Australia, sorghum is mainly used for livestock feed in the poultry, beef and pork industries (Vanderlip 1993) and not for human consumption so livestock mycotoxicosis is of major concern in these countries. For U.S., the regulatory level for aflatoxin is 20 ppb and 100 ppb, for

DON is 1ppm and 5-10 ppm, and for fumonisin is 2-3 ppm and 20 ppm, for human and animal consumption, respectively, while zearalenone does not have a standard regulation (FDA, accessed Jan 6, 2019). In the mid-Atlantic U.S., cropping systems include wheat, corn, and sorghum. Since *Fusarium graminearum* and related trichothecene-producing species are commonly associated with wheat and corn, some of these *Fusarium* species in the may be involved in sorghum grain mold. Furthermore, high levels of deoxynivalenol (DON), a mycotoxin produced by *F. graminearum* and related species, have been detected in some sorghum samples from Virginia and North Carolina (Dr. Mehl, personal communication). In a preliminary study in 2013, Mehl and coworkers (personal communication) observed a high percentage of sorghum head infection indicating a high impact on yield and quality of grains. In the study, average grain mold symptoms for hybrids ranged from 7.5% to 28.5%. Thus, it is important to identify the diversity of *Fusarium* species that are present in the sorghum grain mold complex in mid-Atlantic region and their mycotoxin-producing potential in order to develop management approaches to minimize yield and quality losses in the region.

Research Objectives

The main goal of this research was to develop management strategies to protect yield and maximize farm profitability by controlling anthracnose and grain mold of sorghum using chemicals and/or host resistance. The specific objectives were to: (1) determine the effectiveness of fungicides and their application timing for the management of sorghum foliar anthracnose, (2) compare grain sorghum hybrids for their susceptibility to foliar anthracnose, grain mold and mycotoxin contamination under field conditions, (3) assess the value of integrating host resistance and judicious use of fungicides to manage sorghum anthracnose and grain mold, and

(4) identify *Fusarium* spp. associated with grain mold and mycotoxin contamination of sorghum in the mid-Atlantic U.S.

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CHAPTER 2: Effectiveness of fungicides and their application timing for the management of sorghum foliar anthracnose in the mid-Atlantic U.S.

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Abstract

Sorghum anthracnose (*Colletotrichum sublineola*) reduces grain yield by up to 50%, but suggested management alternatives have not been developed for the mid-Atlantic U.S. where warm, wet conditions favor disease. Under factorial arrangement, five fungicides plus a non-treated and four application timings were compared for foliar anthracnose control, yield, and profitability of fungicide use in grain sorghum over eight site-years in Virginia and North Carolina. Anthracnose severity was rated at the hard dough stage and grain yield was determined at harvest and treatment effects were compared by experiment. Every percent increase in disease severity resulted in yield losses of 27 kg/ha to 85 kg/ha. Pyraclostrobin and pyraclostrobin + fluxapyroxad reduced anthracnose ($P<0.001$), and three applications resulted in less disease and greater yield compared to single applications ($P<0.001$). However, three applications would exceed the labeled maximum application for the fungicides and would not be economical. Among single applications, those at boot or flowering reduced disease and flowering applications resulted in the overall greatest yield. Our results suggest that when disease onset occurs at boot or prior a single application of a pyraclostrobin-containing fungicide no later than flowering reduces anthracnose, protects yield, and increases income. However, in the absence of disease or at low severity until flowering, application of a fungicide may not be profitable.

Introduction

Sorghum [*Sorghum bicolor* (L.)] is a cereal crop grown on nearly 43 million ha worldwide in the tropics, subtropics and semi-arid regions (FAS, USDA, 2018). It is grown for food, feed, forage, fiber, sugar, fuel, and alcoholic and non-alcoholic beverages (Cuevas et al.

2014c; Marley et al. 2005). In the United States, sorghum is used primarily as a feed by poultry, beef and pork industries (Vanderlip 1993) and is grown in semi-arid regions including Kansas and Texas, where a majority of the U.S. sorghum is produced (USDA, 2018). In the mid-Atlantic region of the U.S., specifically in Virginia and North Carolina, sorghum production has increased due to demand for locally sourced animal feed that cannot be met by current corn production. Sorghum yields well relative to corn in harsher climatic conditions and on marginal land (Thakur and Mathur 2000), thus sorghum can be grown in the drought prone areas in the region that are not suitable for corn production. Sorghum acreage in the region is increasing with combined acreage in Virginia and North Carolina increasing from approximately 8,000 ha in 2005 to approximately 24,000 ha in 2016 (Balota et al. 2018).

Anthracnose [*Colletotrichum sublineola* P. Henn in Kabát and Bubk (formerly *C. graminicola* (Ces.) G. W. Wilson)] is an economically important disease of sorghum worldwide. The fungus can infect all aerial parts of the plant including leaves, leaf sheaths, stalks, panicles, and grains (Thakur and Mathur 2000). Foliar anthracnose is considered the most common and economically devastating stage of the disease since it interferes with photosynthate accumulation by covering the leaf surface, thereby reducing yield. Environmental conditions during the growing season in the mid-Atlantic are typically favorable for anthracnose development. Disease outbreaks occur during periods of high rainfall or humidity greater than 90% and when the temperature ranges from 25 to 30^{o}C (Pande et al. 1994). Typical foliar symptoms appear as small, circular, elliptical, or elongated spots that have gray to straw-colored centers with visible setae from acervuli that can coalesce and cover the entire leaf at advanced stages (Thakur and Mathur 2000). Anthracnose is a polycyclic disease and infection is enhanced by rain and contact of leaves with infested soil (Ngugi et al. 2000; Thakur and Mathur 2000). In

severe cases, defoliation and premature senescence occurs (Thakur and Mathur 2000). Under favorable conditions, development of anthracnose is more rapid from flowering through the grain filling stage compared to early developmental stages of sorghum (Li and TeBeest 2009; Ngugi et al. 2000; Pande et al. 1994). The pathogen can overwinter in crop debris as microsclerotia and mycelia, in infected seed and on the alternative host, Johnsongrass [*Sorghum halepense* (L.) Pers.] (Cardwell et al. 1989; D. M. Gwary et al. 2006; Tesso et al. 2012; Thakur and Mathur 2000).

Fungicide use in cereal crops depends on the economic importance of the crop and the impact of the disease on yield (Poole and Arnaudin 2014). Anthracnose severity as low as 20% can impact yield (Ngugi et al. 2000) and yield losses as high as 50% have been reported in susceptible sorghum cultivars (Thakur and Mathur 2000). In other sorghum producing countries such as Nigeria and Brazil, use of fungicides (seed and foliar) have proven successful in managing this disease (Gwary et al. 2008; Marley 2004; Pinto 2003). Gwary et al. (2008) reported that seed-treated with fungicides, metalaxyl + carboxin (Apron plus 50DS) and thiram + thiophanate methyl (Super homai 70% WP) presented reductions in leaf anthracnose severity up to 70 days after planting compared to non-treated controls, but yield differences were not observed. Similarly, three foliar applications of benomyl at 7-day intervals beginning at disease onset resulted in lower anthracnose severity and increased yield compared to the non-treated control (Marley 2004). Pinto et al. (2003) also found that three applications of fungicides; procloraz, carbendazim, benomyl, and azoxystrobin at 10-day intervals were effective in controlling the disease and increasing yield. However, multiple foliar fungicide applications are unlikely to be economical for sorghum growers (Marley et al. 2005), and a single optimum application timing was not determined in these studies.

Diseases in semi-arid regions are managed through breeding for genetic resistance and seed treatment fungicides, but not foliar fungicides (Erpelding 2008b; Thakur and Mathur 2000) due to inconsistent occurrence and low severity of foliar diseases. However, with the subtropical conditions in the mid-Atlantic U.S., anthracnose severity is greater than that in semi-arid regions of U.S. A majority of the sorghum hybrids grown in the U.S. have been bred to be adapted to semi-arid environments, thus, high yield producing hybrids may be susceptible to fungal diseases including anthracnose when grown in humid, wet environments. The effectiveness of foliar fungicides and optimum application timings have not been evaluated for sorghum anthracnose management in the mid-Atlantic region. We hypothesized that at least one fungicide when applied at an appropriate developmental stage will reduce anthracnose severity and protect grain yield and farm profitability. Therefore, the objectives of this study were to: 1) compare five fungicides and four application timings for anthracnose control and yield response; 2) estimate yield losses associated with sorghum anthracnose in the mid-Atlantic region; and 3) evaluate the profitability of foliar fungicide applications in sorghum production in the region.

Materials and Methods

Field trials. Field experiments were conducted over eight site-years in Virginia and North Carolina from 2015 to 2017. Anthracnose susceptible sorghum hybrid, '84P80' (DuPont Pioneer, Johnston, IA), was planted at a rate of approximately 193,000 seeds/ha. Information on previous crop, planting date, fungicide applications, date of anthracnose onset, and harvest date are provided in Table 1. Soil fertility, insects and weeds were managed following standard practices for sorghum in the region (Heiniger et al. 2011). In 2016 and 2017, sugarcane aphids were controlled by using sulfoxaflor (as 0.1 liter/ha of Transform, Dow AgroSciences,

Indianapolis, IN) or flupyradifurone (as 0.4 liter/ha of Sivanto, Bayer CropScience, Research Triangle, NC) and alternating the chemistry when multiple sprays were required.

Fungicides and application timings. The experiment included a 6 × 4 factorial arrangement of treatments in a randomized complete block design. The main factors were fungicide (non-treated control or one of five different fungicides) and application timing. Fungicides and rates were: azoxystrobin (as 0.44 liter/ha of Quadris EC, Syngenta Crop Protection, Greensboro, NC); pyraclostrobin (as 0.44 liter/ha of Headline EC , BASF Corporation, Research Triangle Park, NC); picoxystrobin (as 0.44 liter/ha of Aproach EC, DuPont Crop Protection, Wilmington, DE); pyraclostrobin + fluxapyroxad (as 0.29 liter/ha of Priaxor EC, BASF Corporation, Research Triangle Park, NC); and propiconazole (as 0.29 liter/ha of Tilt EC, Syngenta Crop Protection). The four application timings were boot (head enclosed in a swollen leaf sheath); flower (beginning of flowering in 50% of the plants); late (14-days post flower application), or applications at all three stages (Vanderlip 1993). Each site had three or four replications with 2.4 m fallow alleys between blocks. Each treatment plot consisted of four ro ws that were 9 m long by 3.7 m wide except for experiments at Kinston in 2016 and 2017 (KS-16 and KS-17) which had three rows with dimensions of 9 m by 2.8 m. Anthracnose severity was evaluated biweekly as a visual estimate of the percentage of leaf area with symptoms and signs of sorghum anthracnose starting at the boot stage.

At the sites in Virginia, fungicide treatments were applied with a Lee Spider Sprayer having eight 8002VS nozzles spaced 46 cm apart delivering 186 liter/ha at 38 psi. At the sites in North Carolina, fungicide treatments were applied with a backpack sprayer having eight XR 11002 flat fan nozzles spaced 46 cm apart delivering 140.3 liter/ha at 30 psi. At the end of the season, grain was harvested from the center two rows of each plot for all the sites except for

Kinston, where all the three rows of a plot were harvested, using a mechanical harvester equipped with a grain gauge and moisture meter. Yield, moisture, and test weight were measured from each plot at harvest, and yield/ha was calculated based on a standard moisture content adjusted to 14%.

Weather data collection. The environmental data for Rocky Mount site-years (RM-15, RM-16, RM-17) and Lewiston-Woodville site-year (LW-17) were recorded at the on-site weather stations. Data for KS-16 and KS-17 were recorded at the weather station at Cunningham Research Station located 4.4 miles North East from the experimental site. The weather data for these site-years were obtained from the website of North Carolina Climate Retrieval and Observations Network of the Southeast (NC CRONOS) database (https://climate.ncsu.edu/cronos). For Suffolk site years, SF-15 and SF-17, weather data were obtained from peanut-cotton infonet (https://webipm.ento.vt.edu/cgi-bin/infonet1.cgi).

Profitability calculations. The profitability of fungicide applications in sorghum were estimated based on average local prices of Priaxor ($120.2/liter), Headline ($108.6/liter), Quadris ($48.2/liter), Aproach ($67.0/liter) and Tilt ($22.1/liter). The estimated machinery cost and labor cost for fungicide applications were $49.3/ha and $33.8/ha, respectively, and the projected price of grain sorghum in Virginia and North Carolina for the year 2018 was $0.17/kg (https://cals.ncsu.edu/are-extension/grain-budgets/). The increase in yield required to cover the cost of use of a fungicide (break-even yield) was determined using the following formula, modified from (Weisz et al. 2011):

Yield (kg/ha) required to cover the cost of using a fungicide = [fungicide cost ($) + machinery and labor cost for fungicide spray ($) per hectare]/[grain sorghum price ($/kg)]

Farm profitability of including a fungicide application in grain sorghum production in the mid-Atlantic was calculated by comparing the monetary return with the non-treated per hectare using the following formula (Lopez et al. 2015):

U.S. $ Net-return = grain sorghum price ($/kg) *[Yield (kg/ha) obtained from a fungicide application – yield (kg/ha) obtained from the non-treated control] – [fungicide cost ($/ha) + machinery and labor cost for fungicide spray ($/ha)]

Statistical analyses. In some experiments, disease onset was late in the season so disease severity could not be evaluated until the hard dough stage, when grains changed color from green to brown and were hard when squeezed. Hence, only the final disease severity evaluation at the hard dough stage was used for statistical analysis and comparison. An initial analysis was performed using block, fungicide, application timing, site-year, and interactions among these factors for anthracnose severity in the model. The effect of site-year and its interactions with fungicide and application timing were significant so each experiment was analyzed separately. Percentage anthracnose severity data were arc sine square root transformed prior to analysis to meet the assumptions of ANOVA. Data for anthracnose severity and yield were analyzed using general linear model procedure (glm) in R version 3.2.2 using the package, agricolae (Mendiburu 2015). In addition, regression analysis of anthracnose severity and yield was done using linear model procedure (lm) in agricolae in order to estimate yield loss associated with disease. Anthracnose severity was subjected to analysis of variance (ANOVA) using a model that included block, fungicide, application timing, and their interactions as sources of variation. Means were compared using Tukey's Honest Significant Difference (HSD) test at the significance level of $\alpha = 0.05$, and back-transformed values are presented.

Results

Field trials. Date of disease onset and foliar anthracnose severity varied among experiments (Table 1, Fig. 1). Five site-years had an onset of anthracnose at or prior to boot stage but anthracnose progression was rapid only at the three of the sites (Fig. 1). The remaining three site-years had late onset of disease and relatively low disease severity throughout the growing season (Fig. 1). When anthracnose was observed at or prior to boot stage, severity was higher later in the season compared to when disease onset occurred after flowering (Table 1, Fig. 1). Mean monthly temperatures were similar among site-years, however, total precipitation differed with some sites receiving more precipitation than others during the growing season (Table 2) providing conducive environmental conditions for anthracnose development. Mean monthly temperature of 27^{o}C and high precipitation of 491 mm in July (Table 2) at RM-16 coincided with the boot growth stage resulting in high anthracnose severity while consistent precipitation of more than 100 mm each month during the growing season with mean temperature close to 25^{o}C resulted in higher anthracnose severity at LW-17. For the remaining site-years, low but frequent precipitation coupled with favorable temperature conditions favored anthracnose severity with an exception of two site-years that had comparatively lower anthracnose severity despite favorable environmental conditions (Table 2, Fig. 1).

Fungicides and application timings

Effect on anthracnose severity. Anthracnose severity varied among experiments (P<0.0001), fungicides (P<0.0001), and application timings (P<0.0001). For eight experiments, disease severity of the non-treated at the hard dough stage of the crop ranged from 4.7% to

71.9% of the leaf area (Table 3). Overall, pyraclostrobin + fluxapyroxad and pyraclostrobin provided the greatest reduction in anthracnose severity followed by azoxystrobin; picoxystrobin and propiconazole treatments were not significantly different from the non-treated (Table 3). Three fungicide applications provided the greatest level of disease control and fungicides applied two weeks after flowering resulted in the least control. In two experiments, there was a significant fungicide by application timing interaction (Table 3). Pyraclostrobin + fluxapyroxad and pyraclostrobin reduced disease severity when applied at the boot, flower, or all three timings, but fungicides did not reduce anthracnose severity when applied two weeks after flowering (Fig.2).

Effect on yield. Yield response to fungicides and application timings varied by experiment. Yield of the non-treated ranged from 1250 kg/ha to 5427 kg/ha (Table 4). Pyraclostrobin + fluxapyroxad and pyraclostrobin were the only fungicides that protected yield (P<0.001) compared to the non-treated, and three applications resulted in higher yield protection compared to a single application (Table 4). In one of the experiments, there was a significant fungicide by application timing interaction and pyraclostrobin + fluxapyroxad and pyraclostrobin protected yield when applied at flower or at all three timings, but did not protect yield when applied at boot or two weeks after flowering (Fig. 3).

Yield losses and anthracnose severity. For three experiments, there was not a relationship between leaf anthracnose severity and yield (Table 5). For the Kinston experiments in 2016 and 2017, there was a significant but poor correlation between disease severity and grain yield. There was a negative linear correlation (P<0.001) of leaf anthracnose severity and yield for three experiments with correlation coefficient (r) values ranging from -0.75 to -0.86

and yield losses ranging from 27 kg/ha to 85 kg/ha due to each percentage of anthracnose on the leaf (Table 5).

Profitability of fungicide applications. Two fungicides, pyraclostrobin + fluxapyroxad and pyraclostrobin, protected yield compared to the non-treated and other fungicides tested in this study (Table 4, Fig.3). Hence, the profitability calculations were performed for a single application of these two fungicides. Since the late application of fungicides did not reduce anthracnose severity or protect yield compared to non-treated (Fig. 2 and Fig. 3), the profitability calculations were conducted for boot and flower applications only. A single application of pyraclostrobin + fluxapyroxad and pyraclostrobin protected yield compared to non-treated and ranged from 111 kg/ha to 2,694 kg/ha and 49 kg/ha to 2,448 kg/ha higher, respectively (Table 4). To cover the cost of a single fungicide application, a yield increase of 693 kg/ha ($117.8) and 769 kg/ha ($130.7) was needed for pyraclostrobin + fluxapyroxad and pyraclostrobin, respectively. For four of the experiments, application of pyraclostrobin + fluxapyroxad or pyraclostrobin at boot stage resulted in a positive return on investment (Fig. 4). When these fungicides were applied at the flowering stage, four experiments had higher yield protection compared to the non-treated that would have covered the cost for a single application. For experiments RM-16 and SF-17, a single application of either fungicide at flowering protected grain yield and crop value significantly compared to non-treated.

Discussion

In this study, it was documented that foliar anthracnose has the potential to limit sorghum grain yield in wet, humid regions such as in the mid-Atlantic U.S. We evaluated five fungicides and four application timings for efficacy in managing leaf anthracnose and protecting grain yield

in sorghum. Three applications of either pyraclostrobin + fluxapyroxad or pyraclostrobin were effective in reducing anthracnose severity and protecting yield. However, these products are not labeled for more than one application in a growing season, and more than one fungicide application is unlikely to be economical for growers. The labeled application timing for both the fungicides is no later than 25% flowering. Among the single applications, boot or flowering timings provided the greatest of disease control, but a yield protection was only observed when fungicides were applied at flowering.

Time of disease onset and environmental conditions varied among experiments, and this likely impacted the levels of anthracnose control and yield response to fungicides observed in this study. Five experiments (SF-15, RM-15, RM-16, SF-17 and LW-17) had early onset of disease resulting in high disease severity later in the season. RM-16 received the greatest amount of rainfall during the growing season compared to any other site resulting into greater anthracnose severity and lower overall yield. Dissemination and germination of *C. sublineola* is water dependent (Leite and Nicholson 1992; Ngugi et al. 2000; Thakur and Mathur 2000) and expansion of the anthracnose lesion and formation of acervuli is favored by temperatures close to 30^{o}C (Crouch and Beirn 2009). Average monthly temperature of 25^{o}C and higher and high precipitation events likely contributed to early onset and progression of disease at RM-16. For experiments SF-17 and LW-17, disease onset was early, but the progression was slow until flowering. As the crop entered reproductive stages, disease severity increased rapidly which is similar to previous reports (Li and TeBeest 2009; Ngugi et al. 2000) where slow anthracnose development was observed prior to flowering. That is, if the disease onset is early, severity may be high later in the season in the absence of control measures. In our study, a single application of azoxystrobin reduced anthracnose severity in some experiments, but disease control was less

consistent than with pyraclostrobin or pyraclostrobin + fluxapyroxad. This is in contrast to a study in Brazil that identified three applications of azoxystrobin as one of the most effective fungicides to control sorghum anthracnose (Pinto 2003) in which effect of single application was not evaluated. Hence, azoxystrobin may be an alternative where pyraclostrobin + fluxapyroxad and pyraclostrobin are not available while propiconazole and picoxystrobin were not effective at all in controlling anthracnose under field conditions, even after three applications.

Yield response to fungicide treatments was only observed for three experiments that had high levels of anthracnose. For SF-15 and RM-15, anthracnose severity was high, but yield did not differ among fungicide treatments or timings, which could be due to heavy precipitation towards the end of the growing season that resulted in lodging, shattering of grains and sprouting in heads (personal observation). For KS-16 and RM-17 experiments, there was no difference in yield among fungicide treatments which could be because of lower disease severity. The hybrid, '84P80', used in this study is highly susceptible to anthracnose, but is also a high yield producing hybrid (Balota and Oakes 2018). Hence, under low disease severity conditions, the hybrid generally produced high grain yield. This indicates that application of fungicides in the absence of disease or when disease pressure is low does not have a yield benefit. At KS-17, the overall disease severity as well as yield was low compared to KS-16 and RM-17 which might be due to drought stress early in the growing season followed by sugarcane aphid damage at this location.

Leaf anthracnose as low as 20% is reported to impact yield (Ngugi et al. 2000). Our study also shows a significant negative correlation between leaf anthracnose and yield. Based on our results, for every percent increase in leaf anthracnose, one may expect 27 to 85 kg/ha reduction

in yield which is a wider range compared to a recent study from Brazil that reported 11.2 to 38.9 kg/ha reduction in yield in susceptible sorghum genotypes (Cota et al. 2017). The wider range in yield reduction might be due to higher number of experimental sites in this study. This signifies the importance of managing sorghum anthracnose in the mid-Atlantic and other regions where environmental conditions favor fungal disease development.

Pyraclostrobin + fluxapyroxad and pyraclostrobin were the only two fungicides that reduced anthracnose severity, increased yield and increased farm profitability with a single application at flowering. Hence, inclusion of a single application of pyraclostrobin + fluxapyroxad or pyraclostrobin is beneficial under high disease severity conditions that result from early onset of the disease. Our study also demonstrated that application of these fungicides after flowering did not have any impact on yield, and late applications are unlikely to be profitable to growers. The use of pyraclostrobin + fluxapyroxad is more economical than pyraclostrobin, even though the price of pyraclostrobin + fluxapyroxad per liter ($120.2/liter) is greater compared to that of pyraclostrobin ($111.0/liter). This is because a smaller quantity, 0.29 liter/ha of pyraclostrobin + fluxapyroxad is enough to reduce anthracnose severity, increase yield and increase farm income compared to 0.44 liter/ha of pyraclostrobin. Pyraclostrobin, a quinone outside inhibitor with single mode of action, is the common active ingredient in both of these fungicides indicating that the pyraclostrobin component in Priaxor is responsible for reducing anthracnose severity. Based on our study, propiconazole did not reduce anthracnose severity, and a preliminary in vitro study indicated that fluxapyroxad does not reduce anthracnose growth (data not presented). Fluxapyroxad was not evaluated separately to control anthracnose in our study, therefore, further research is needed to determine if fluxapyroxad is effective against this disease and determining the baseline sensitivities of *C. sublineola* isolates

to different fungicide chemistries used in this study will help further elucidate fungicide efficacy in managing anthracnose. Pyraclostrobin is categorized as a high risk fungicide that may rapidly induce resistance in a heterogenous species with diverse pathotypes if not rotated with different modes of actions (Costa et al. 2011; Moore et al. 2010a; Rosewich et al. 1998). Fungicides, pre-mixes, or tank mixes with different modes of action to rotate with pyraclostrobin should be explored to reduce fungicide resistance development. Studies from Africa have reported the effectiveness of mixing fungicides with different modes of action in controlling sorghum anthracnose (Gwary et al. 2008; Marley 2004).

The climate of the mid-Atlantic is warm and humid compared to major sorghum growing regions in the U.S. providing suitable conditions for anthracnose epidemics that may severely impact yield and farm profitability. Anthracnose development and dispersal is temperature and precipitation dependent. When the average temperature is 25^{o}C or greater and is accompanied by frequent or heavy rainfall events, there is a high chance for disease development. Based on our study, if anthracnose symptoms and signs are observed at or before boot stage, there is a greater chance of disease outbreak later in the season. Hence, regular scouting is suggested at early developmental stages when plants are close to boot stage to avoid a disease outbreak. Results from this study indicate that fungicide application at the flowering stage is critical for disease control and to protect yield. Application of these fungicides may not be profitable if disease is absent prior to the flowering stage as we did not see any significant yield differences between non-treated and treated plots under low disease severity. Our results are in accordance with two other studies (Fromme et al. 2017; Weisz et al. 2011) that showed application of fungicides at low or no disease conditions do not increase grain yields in agronomic crops.

In conclusion, sorghum anthracnose occurrence and severity depends on the timing of disease onset and environmental conditions during the growing season. So, fungicide application decisions must be based on these factors. The crop should be scouted for disease frequently when the crop is close to the boot stage. A single application of fungicide (Pyraclostrobin + fluxapyroxad or pyraclostrobin) is warranted if anthracnose onset is at boot stage or proir and should be applied no later than flowering to reduce anthracnose severity, increase yield, and increase farm profitability, particularly when a susceptible hybrid is being grown. In the absence of disease prior to flowering, which results in low anthracnose severity later in the season, fungicide application is unlikely to protect yield or farm profitability. In this study, we did not consider the effect of host resistance on management of sorghum anthracnose. Therefore, future studies will focus on the integrated effect of host resistance and fungicide applications on anthracnose management and yield in sorghum production in the mid-Atlantic region of the U.S.

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Table 1. Location, previous crop, planting date, fungicide timing, anthracnose onset and harvest date for each experiment.

Site-Year[a]	Previous crop	Planting date	Fungicide, DAP[b]			Disease onset, DAP[b]	Harvest date
			Boot	Flower	Late		
SF-15	Soybean	6 Jun	53	61	75	51	20 Oct
RM-15	Soybean	28 May	60	67	81	64	21 Oct
RM-16	Soybean	27 May	49	61	74	43	3 Oct
KS-16	Soybean	14 Jun	44	59	73	72	4 Oct
SF-17	Cotton	26 Jun	51	58	71	48	2 Nov
RM-17	Soybean	12 Jun	53	64	79	66	19 Oct
LW-17	Sorghum	8 Jun	62	68	84	50	4 Oct
KS-17	Soybean	7 Jun	62	70	90	71	23 Oct

[a] Location of experiments: SF= Suffolk,VA (Tidewater Agricultural Research and Extension Center); RM = Rocky Mount, NC (Upper Coastal Plain Research Station); LW = Lewiston-Woodville, NC (Peanut Belt Research Station); KS = Kinston, NC (Casewell Development Center Research Farm). Numbers following the location abbreviation indicate the year of the experiment (2015-2017).
[b]DAP = Days after planting.

Table 2. Total monthly rainfall and average temperatures per month for eight site-years in VA and NC.

	May	Jun	Jul	Aug	Sep	Oct
Site-Year[a]	Rainfall, mm					
SF-15	12	161	96	55	115	74
RM-15	60	84	54	82	167	137
RM-16	94	111	491	85	246	259
KS-16	175	112	132	128	240	84
SF-17	120	89	60	186	94	88
RM-17	125	122	151	176	76	87
LW-17	148	143	189	128	82	69
KS-17	93	120	43	86	108	77
	Mean temperature, ^{o}C					
SF-15	21	26	26	25	23	16
RM-15	21	26	26	25	23	16
RM-16	20	24	27	27	23	18
KS-16	20	25	27	27	24	19
SF-17	20	26	28	25	22	21
RM-17	21	24	26	25	22	18
LW-17	20	24	26	24	22	18
KS-17	22	25	27	25	22	18

[a]SF= Suffolk,VA (Tidewater Agricultural Research and Extension Center); RM = Rocky Mount, NC (Upper Coastal Plain Research Station); LW = Lewiston-Woodville, NC (Peanut Belt Research Station); KS = Kinston, NC (Casewell Development Center Research Farm). Numbers following the location abbreviation indicate the year of the experiment (2015-2017).

Table 3. Impact of fungicide treatments on foliar anthracnose severity in sorghum hybrid 'P84P80' for eight site-years in VA and NC.

	Anthracnose severity, %[y, x, w]							
Treatment[z]	SF-15	RM-15	RM-16	KS-16	SF-17	RM-17	LW-17	KS-17
Fungicide (F)								
None	71.9 a	63.1 a	44.7 a	16.7 a	43.4 a	27.4 a	82.5 a	4.7 a
Tilt	71.2 a	61.8 a	42.8 ab	12.9 a	42.2 a	24.2 ab	79.4 ab	8.4 a
Aproach	72.6 a	65.9 a	42.1 ab	10.5 a	37.0 ab	18.3 ab	77.8 ab	3.7 ab
Quadris	72.6 a	62.4 a	38.7 b	9.9 ab	27.3 b	14.7 b	64.4 b	3.5 ab
Priaxor	63.8 b	52.8 b	22.4 c	2.5 bc	8.6 c	2.8 c	28.3 c	0.5 b
Headline	70.6 a	62.4 a	25.9 c	1.5 c	2.3 d	2.3 c	23.7 c	0.5 b
P-value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Application (A)								
Boot	70.0	63.1	39.0 ab	10.1	25.6 ab	16.7 a	56.0 b	3.6
Flower	71.7	59.9	36.5 b	9.4	24.2 ab	14.5 ab	61.6 b	2.7
Late	71.7	60.8	40.6 a	8.6	29.7 a	13.3 ab	78.7 a	4.2
All	68.6	61.8	27.5 c	4.5	17.5 b	8.5 b	42.2 c	1.6
P-value	0.21	0.52	<0.01	0.09	<0.01	0.02	<0.01	0.18
F × A, *P*-value	0.42	0.35	<0.01	0.24	0.60	0.08	<0.01	0.57

[z]Treatments consisted of fungicides (F) and application timings (A). Significant treatment effects and treatment (F × A) interactions were determined using a factorial analysis of variance.

[y]Anthracnose severity was rated as a visual estimate of percent leaf area with signs and symptoms of sorghum anthracnose.

[x]Location of experiments: SF= Suffolk,VA (Tidewater Agricultural Research and Extension Center); RM = Rocky Mount, NC (Upper Coastal Plain Research Station); LW = Lewiston-Woodville, NC (Peanut Belt Research Station); KS = Kinston, NC (Casewell Development Center Research Farm). Numbers following the location abbreviation indicate the year of the experiment (2015 to 2017).

[w]Within a treatment factor, means followed by the same letter are not significantly different from each other according to Tukey's HSD test.

Table 4. Impact of fungicide treatments on grain yield in sorghum hybrid 'P84P80' at eight site-years in VA and NC.

	Yield, kg/ha [y, x]							
Treatment[z]	SF-15	RM-15	RM-16	KS-16	SF-17	RM-17	LW-17	KS-17
Fungicide (F)								
None	4,910	3,880	1,250 b	5,195	2,398 b	5,427	2,970 b	3,666
Tilt	4,840	3,573	1,408 b	5,211	2,697 b	5,850	2,523 b	3,515
Aproach	4,625	3,274	1,506 b	5,180	2,892 b	5,594	2,809 b	4,075
Quadris	4,810	3,061	1,799 b	5,534	3,195 b	5,861	2,713 b	4,161
Priaxor	4,787	3,991	2,804 a	4,980	5,092 a	6,053	4,191 a	4,266
Headline	4,751	4,342	2,771 a	5,244	4,846 a	6,134	3,977 a	4,020
P-value	0.66	0.05	<0.01	0.55	<0.01	0.16	<0.01	0.11
Application (A)								
Boot	4,874	3,561	1,770 b	5,325	3,548 ab	5810	3,318 a	3,954
Flower	4,783	3,580	1,796 b	5,405	3,472 b	5893	3,366 a	3,819
Late	4,700	3,961	1,524 b	5,065	2,929 b	5598	2,535 b	3,928
All	4,793	3,602	2,602 a	5,101	4,132 a	5978	3,569 a	4,102
P-value	0.66	0.59	<0.01	0.37	<0.01	0.44	<0.01	0.72
F x A								
P-value	0.91	0.95	<0.01	0.74	0.24	0.45	0.19	0.35

[z]Treatments consisted of fungicides (F) and application timings (A). Significant treatment effects and treatment (F × A) interactions were determined using a factorial analysis of variance.
[y]Location of experiments: SF= Suffolk,VA (Tidewater Agricultural Research and Extension Center); RM = Rocky Mount, NC (Upper Coastal Plain Research Station); LW = Lewiston-Woodville, NC (Peanut Belt Research Station); KS = Kinston, NC (Casewell Development Center Research Farm). Numbers following the location abbreviation indicate the year of the experiment (2015-2017).
[x]Within a treatment factor, means followed by the same letter are not significantly different from each other according to Tukey's HSD test.

Table 5. Correlation between foliar anthracnose severity and yield, and minimum and maximum disease severity and yield at eight site-years in VA and NC.

	Correlation, disease severity *vs* yield				Disease severity, %[c]		Yield, kg/ha	
Site[a]	r[b]	*P*-value	Slope	Intercept	Min	Max	Min	Max
SF-15	0.04	0.67	3	4,584	53	75	3,117	5,838
RM-15	-0.01	0.93	-1	3,755	43	75	1,814	8,317
RM-16	-0.79	<0.01	-85	5,130	5	50	814	5,205
KS-16	0.27	0.01	16	5,046	1	55	4,008	7,754
SF-17	-0.86	<0.01	-66	5,351	0	60	1,065	7,546
RM-17	-0.17	0.09	-12	6,016	0	45	3,456	7,748
LW-17	-0.75	<0.01	-27	4,764	2	95	1,791	6,822
KS-17	-0.22	0.03	-27	4,058	0	40	1,501	6,655

[a]SF= Suffolk,VA (Tidewater Agricultural Research and Extension Center); RM = Rocky Mount, NC (Upper Coastal Plain Research Station); LW = Lewiston-Woodville, NC (Peanut Belt Research Station); KS = Kinston, NC (Casewell Development Center Research Farm). The number following the location abbreviation indicates the year.
[b]Pearson's correlation coefficient.
[c]Anthracnose severity was rated as a visual estimate of percent leaf area with signs and symptoms of sorghum anthracnose.

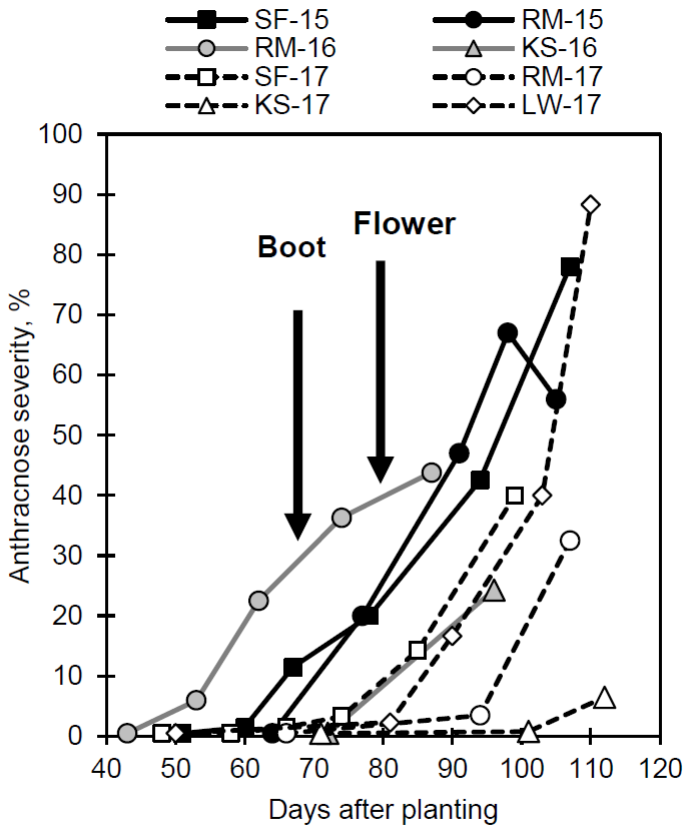

Figure 1. Progression of foliar anthracnose severity on anthracnose susceptible sorghum hybrid, '84P80', in non-treated plots at different site-years. The first data point for each site-year indicates the time of disease onset. Anthracnose severity was measured as the percent leaf area with symptoms and signs of the disease. SF= Suffolk,VA (Tidewater Agricultural Research and Extension Center); RM = Rocky Mount, NC (Upper Coastal Plain Research Station); LW = Lewiston-Woodville, NC (Peanut Belt Research Station); KS = Kinston, NC (Casewell Development Center Research Farm). The number following the location abbreviation indicates the year.

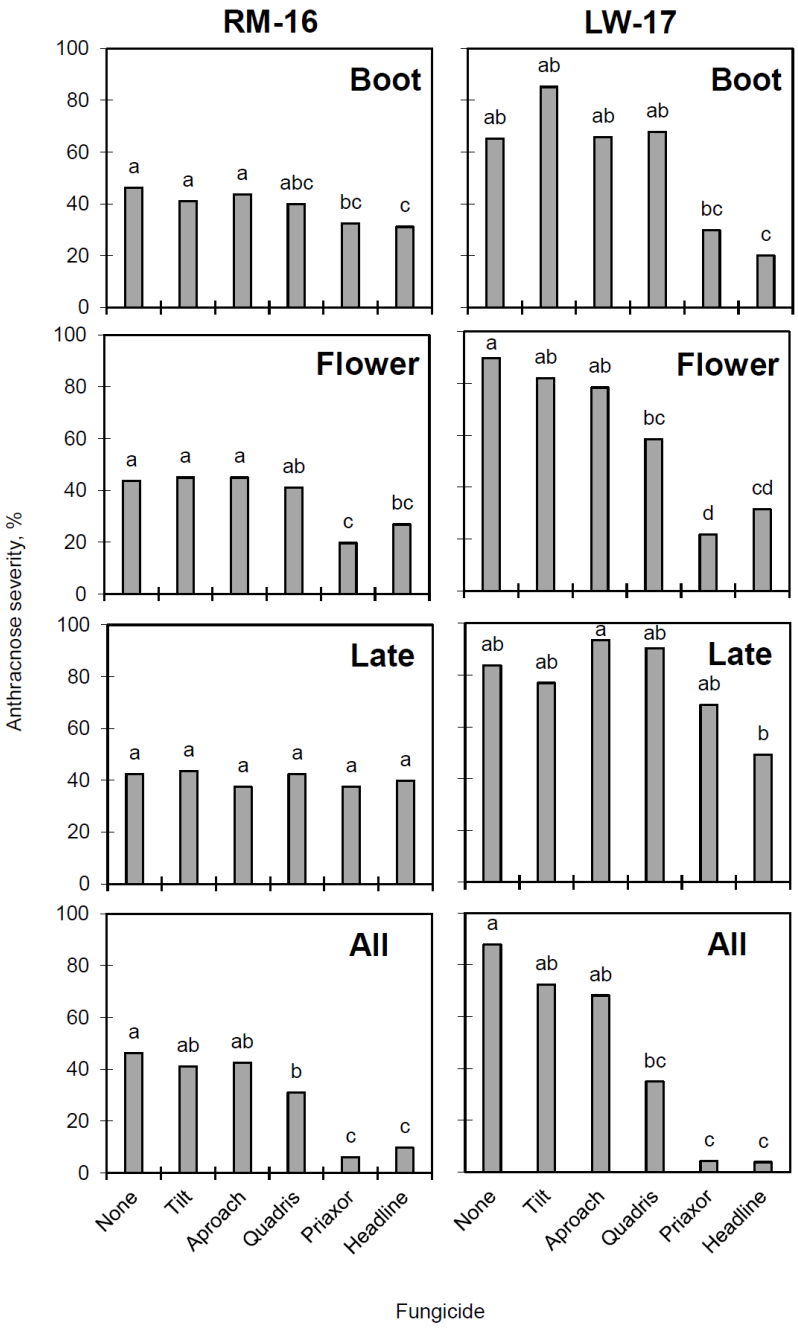

Fig. 2. Variation in severity of leaf anthracnose in sorghum hybrid ‘P84P80’ due to interaction of fungicides and application timings at Rocky Mount, NC in 2016 (RM-16) and Lewiston, NC in 2017 (LW-17). Fungicides were applied at the boot stage, flowering stage, 14 days after the flowering stage (late), or at all three timings (all). Bars with the same letter are not significantly different from each other according to Tukey’s HSD test.

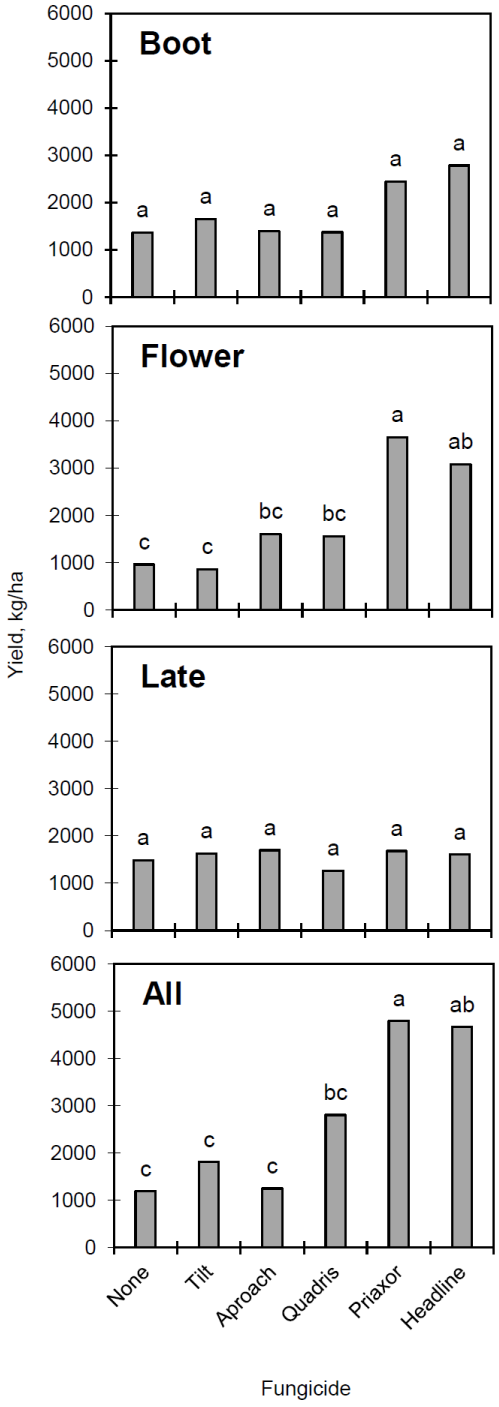

Fig. 3. Variation in grain yield of sorghum hybrid, 'P84P80', due to interaction of fungicides and application timings at Rocky Mount, NC in 2016 (RM-16). Fungicides were applied at the boot stage, flowering stage, 14 days after the flowering stage (late), or at all three timings (all). Bars with the same letter are not significantly different from each other according to Tukey's HSD test.

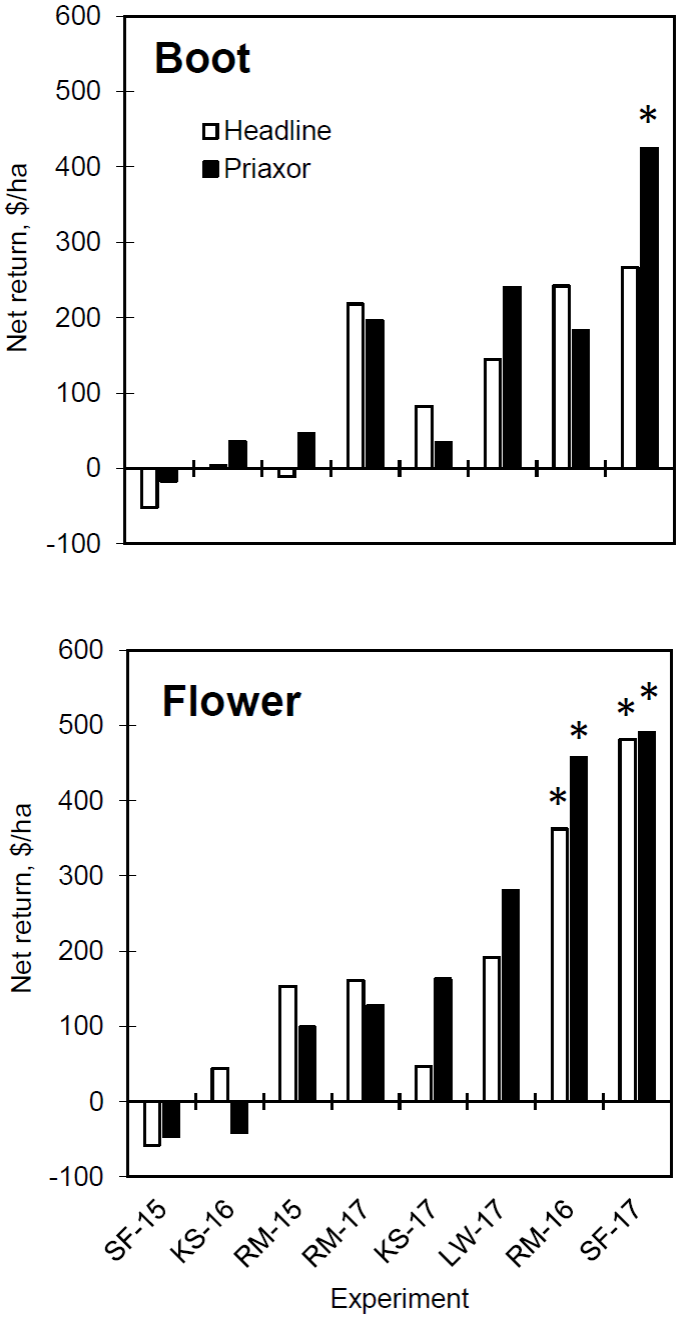

Fig. 4. Net return from single application of Headline and Priaxor applied to sorghum across eight experiments. Net return was calculated as the value ($/ha) of the difference in yield between the fungicide treated plots and the non-treated. Bars with an asterisk (*) are from treatments that had significantly greater grain yield compared to the non-treated control within the experiment.

CHAPTER 3: Comparison of grain sorghum hybrids for production in the mid-Atlantic region based on disease susceptibility and yield

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B. Acharya conducted the experiments. M. Balota, R. Heiniger, and H. L. Mehl planned and coordinated the experiments in Virginia and North Carolina. W. Thomason helped with the statistical analysis of the data. B. Acharya and H. L. Mehl coordinated the writing of the manuscript. All the authors contributed to the edits of the manuscript.

Keywords: Sorghum, Anthracnose, Grain mold, DON, Fumonisin, Aflatoxin

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Abstract

Sorghum production in the mid-Atlantic region of the U.S. has increased since 2012 due to industry demand for local sources of grain for animal feed. Anthracnose (causal agent *Colletotrichum sublineola*) and the grain mold complex, which includes mycotoxin-producing *Fusarium* spp., limit the yield and quality of grain sorghum in humid climates worldwide. Since a majority of U.S. grain sorghum production is in arid regions, suitability of hybrids for semi-tropical conditions of the mid-Atlantic needs to be assessed. The objective of this study was to evaluate five grain sorghum hybrids for yield and susceptibility to anthracnose, grain mold, and mycotoxin contamination over multiple years and locations. Anthracnose and grain mold severity varied among experiments, but some hybrids had consistently high or low levels of disease. However, reduced susceptibility to disease did not necessarily correspond to higher grain yield or test weight. Deoxynivalenol, fumonisin, and aflatoxin were detected from grain samples, but overall mycotoxin concentrations were low. Results of this study indicate that hybrids with high yield potential and moderate disease resistance should be selected for mid-Atlantic sorghum production in order to maximize grain yield and quality while minimizing the need for fungicide inputs.

Grain sorghum [*Sorghum bicolor* (L.)] is the fifth most important cereal crop worldwide, grown on nearly 44.3 million hectares that produce 63.3 million metric tons of grain (FAS, USDA, 2018). Sorghum is widely grown in the tropics, sub-tropics and semi-arid climates throughout the world. In the U.S., grain sorghum is primarily used as animal feed (Vanderlip 1993), and the climatic conditions of the southern U.S. are favorable for sorghum production. However, in the southeastern and mid-Atlantic states where wet, humid conditions are common during the growing season, fungal diseases are a major constraint to sorghum production that reduce both grain yield and quality.

Sorghum anthracnose (*Colletotrichum sublineola* Henn. ex Sacc. & Trotter) is a destructive fungal disease that reduces yield of susceptible hybrids up to 50% (Thakur and Mathur 2000). The fungus can infect all above ground parts of the plant causing stalk rot, as well as foliar, panicle and grain anthracnose in sorghum. The disease degrades both the quantity and quality of grain and stover (Prom et al. 2012; Tesso et al. 2012). Foliar anthracnose can cause defoliation and premature death of plants and is the phase of the disease with the greatest potential to significantly reduce grain yield (Thakur and Mathur 2000). Foliar anthracnose epidemics occur with temperatures between 25 and 30^{o}C during extended periods of high rainfall and/or relative humidity greater than 90% (Pande et al. 1994; Thakur and Mathur 2000). Infection is enhanced by rain splash and contact of leaves with infested soil (Ngugi et al. 2000; Thakur and Mathur 2000).

The grain mold complex is another major disease of sorghum that can be caused by more than 40 different fungal genera that infect and degrade the quality of grains (Singh and Bandyopadhyay 2000). Fungi with the potential to cause grain mold are widespread in the environment in association with plants, organic debris, and soil, and spores can initiate disease

epidemics under favorable environmental conditions (Bandyopadhyay et al. 1991; Ratnadass et al. 2003). *Fusarium, Curvularia, Alternaria,* and *Phoma* are frequently reported as causal agents of grain mold in sorghum, but species assemblages vary by geographic location (Mpofu and McLaren 2014; Navi et al. 2005; Prom et al. 2015). Grain mold infection is favored by above average temperatures and precipitation with a wetness duration of at least 40 h (Montes-Belmont et al. 2003; Navi et al. 2005; Tarekegn et al. 2006). In addition, high relative humidity between the end of flowering and harvest is associated with high grain mold incidence and severity (Ratnadass et al. 2003). Grain mold can cause yield loss from 30 to 100% (Singh and Bandyopadhyay 2000). Certain fungal species causing grain mold produce mycotoxins, chemically diverse and harmful secondary metabolites that are toxic to animals and humans (Waliyar et al. 2008). Trichothecenes, zearalenone, fumonisins, and aflatoxins are some important mycotoxins produced by grain mold fungi (Waliyar et al. 2008). Among these, deoxynivalenol (DON), fumonisins, and aflatoxins are of major concern in grain sorghum in different parts of the world (Apeh et al. 2016; Bluhm and Faske 2015; Hussaini et al. 2009; Isakeit et al. 2008; Kange et al. 2015; Osman et al. 2017; Yassin et al. 2010). Grain sorghum contaminated with DON and zearalenone were previously reported from North Carolina (Hagler et al. 1987), but current information on the occurrence and severity of sorghum mycotoxin contamination in the mid-Atlantic is lacking.

In recent years, demand for locally sourced animal feed by swine and poultry industries and the resulting premium offered for grain sorghum has increased production in the mid-Atlantic (Balota et al. 2018). Relative to corn, sorghum is drought tolerant and performs well on marginal land (Thakur and Mathur 2000) making it suitable for areas in mid-Atlantic where corn production is not profitable. However, warm, wet conditions during the growing season in the

region promote anthracnose and grain mold development, and high levels of disease can result in significant grain yield and quality losses. Hence, in order to maximize profitability of sorghum production in the mid-Atlantic region, high yielding sorghum hybrids with resistance to locally important diseases need to be identified. Therefore, we hypothesized that sorghum hybrids with some type of resistance to diseases and mycotoxins, that are adapted to the mid-Atlantic conditions are commercially available. The objective of this study was to evaluate five grain sorghum hybrids for yield and susceptibility to anthracnose, grain mold, and mycotoxin contamination over multiple years and locations in order to identify hybrids well-adapted to production in the mid-Atlantic region.

Field evaluation of sorghum hybrids

Field experiments were conducted at three locations over two years (2015 and 2016) in Virginia (Suffolk and Isle of Wight) and North Carolina (Rocky Mount). Dates of planting, disease evaluation, and harvest are provided in Table 1. Two Pioneer hybrids (83P17, 84P80) and three DEKALB hybrids (DKS51-01, DKS53-53, DKS54-00) that were part of the mid-Atlantic grain sorghum official variety trials were selected for comparison because they were the five hybrids that were planted across all three locations in both years. Each experiment had three to four randomized replications, and each plot was 9 m long by 3.7 m wide with 2.4 m alleys between blocks. Soil fertility, insects and weeds were managed following standard practices for sorghum in Virginia and North Carolina (Heiniger et al. 2011). Sugarcane aphids were controlled by using sulfoxaflor (as 0.1 liter/ha of Transform, Dow AgroSciences, Indianapolis, IN) or flupyradifurone (as 0.4 liter/ha of Sivanto, Bayer CropScience, Research Triangle, NC) and alternating the chemistry when multiple sprays were required.

Disease assessment and statistical analysis

Foliar anthracnose and grain mold severity were assessed when the sorghum crop reached the hard dough stage (i.e., when grains are brown in color and difficult to squeeze). Anthracnose severity was evaluated as a visual estimate of percent leaf area with symptoms and signs of anthracnose, and grain mold was evaluated as the percent sorghum head with visible symptoms of fungal growth on the grains. The center two rows of each plot were harvested using a combine harvester at the end of the season. Yield from each plot was measured at harvest, and moisture and test weight were measured post-harvest (GAC2000, DICKY-john Corporation, IL). Yield/ha was calculated based on a standard moisture content adjusted to 14%.

Deoxynivalenol (DON), fumonisin, and aflatoxin were measured from grain samples collected at harvest except for the experiment conducted at Rocky Mount, NC in 2015 for which grain samples were not available post-harvest. For each hybrid within an experiment, 500 g subsamples from each of three replicates were combined and 100 g from the combined subsample was used for the analysis. Mycotoxins were quantified by ELISA using Veratox® kits for each mycotoxin (Neogen, Lansing, MI). The limits of detection were 0.1 ppm, 0.2 ppm, and 0.5 ppb for DON, fumonisin, and aflatoxin, respectively.

An initial analysis was performed using hybrid, year, location, and block as fixed variables, and their interactions, and anthracnose and grain mold as dependent variable in the model. The effect of hybrid, year, location and their interactions were significant ($P<0.05$) so each experiment was analyzed separately. Data for anthracnose severity, grain mold severity, yield, and test weight were analyzed using general linear model procedure (GLM) in R version 3.5.1 using the package, agricolae (Mendiburu 2017) and analysis of variance (ANOVA) was

performed using a model that included block and hybrid as sources of variation for each experiment. Mycotoxin contamination for DON, aflatoxin and fumonisin were analyzed using a model that included location (as replication) and hybrid as sources of variation. The normality and homogeneity of variance were confirmed using Shapiro-Wilk's test and Levene test prior to analysis. Means were compared using Fisher-protected Least Significant Differences (LSD) test at the significance level of $\alpha = 0.05$ using the package, car (Fox and Weisberg 2011).

Susceptibility of sorghum hybrids to disease and mycotoxin contamination

Warm ($\geq$23^{o}C) average temperature accompanied by frequent precipitation provided conducive conditions for anthracnose development across all locations in both years (Table 2). There was less precipitation in the early growing season at Rocky Mount in 2015 where anthracnose severity was relatively low (Table 2, 3). Anthracnose severity varied by hybrid ($P<0.001$) and experiment ($P<0.001$). The highest levels of foliar anthracnose were at Isle of Wight and Suffolk in 2015 (mean = 31% and 38%, respectively) and the lowest level of disease was at Rocky Mount in 2016 (mean = 16%). There was a hybrid by experiment interaction ($P<0.001$), so hybrids were compared for each site-year separately (Table 3). Anthracnose severity was consistently high on hybrids '84P80' and 'DKS53-53', whereas disease on 'DKS54-00' was consistently low among locations and years (Table 3).

Overall grain mold severity was low, but it varied by hybrid ($P=0.002$), experiment ($P<0.0001$), and there was a hybrid by experiment interaction ($P =0.002$). Across experimental sites, the precipitation was low in the month of August (Table 2) which coincides with the grain filling, the stage most vulnerable to grain mold infection; this may explain the relatively low grain mold severity in this study. Differences in grain mold severity among hybrids were only

detected at Rocky Mount in 2015 and Isle of Wight in 2015 and 2016, the three site-years with the highest overall grain mold severity (Table 4). Among the five hybrids, '84P80' had the highest grain mold severity at two of the locations, and 'DKS53-53' had the highest severity at the third location. These two hybrids also had consistently high severity of foliar anthracnose symptoms across experiments. In contrast, 'DKS54-00', which among the five hybrids was the least susceptible to foliar anthracnose, had low grain mold severity across all experiments.

Though grain mold was observed on all hybrids, severity of grain infection was not associated with increased levels of mycotoxin contamination (r^2 =0.003, P =0.78 for DON; r^2 =0.079, P =0.17 for aflatoxin; r^2 =0.079, P =0.78 for fumonisin). For each experiment, a single pooled grain sample was analyzed for each hybrid, and the five hybrids did not differ in contamination by DON (P=0.067), aflatoxin (P=0.901) or fumonisin (P=0.826). All of the samples had <4 ppb aflatoxin, thus none of the grain samples exceeded the action level of 20 ppb for aflatoxin. *Fusarium* spp. were observed infecting grains, but concentrations of DON and fumonisin were below advisory (<5 ppm DON) and guidance (<20 ppm fumonisin) levels for these two toxins in grain used for animal feed (Fig. 1). However, the range of mycotoxin concentrations in grain was variable across experiments and hybrids (Fig. 1 and 2), suggesting that field-specific climate conditions and hybrid susceptibility may influence the potential for high levels of DON or fumonisin contamination. For example, the maximum DON concentration (1.1 ppm) was from hybrid 'DKS54-00', and Suffolk and Isle of Wight in 2015 had higher maximum concentrations of DON than the other locations (Fig. 1, 2).

Variability in grain yield among hybrids, years, and locations

Grain yields ranged from 852 kg/ha to 7601 kg/ha and varied among hybrids (P=0.033), experiments (P<0.001), and there was a hybrid by experiment interaction (P<0.001). Overall yields were highest at the Suffolk and Isle of Wight locations in 2015 and lowest at Suffolk and Rocky Mount in 2016 (Table 5). Yield varied among hybrids at Rocky Mount in both years, but yields were similar for the five hybrids in the other four experiments. Hybrid '84P80' and 'DKS51-01' were the highest yielding hybrids at Rocky Mount in 2015 and 2016, respectively, and 'DKS54-00' had the lowest yield among the five hybrids in both experiments. When yields were standardized to percent of the average yield for each experiment (Fig. 3), hybrids '83P17' and 'DKS51-01' had the most consistent relative yields across experiments, whereas '84P80' and 'DKS54-00' were the most variable. Yield was negatively correlated with anthracnose only at Suffolk in 2015 (r^2 = 0.31, P = 0.0406) and 2016 (r^2 = 0.37, P = 0.0166), the experiments for which severity on the most susceptible hybrid ('84P80') exceeded 50% (Table 5). When analyzed by hybrid instead of experiment, yield was positively correlated with anthracnose severity for '83P17' (r^2 =0.44 P=0.002), 'DKS51-01' (r^2 = 0.49, P = 0.0008), and 'DKS54-00' (R^2 = 0.21, P = 0.0429). This suggests that environmental conditions favoring disease development (warm temperatures, rainfall) also favor crop growth, and field environment may contribute more to increased yield potential than anthracnose contributes to suppressed yield for these hybrids. There was a positive correlation between grain mold and yield for hybrid '84P80' but not for any other hybrid; again this suggests warm, wet conditions favoring disease also favor high yield for some hybrids. Test weight varied among hybrids for three of the experiments (Table 6). On average across all experiments, 'DKS51-01' had both the highest grain yield and test weight whereas 'DKS54-00' had the lowest average grain yield and test

weight despite being the hybrid with the lowest overall disease severity (Table 3, 4). There was not a significant correlation between test weight and grain mold severity across experiments ($P>0.05$).

Suitability of sorghum hybrids for grain production in the mid-Atlantic U.S.

In this study, we evaluated the suitability of five grain sorghum hybrids for production in the mid-Atlantic based on disease susceptibility, yield, and grain quality. None of the hybrids could be considered resistant to anthracnose, but some hybrids had consistently moderate to low anthracnose severity across experiments suggesting these hybrids are partially or moderately resistant. The genetic background and presence or absence of resistance genes within hybrids was not known, but previous studies have demonstrated that sorghum resistance to anthracnose is not durable across regions (Ali and Warren 1987; Wharton et al. 2001). Based on anthracnose severity, the observed susceptibility of some hybrids in the study varied among locations and years. This may have been due to variation in environmental conditions or due to the variation in *C. sublineola* populations and pathotypes across experiments (Prom et al. 2012; Were and Ochuodho 2012). Dominate pathotypes vary across locations, and sorghum resistance to anthracnose is pathotype-specific (Casela et al. 2001). Assemblages of *C. sublineola* pathotypes present in the mid-Atlantic region have not been characterized, but they may be different from those present in regions for which most sorghum hybrids have been developed.

Grain mold can greatly reduce the quality of sorghum grain, especially if infections are associated with mycotoxin contamination. Despite overall high humidity and rainfall during the growing seasons in this study, grain mold was a minor issue in most of the experiments. The overall lower grain mold severity in this study could be due to less precipitation during the most

susceptible developmental stage, the grain filling stage, of sorghum. The species of grain mold fungi present in a field also influence levels of grain mold severity (Prom et al. 2003). Though the genera and species of grain mold were not thoroughly characterized as part of this study, *Curvularia spp., Alternaria spp., Colletotrichum sublineola*, and *Fusarium spp.* were commonly observed. In contrast, a study from Nigeria where high grain mold severity in sorghum was common, *Aspergillus niger, Rhizopus oryzae* and *A. flavus* were the major contaminants of sorghum while *Fusarium spp*. (*F. equiseti, F. oxysporum, F. semitectum, F. solani and Fusarium spp.*) were less frequent (Hussaini et al. 2009). In Argentina, genus *Fusarium* (*F. moniliforme, F. graminearum, F. equiseti, F. proliferatum and F. semitectum*) was the most prevalent followed by *Alternaria, Phoma, Penicillium* and *Aspergillus* (González et al. 1997). In South Texas, *Alternaria* was the dominant genus followed by *Bipolaris* while *Fusarium One* was isolated at low frequencies (Prom et al. 2015). Little is known about the extent of gain mold damage caused by each species in the gain mold complex, but of the different species of fungi causing grain mold, presence of mycotoxin-producing fungi such as *Fusarium spp.* and *Aspergillus spp.* are of major concern due to the high risk of mycotoxin contamination of the grains. Among the three mycotoxins tested, aflatoxin level was very low across all the sites. Our results concur with other studies in the U.S. where very low levels of aflatoxin were reported in grain sorghum (Hagler et al. 1987; Bluhm and Faske 2015). Small-sized antifungal proteins (AFPs) are found in sorghum grains that inhibit spore germination and hyphal extension of mold fungi including *Aspergillus flavus* (Gosh and Ulaganathan 1996; Seetharaman et al. 1997). This might be one of the reasons why aflatoxin contamination was low in sorghum grains in our study. In addition, aflatoxin is associated with higher temperatures and drought stress (Williams et al. 2004) and weather conditions in the mid-Atlantic are typically not favorable for high levels

of aflatoxin contamination. Grain from only one hybrid exceeded the regulatory limit for human consumption of 1 ppm DON at Suffolk in 2015. DON has been found to be associated with grain sorghum in the region previously but in lower concentrations (Hagler et al. 1987) and information on fumonisin contamination of sorghum is limited. Grain mold severity and mycotoxin contamination are not only influenced by hybrid susceptibility but also by insect pests of sorghum that can open up avenues for grain mold fungi infection (Ratnadass et al. 2003; Sharma et al. 2000). High sorghum head worm damage and stink bug activities observed at Rocky Mount and Isle of Wight in 2015 (personal observation) may have contributed to the higher grain mold severity at these sites.

Foliar anthracnose and grain mold both have the potential to severely decrease sorghum grain yield and test weight (Singh and Bandyopadhyay 2000; Thakur and Mathur 2000). A major goal of this study was to identify high-yielding sorghum hybrids with reduced susceptibility to major diseases present in the mid-Atlantic, and thus lower risk of yield loss when environmental conditions favor disease development. Ideally, a hybrid should have 1) high yield potential across variable environments, 2) consistently high quality grain characteristics that meet the standard for the target market, and 3) at least moderate resistance to potentially yield-reducing diseases so that fungicide inputs and associated costs can be minimized (Acharya et al., submitted). These three factors all contribute to the overall profitability of crop production, and sorghum hybrids that meet these criteria need to be identified for the mid-Atlantic in order for grain sorghum production to be successful in the region (Balota et al. 2018). Some disease resistant hybrids do not yield well, but in this study, hybrid ‘DKS51-01’ consistently had moderate resistance to both anthracnose and grain mold and consistently high yield and test weight relative to other hybrids across the experimental

sites. Fungal disease pressure is high in the mid-Atlantic, so it may be possible to further optimize sorghum yield and quality in the region through breeding of region-adapted hybrids with improved disease resistance and integrated disease management including crop rotation and judicious use of fungicides.

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Table 1. Location and date of planting, harvest, and disease evaluation for experiments comparing five sorghum hybrids.

Location	Year	Planting	Harvest	Disease evaluation
Rocky Mount (RM)	2015	26 May	24 Oct	26 Aug
	2016	26 May	19 Oct	26 Aug
Suffolk (SF)	2015	6 Jun	16 Oct	1 Sep
	2016	9 Jun	4 Oct	12 Sep
Isle of Wight (IOW)	2015	9 Jun	18 Oct	4 Sep
	2016	14 Jun	8 Nov	16 Oct

Table 2. Total monthly rainfall and average air temperatures for locations where sorghum hybrids were grown over two years in Virginia and North Carolina.

		Total monthly rainfall (mm)				
Year	Location[a]	Jun	Jul	Aug	Sep	Oct
2015	RM	84	54	82	167	137
	SF	188	115	65	132	90
	IOW	115	78	0	162	78
2016	RM	111	491	85	246	259
	SF	88	212	63	305	255
	IOW	70	180	20	248	283
		Average monthly temperature (^{o}C)				
2015	RM	26	26	25	23	16
	SF	26	26	24	23	16
	IOW	25	26	24	22	15
2016	RM	24	27	27	23	18
	SF	24	27	27	23	17
	IOW	23	27	26	23	17

[a]Experimental locations: RM = Rocky Mount, NC (Upper Coastal Plain Research Station); SF= Suffolk, VA (Tidewater Agricultural Research and Extension Center); IOW = Isle of Wight (Windsor, VA).

Table 3. Comparison of anthracnose severity on five sorghum hybrids planted at three locations over two years.

Hybrids	Anthracnose severity, %[x]								
	2015				2016				
	RM[y]	SF		IOW		RM	SF		IOW	
83P17	23	46	a[z]	22	bc	5 b	22	c	27	bc
84P80	26	51	a	39	ab	33 a	52	a	37	ab
DKS51-01	28	36	b	32	abc	6 b	5	d	15	cd
DKS53-53	23	36	b	51	a	34 a	38	b	42	a
DKS54-00	19	22	c	13	c	5 b	4	d	13	d
P-value	0.385	0.001		0.035		<0.001	<0.001		0.005	

[x]Anthracnose severity was evaluated as the visual estimate of percentage of leaf area with symptoms and signs of anthracnose.
[y] Experimental locations:RM= Rocky Mount, NC (Upper Coastal Plain Research Station);SF=Suffolk, VA (Tidewater Agricultural Research and Extension Center); IOW = Isle of Wight (Windsor, VA).
[z]Means within a column followed by the same letter are not significantly different from each other according to Fisher- protected LSD test at the significance level of 0.05.

Table 4. Comparison of grain mold severity on five sorghum hybrids planted at three locations over two years.

	Grain mold severity, %[x]					
	2015			2016		
Hybrid	RM[y]	SF	IOW	RM	SF	IOW
83P17	23 ab[z]	8	12 b	2	2	8 b
84P80	25 a	8	23 a	1	3	7 b
DKS51-01	20 a-c	3	13 b	1	1	7 b
DKS53-53	13 c	2	13 b	3	2	17 a
DKS54-00	14 bc	2	10 b	2	1	8b
P-value	0.040	0.174	0.052	0.194	0.079	0.004

[x]Grain mold severity was evaluated as the estimate of the percentage of sorghum head with grain mold.
[y]Experimental locations: RM = Rocky Mount, NC (Upper Coastal Plain Research Station); SF= Suffolk, VA (Tidewater Agricultural Research and Extension Center); IOW = Isle of Wight (Windsor, VA).
[z]Means within a column followed by the same letter are not significantly different from each other according to Fisher- protected LSD test at the significance level of 0.05.

Table 5. Variation in yield among five sorghum hybrids planted at three locations over two years.

	Yield, kg/ha					
	2015			2016		
Hybrid	RM[y]	SF	IOW	RM	SF	IOW
83P17	3265 b[z]	7521	5648	1928 b	2764	4785
84P80	5033 a	4349	6075	1941 b	1866	4961
DKS51-01	3197 b	6698	7601	2590 a	2532	4677
DKS53-53	3292 b	6207	5285	1878 b	1357	4508
DKS54-00	2947 b	7522	4521	852 c	2687	2815
P-value	0.015	0.260	0.272	<0.001	0.115	0.172

[y]Experimental locations: RM = Rocky Mount, NC (Upper Coastal Plain Research Station); SF= Suffolk, VA (Tidewater Agricultural Research and Extension Center); IOW = Isle of Wight (Windsor, VA).

[z] Means within a column followed by the same letter are not significantly different from each other according to Fisher- protected LSD test at the significance level of 0.05.

Table 6. Variation in grain test weight among five sorghum hybrids planted at three locations over two years.

	Test weight, kg/hL					
	2015			2016		
Hybrid	RM[y]	SF	IOW	RM	SF	IOW
83P17	71 b[z]	59 b	63	48	60 a-c	70
84P80	75 a	62 b	60	47	58 c	70
DKS51-01	74 a	67 a	56	47	65 a	72
DKS53-53	72 b	68 a	55	47	59 bc	70
DKS54-00	74 a	61 b	55	48	65 ab	67
P-value	<0.001	0.003	0.263	0.982	0.052	0.176

[y]Experimental locations: RM = Rocky Mount, NC (Upper Coastal Plain Research Station); SF= Suffolk, VA (Tidewater Agricultural Research and Extension Center); IOW = Isle of Wight (Windsor, VA).
[z]Means within a column followed by the same letter are not significantly different from each other according to Fisher- protected LSD test at the significance level of 0.05.

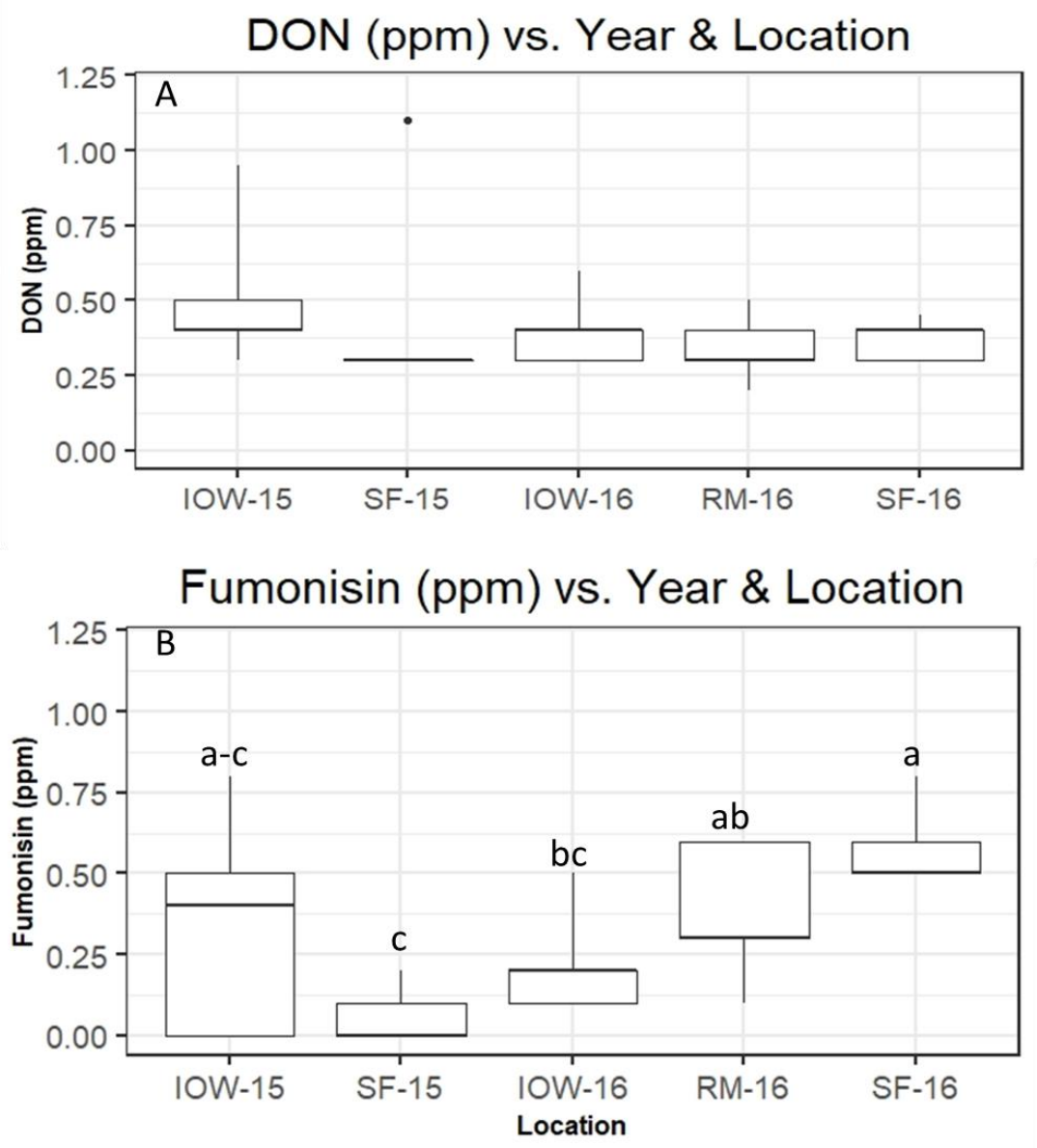

Fig. 1. Box and whisker pot of variation in mycotoxin contamination of A. DON (P=0.602), and B. Fumonisin (P=0.024) in grain samples across experiments. DON stands for mycotoxin deoxynivalenol, ppm = parts per million. Experimental locations were IOW = Isle of Wight (Windsor, VA); RM = Rocky Mount, NC (Upper Coastal Plain Research Station); SF= Suffolk, VA (Tidewater Agricultural Research and Extension Center). The numbers following the location are the year of the experiment (2015 or 2016). The horizontal line within the box indicates the median, boundaries of the box indicate the first and third quartile, black dot indicates an outlier, and the whiskers indicate the highest and lowest values of the mycotoxins. Box and whisker plot with the same letters are not significantly different from each other according to Fisher- protected LSD test at the significance level of 0.05.

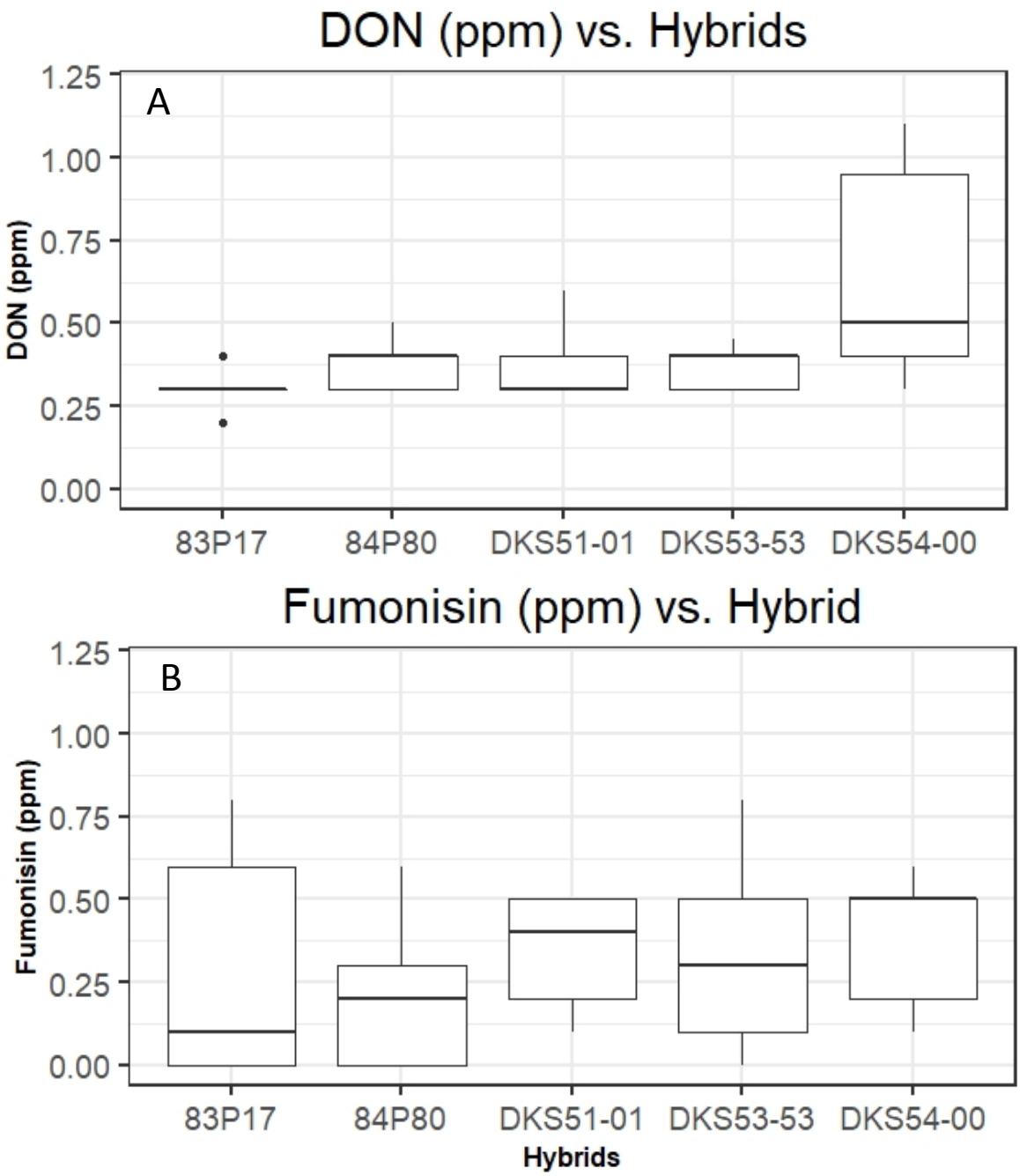

Fig. 2. Box and whisker plot of variation in mycotoxin contamination of A. DON (P=0.068), and B. Fumonisin (P=0.826) in grain samples of different sorghum hybrids in 2015 and 2016. DON stands for mycotoxin deoxynivalenol, ppm = parts per million. The horizontal line within the box indicates the median, boundaries of the box indicate the first and third quartile, black dot indicate outliers, and the whiskers indicate the highest and lowest values of the mycotoxins.

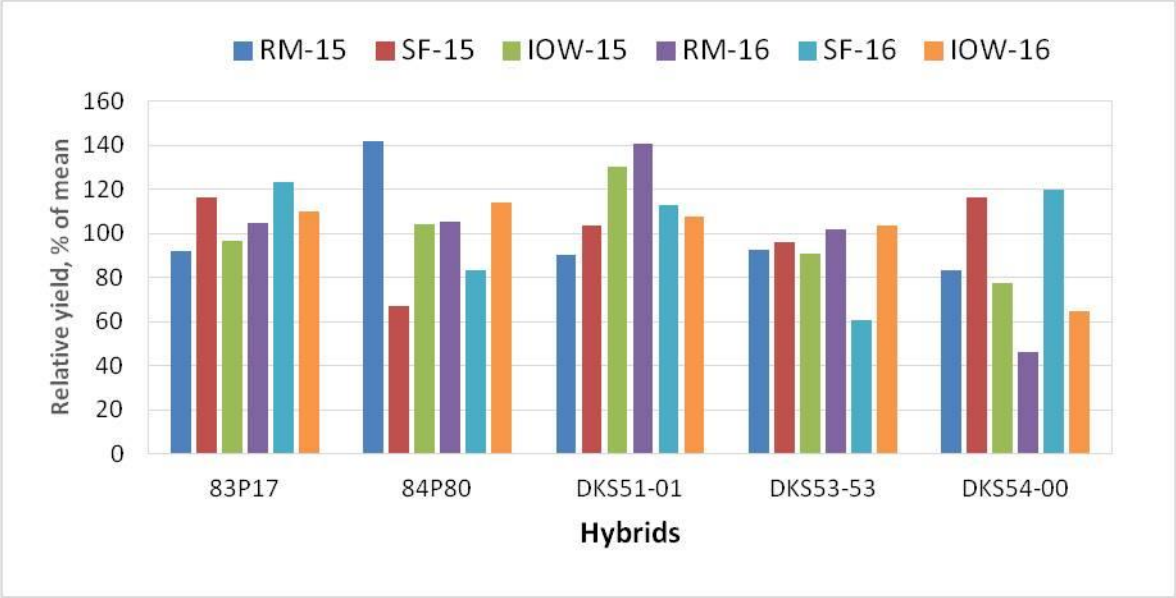

Fig. 3. Relative yield of five grain sorghum hybrids across experiments. Percent relative yield was calculated as the yield of the hybrid divided by the average yield for the experiment times 100%. Experimental locations were RM = Rocky Mount, NC (Upper Coastal Plain Research Station); SF= Suffolk, VA (Tidewater Agricultural Research and Extension Center); and IOW = Isle of Wight (Windsor, VA). The numbers following the location are the year of the experiment (2015 or 2016).

Chapter 4: Integration of host resistance and fungicide application to manage anthracnose (*C. sublineola*) and grain mold in the mid-Atlantic.

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B. Acharya and H. L. Mehl designed and conducted the experiments and coordinated the writing of the manuscript. W. Everman coordinated and conducted field experiments in North Carolina.

Keywords: Grain sorghum, anthracnose, grain mold, hybrids, fungicide

Abstract

Environmental conditions in the mid-Atlantic favor sorghum anthracnose and grain mold development that impact yield and quality of harvested grain. This necessitated development of an effective and economical approaches to manage disease. The objective of this study was to integrate host resistance and fungicide application timing to manage anthracnose and grain mold in sorghum. A disease susceptible ('84P80') and moderately resistant ('765B') hybrid, and two application timings of pyraclostrobin + fluxapyroxad (as Priaxor) at heading or flowering and a non-ttreated were evaluated at two locations for two years in a factorial randomized complete block design with four replications. The untreated moderately resistant hybrid had lower anthracnose and grain mold severity compared to susceptible hybrid. For susceptible hybrid, fungicide applied at heading or flowering reduced anthracnose severity in three experiments and grain mold severity was reduced only in one experiment, when the severity was high. However, fungicide applied at heading resulted in better yield only in one experiment. For moderately resistant hybrid, fungicide reduced anthracnose severity only in two experiments that had high severity. Hence, a single fungicide application reduces disease severity and protects yield of susceptible sorghum hybrids. However, utilization of moderately resistant hybrids reduces disease, minimizes yield loss, and reduces the need for fungicide applications in sorghum production.

Introduction

Sorghum [*Sorghum bicolor* (L.)] is widely grown on nearly 41 million hectares worldwide producing about 59 million metric tons of grains (FAS, USDA, 2019) for food, feed, fiber, fuel and beverages (Cuevas et al. 2014b; Marley et al. 2005). In the United States,

sorghum is grown on nearly 2 million hectares that produce about 9 million metric tons of grains (Foreign Agricultural Services, USDA, 2019) and is used as animal feed within the country (Vanderlip 1993). Majority of the grain sorghum produced in the United States is exported and ranks first among grain sorghum exporters (Foreign Agricultural Services, USDA, 2019).

In the mid-Atlantic, demand of swine and poultry industries for locally sourced animal feed and the premium offered for grain sorghum by these industries has increased sorghum production in the mid-Atlantic (Balota et al. 2018). However, warm, humid conditions during the growing season promote disease development in the region. Anthracnose (*Colletotrichum sublineola* Henn. ex Sacc. & Trotter) and grain mold are two major constraints of grain sorghum production in the region due to the yield loss and mycotoxin contamination associated with the diseases (Chapter 2 and 3). When the anthracnose severity is as low as 20%, yield is impacted (Ngugi et al. 2000) and yield loss can be 50% or more when susceptible cultivars are used (Thakur and Mathur 2000). Anthracnose can infect all the above ground parts of the plant degrading both the quantity and quality of grain and stover (Prom et al. 2012b; Tesso et al. 2012) but foliar anthracnose has the greatest potential to reduce yield from defoliation and premature death of plants (Thakur and Mathur 2000). Yield reduction due to grain mold can range from 30 to 100% depending on the hybrids/cultivars used and environmental conditions during the growing season (Singh and Bandyopadhyay 2000). More than 40 different genera of fungi cause grain mold that degrade the quality of grains (Singh and Bandyopadhyay 2000) and some species produce mycotoxins, toxic to humans and animals (Waliyar et al. 2008). Among different mycotoxins, deoxynivalenol (DON), zearalenone and fumonisin are of major concern in the mid-Atlantic (Chapter 5, (Hagler et al. 1987).

To manage these diseases and avoid mycotoxin contamination, hundreds of sorghum germplasm have been evaluated and some of them have been identified as potential sources of resistance (Buiate et al. 2010; Cuevas et al. 2016; Cuevas et al. 2014a; Cuevas et al. 2014b; Erpelding 2008a, 2010a; Erpelding 2010b; Erpelding 2011; Erpelding and Prom 2004, 2006; Prom and Erpelding 2009; Prom et al. 2012a; Prom et al. 2011; Prom et al. 2014; Sharma et al. 2010; Tesso et al. 2012). However, diversity in the anthracnose pathogen and large number of fungal genera involved in grain mold has limited the breeding effort of developing resistant hybrids or cultivars suitable to different environmental conditions (Ambekar et al. 2011; Prom et al. 2012; Thakur and Mathur 2000). Limited information is available on the integration of host resistance and chemical control with fungicides to effectively manage these diseases. Two studies from Nigeria showed that integration of host resistance with foliar or seed treatment fungicides or both are beneficial in managing anthracnose and protecting yield (Gwary et al. 2008; Marley 2004). The hybrid resistant to anthracnose was resistant to grain mold in one of the studies but use of fungicides did not impact grain mold severity (Marley 2004). We hypothesized that integration of host resistance with a single application of pyraclostrobin-based fungicide will help reduce sorghum anthracnose and grain mold seveity and protect yield. The objective of this study was to evaluate the integrated effect of host resistance and a fungicide to manage sorghum anthracnose and grain mold in the mid-Atlantic.

Materials and Methods

Field experiments. Field experiments were conducted at two locations (Suffolk, VA and Rocky Mount, NC) for two years, 2017 and 2018. Two sorghum hybrids, '84P80' (DuPont Pioneer, Johnston, IA) and '765B' (Dyna-Gro, Geneseo, IL), previously identified as a susceptible (S) and moderately resistant hybrid (MR) in mid-Atlantic sorghum variety trials

(Balota et al. 2017) with similar maturity, were selected to represent the different levels of disease severity to both the diseases, anthracnose and grain mold. Sorghum was planted at a rate of approximately 193,000 seeds/ha. Information on experimental locations; year; and date of planting, fungicide application, anthracnose onset, disease evaluation, and harvest are provided in Table 1. Soil fertility, insects and weeds were managed following standard practices for sorghum in the region (Heiniger et al. 2011). In both the years, sugarcane aphids were controlled by using sulfoxaflor (as 0.1 liter/ha of Transform, Dow AgroSciences, Indianapolis, IN) or flupyradifurone (as 0.4 liter/ha of Sivanto, Bayer CropScience, Research Triangle, NC) and alternating the chemistry when multiple sprays were required.

Hybrids and fungicide application timing. The experiment included a 2 × 3 factorial arrangement of treatments in a randomized complete block design. The treatment factors were hybrids (‘84P80’ and ‘765B’) and fungicide (pyraclostrobin + fluxapyroxad as 0.29 liter/ha of Priaxor EC, BASF Corporation, Research Triangle Park, NC) application timing. The two application timings were heading (beginning of heading in 50% of the plants) and flowering (beginning of flowering in 50% of the plants) (Vanderlip 1993), and a non-treated control. Each site had four replications with 2.4 m fallow alleys between blocks. Each treatment plot consisted of four rows that were 9 m long by 3.7 m wide. Anthracnose severity and grain mold severity were evaluated at hard dough stage, when seeds changed color from green to brown and are hard to squeeze. Anthracnose severity was evaluated as a visual estimate of percent leaf area with symptoms and signs of anthracnose, and grain mold severity was evaluated as the percent sorghum head with visible symptoms of fungal growth on the grains.

At Suffolk, VA, fungicide was applied with a Lee Spider Sprayer having eight 8002VS nozzles spaced 46 cm apart delivering 186 liter/ha at 38 psi with 0.125% v/v of non-ionic

surfactant (Induce). At Rocky Mount, NC fungicide treatments were applied with a backpack sprayer having eight XR 11002 flat fan nozzles spaced 46 cm apart delivering 140.3 liter/ha at 30 psi with 0.125% v/v of non-ionic surfactant (Induce). At the end of the season, grain was harvested from the center two rows of each plot using a mechanical harvester at both the locations in 2017. In 2018, at Suffolk, VA harvesting was done using a mechanical harvester and at Rocky Mount, NC it was hand harvested at physiological maturity due to lodging. Yield was measured at harvest and moisture was measured post-harvest (GAC2000, DICKY-john Corporation, IL) to calculate yield/ha based on a standard moisture content adjusted to 14%.

Statistical analyses. An initial analysis was performed using block, hybrid, fungicide application timing, site, and interactions for anthracnose and grain mold severity in the model. The effect of site and its interactions with hybrid and application timing were significant so each experiment was analyzed separately. Anthracnose severity and grain mold severity data in percentage were arcsine square root transformed when the assumptions of ANOVA were not met. Data for anthracnose severity, grain mold severity and yield were analyzed using general linear model procedure (glm) in R version 3.2.2 using the package, agricolae (Mendiburu 2015). Anthracnose severity and grain mold severity was subjected to analysis of variance (ANOVA) using a model that included block, hybrid, fungicide application timing, and their interactions as sources of variation. Means were compared using Tukey's Honest Significant Difference (HSD) test at the significance level of $\alpha = 0.05$, and back-transformed values are presented for the arc sine transformed data.

Weather data collection. The weather data for Rocky Mount were obtained from the website of North Carolina Climate Retrieval and Observations Network of the Southeast (NC

CRONOS) database (https://climate.ncsu.edu/cronos). The weather data for Suffolk were obtained from peanut-cotton infonet (https://webipm.ento.vt.edu/cgi-bin/infonet1.cgi).

Results

Anthracnose severity. Warmer temperature (≥24^{o}C) and frequent precipitation provided the conducive environmental conditions for disease development (Fig.1). In 2018, both the experimental locations received more frequent precipitation compared to 2017. The precipitation frequency was higher in Rocky Mount, 2017 compared to Suffolk, 2017. The effect of hybrid ($P<0.001$), fungicide application timing ($P<0.001$), and the interaction of these two factors were significant ($P<0.001$) for all the experiments except for Suffolk 2018 where only main effects were significant (Table 2). The untreated susceptible hybrid '84P80' had the highest anthracnose severity in each experiment and the rest of the treatment combinations had significantly lower severity (Fig. 2). The untreated moderately resistant hybrid '765B' had lower anthracnose severity in all the experiments compared to the susceptible hybrid. Application of fungicides reduced anthracnose severity in both the hybrids at all the experiments and application at heading had lower severity compared to the flower (Fig. 2). The use of fungicide had a greater effect in reducing anthracnose severity in the susceptible hybrid compared to that of moderately resistant hybrid. Overall, Rocky Mount had lower anthracnose severity compared to that of Suffolk despite more favorable environmental conditions for anthracnose development (Fig. 1, Fig. 2) which could be due to less initial inoculum present in those fields that did not have any history of sorghum cultivation.

Effect on grain mold severity. There are no fungicides registered for use to manage grain mold. However, an indirect protective effect which could be due to the fungicide's residual activity on grain mold was evaluated. In the experiments at Suffolk, the hybrids differed

in their response to the fungicides ($P<0.001$) and the interaction of hybrids and fungicide application timing was significant ($P<0.023$), in both years (Table 2). In both the experiments at Rocky Mount, the hybrids or the fungicide application timing or their interaction were not significant for grain mold severity (Table 2). At Suffolk, the grain mold severity was greater in 2018 compared to 2017 (Table 3). The use of fungicide at either heading or flower reduced the severity significantly in the susceptible hybrid at Suffolk, 2018. For the moderately resistant hybrid, the use of fungicide did not reduce the severity significantly compared to that of the control (Table 3). Favorable environmental conditions of $\geq 25^{o}$C and higher precipitation in August and September, which coincides with the grain filling, the stage most vulnerable to grain mold infection resulted in the greater grain mold severity in 2018 at Suffolk.

Effect on yield. The yield response of the hybrids differed at two experiments in 2017 and also for application timing at two experiments in Suffolk (Table 2). At Suffolk in 2017 where the severity of anthracnose was high, moderately resistant hybrid '765B' had higher yield compared to the susceptible hybrid '84P80' (Fig.3). At Rocky Mount in 2017 where the severity of both the diseases was low, the susceptible hybrid had higher yield compared to the moderately resistant. The hybrids did not differ significantly in yield at both the experiments at Rocky Mount in 2018 (Fig. 3). For the fungicide application timing, untreated had the lower yield and the heading application consistently resulted in better yield in all the experiments. At Suffolk in 2017, yield did not differ significantly between heading and flowering application in a susceptible hybrid, while in 2018, yield from the flower application and untreated did not differ (Fig. 3). At Suffolk locations when the disease severity of anthracnose and/or grain mold was high, yield of susceptible hybrid was higher when fungicide was applied at heading.

Discussion

In this study, efficacy of single fungicide (pyraclostrobin + fluxapyroxad as Priaxor) application timing when a susceptible and a moderately resistant grain sorghum hybrids were evaluated. In the untreated susceptible hybrid, anthracnose severity ranged from 0 to 60% and grain mold severity ranged from traces to 40%. In the untreated moderately resistant hybrid, anthracnose severity ranged from 0 to 45% and grain mold severity ranged from 1% to 10%. Single application of fungicide at heading or flower reduced anthracnose severity and at one location reduced grain mold severity in a susceptible hybrid compared to untreated. In our previous study (Chapter 2), fungicide applications at boot, flower and two weeks post flower were evaluated but application at heading was not. Comparison of the two fungicide application timings in this study indicated that heading application provides better control of diseases and yield protection compared to flowering. The untreated moderately resistant hybrid consistently had lower anthracnose and grain mold severity compared to the susceptible hybrid indicating its disease resistance across experimental sites.

Dissemination and germination of *C. sublineola* is water dependent (Leite and Nicholson 1992; Ngugi et al. 2000; Thakur and Mathur 2000) and expansion of the anthracnose lesion and sporulation is favored by warmer temperatures (22^{o}C to 30^{o}C) (Thakur and Mathur 2000). The anthracnose onset was early at Suffolk in 2018 compared to other experiments resulting in higher anthracnose severity later in the season due to the favorable environmental conditions ($\geq$25^{o}C and >100mm of precipitation) during the growing season. The inoculum for grain mold is ubiquitous, found in soil, crop debris, alternate host or atmosphere above the sorghum field (Bandyopadhyay et al. 1991a; Funnell-Harris and Pedersen 2011; Ratnadass et al. 2003). In addition, high relative humidity (RH) between the end of flowering and harvest was strongly

correlated with mold incidence (Ratnadass et al. 2003) and a wetness duration of 16 to 72 h was ideal for grain mold infection (Bandyopadhyay et al. 2000). Hence, high grain mold severity in this experiment should be due to favorable environmental conditions during the reproductive developmental stages in the month of August and September compared to other experiments. However, at Rocky Mount in 2017 despite the favorable environmental conditions (≥24ºC and >100mm of precipitation), severity of both the diseases was low which could be due to lower levels of primary inoculum at this location.

Integration of host resistance and fungicide use has been successful in managing diseases in various crops (Crute 1984; Huzar-Novakowiski et al. 2017; Rogers and Stevenson 2006; Shtienberg et al. 2000; Wegulo et al. 2010; Willyerd et al. 2011) and is believed to provide greater efficiency and economy in disease control (Wolfe and Jeger 1981). In addition, integration of host resistance and fungicide use lessen the chances of selecting resistant pathogen forms and provide stable and durable control of diseases (Crute 1984; Wolfe and Jeger 1981). Limited information is available on the effect of integrating host resistance and fungicide use to manage diseases in sorghum. Integration of host plant resistance with fungicides (foliar alone or combined with seed treatment) was effective in managing anthracnose and protecting yield in Nigeria (Marley 2004). In the same study, use of fungicides reduced anthrcanose severity in the test hybrids irrespective of their susceptibility to anthracnose. In the present study, use of a moderately resistant hybrid alone was effective in reducing anthracnose and grain mold severity, and protecting yield. The study by Marley et al. (2004) also reported anthracnose resistant variety to have similar anthracnose rating with or without fungicide treatments but yield was high with fungicide treatment than in the untreated. For the susceptible hybrid, integration of a single application of fungicide (pyraclostrobin +

fluxapyroxad as Priaxor) at heading controlled diseases and protected yield, similar to a study that found reduced anthracnose severity in the susceptible variety compared to the control when coupled with seed dressing fungicides (Gwary et al. 2008).

Based on the findings in this study, the use of moderately resistant hybrid alone is effective to reduce anthracnose and grain mold severity in mid-Atlantic and eliminate the need of fungicide application for yield protection. If susceptible hybrids are grown in the fields with a history of these diseases, a fungicide application (pyraclostrobin-based) optimally at heading and no later than beginning of flowering will reduce disease and protect yield when the environmental conditions are favorable for disease development.

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Table 1. Location, date of planting, fungicide application, anthracnose onset, disease evaluation and harvest for experiments comparing two sorghum hybrids and three fungicide application timings.

Experimental Location[a]	Year	Planting	Fungicide application[b]	Anthracnose Onset[c]	Disease evaluation[d]	Harvest
Rocky Mount, NC	2017	12 Jun	22 Aug (Heading) 30 Aug (Flower)	17 Aug	28 Sep	23 Oct
	2018	6 Jun	10 Aug (Heading) 14 Aug (Flower)	9 Aug	20 Sep	1 Oct
Suffolk, VA	2017	26 Jun	24 Aug (Heading) 28 Aug (Flower)	13 Aug	6 Oct	2 Nov
	2018	8 Jun	8 Aug (Heading) 13 Aug (Flower)	18 Jul	19 Sep	21 Sep

[a]Experimental locations: Rocky Mount, NC (Upper Coastal Plain Research Station); Suffolk, VA (Tidewater Agricultural Research and Extension Center).
[b]Application timing of pyraclostrobin + fluxapyroxad (as 0.29 liter/ha of Priaxor). Heading refers to the sorghum developmental stage, beginning of heading in 50% of the plants and flower refers to the sorghum developmental stage, beginning of flowering in 50% of the plants.
[c]Anthracnose onset refers to the date when first symptoms and/or signs of anthracnose were visually observed.
[d]Disease evaluation refers to the date when anthracnose severity and grain mold severity were visually rated.

Table 2. *P*-values for experiments in Suffolk and Rocky Mount for two years 2017 and 2018.

	Suffolk, 2017			Rocky Mount, 2017			Suffolk, 2018			Rocky Mount, 2018		
Source	Anth[a]	GM[b]	Yield, kg/ha	Anth	GM	Yield, kg/ha	Anth	GM	Yield, kg/ha	Anth	GM	Yield, kg/ha
Block	0.159	0.019	0.469	0.528	0.283	0.901	0.002	0.442	<0.001	0.009	0.124	0.428
Hybrid (H)	<0.001	<0.001	0.005	<0.001	0.395	0.016	<0.001	<0.001	0.066	<0.001	0.184	0.252
App.Timing (A)	<0.001	0.118	<0.001	<0.001	0.364	0.430	<0.001	0.002	<0.001	0.018	0.448	0.341
H x A	<0.001	0.017	0.154	<0.001	0.786	0.722	0.736	0.023	0.386	0.017	0.448	0.861

[a]Anth= Sorghum anthracnose
[b]GM= Grain mold

Table 3. Comparison of grain mold severity of hybrids and three application timing of pyraclostrobin + fluxapyroxad at four experimental sites.

	Experimental locations			
Factor	Suffolk, 2017	Rocky Mount, 2017	Suffolk, 2018	Rocky Mount, 2018
Hybrid **(H)** [x]				
84P80 (S)	3.8 a [y]	4.3	22.5 a	1.8
765B (MR)	1.3 b	3.7	3.7 b	1.0
Fungicide application **(F)** [z]				
None	2.8 a	4.7	19.2 a	1.9
Heading	2.1 a	3.5	7.8 b	1.3
Flower	2.1 a	3.8	7.8 b	1.0
H x F				
	84P80 765B	84P80 765B	84P80 765B	84P80 765B
None	5.0 a 1.2 b	5.3 4.1	44.4 a 5.1 bcd	1.0 1.0
Heading	2.8 ab 1.5 b	3.8 3.2	12.8 bc 4.1 cd	1.6 1.0
Flower	2.8 ab 1.5 b	3.6 3.9	16.9 b 2.3 d	3.0 1.0

[x]Hybrids used in this study. A susceptible (S) hybrid '84P80' and a moderately resistant (MR) hybrid, '765B'.
[y]Means within a column followed by the same letter are not significantly different from each other according to Tukey's HSD test at the significance level of 0.05.
[z]Three application timing used in this study. None = untreated; Heading = beginning of heading in 50% of the plants; flower = beginning of flowering in 50% of the plants
Grain mold severity was evaluated as the estimate of the percentage of sorghum head with grain mold.

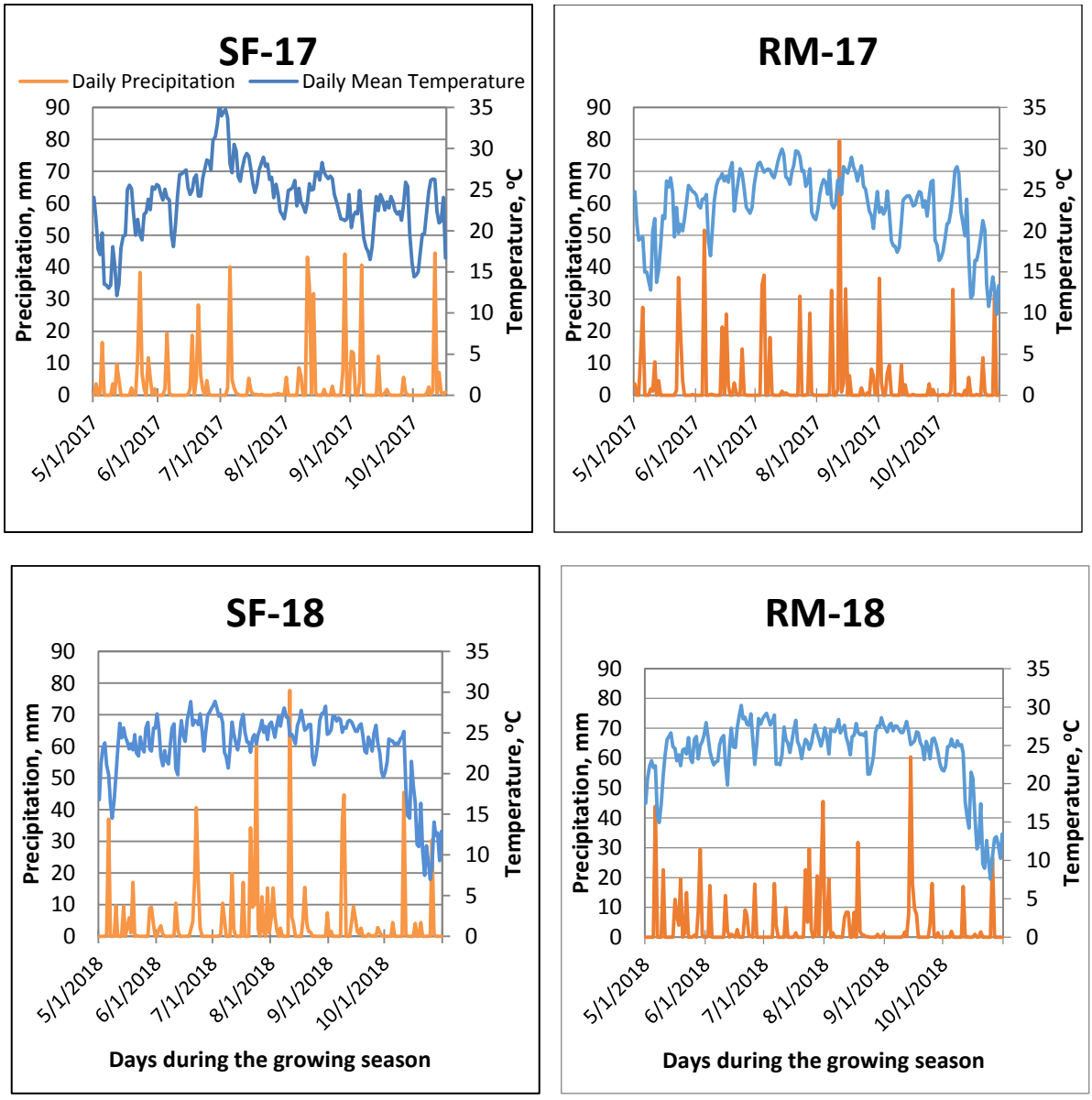

Fig. 1. Daily rainfall and average air temperatures for locations where the experiments were conducted over two years in Virginia and North Carolina. Experimental locations: RM = Rocky Mount, NC (Upper Coastal Plain Research Station); and SF= Suffolk, VA (Tidewater Agricultural Research and Extension Center). Numbers following the location abbreviation indicate the year of the experiment (2015-2017).

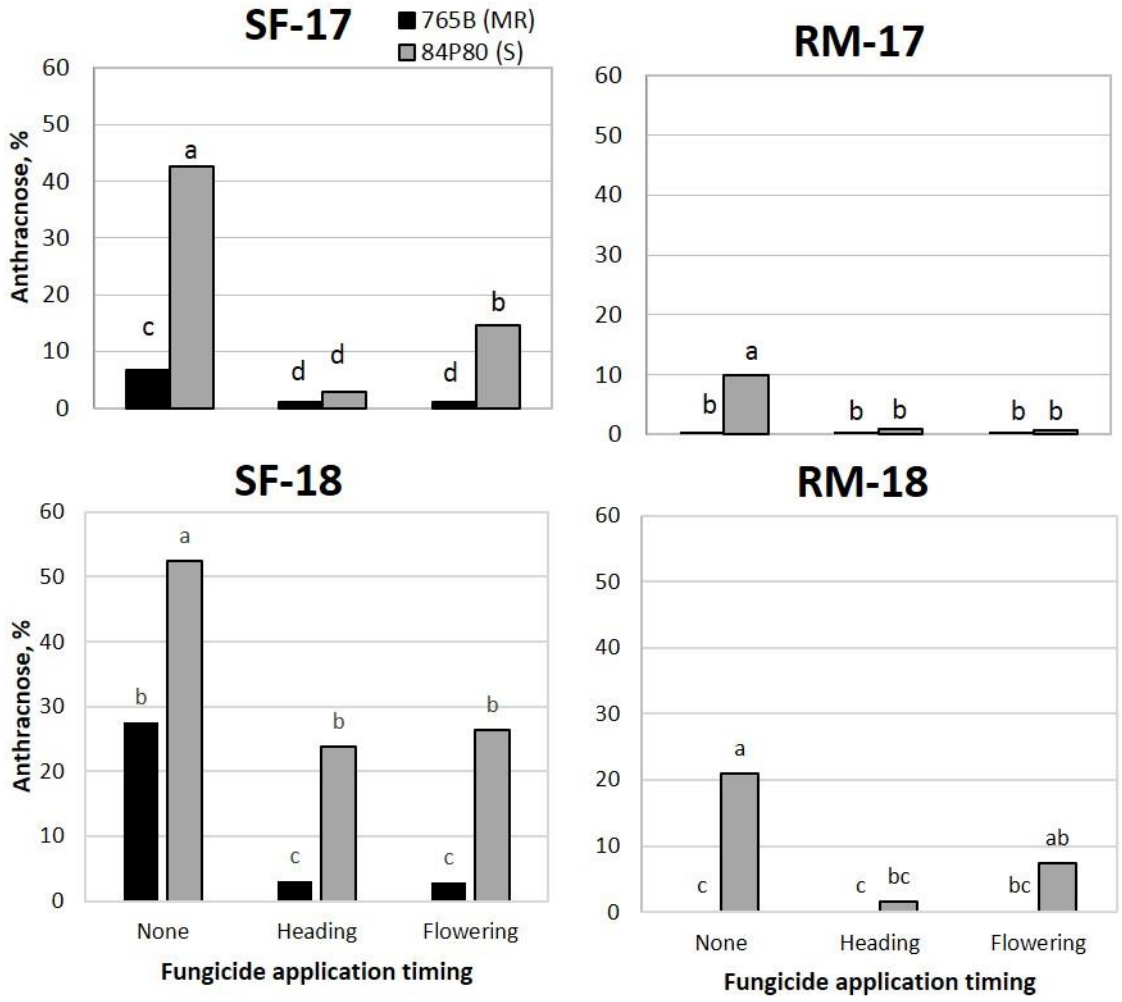

Fig. 2. Comparison of anthracnose severity of hybrids and three application timing of pyraclostrobin + fluxapyroxad (as 0.29 liter/ha of Priaxor) at four experimental locations. Experimental locations: SF=Suffolk, VA (Tidewater Agricultural Research and Extension Center) and RM= Rocky Mount, NC (Upper Coastal Plain Research Station). Two hybrids were compared and MR= Moderately Resistant and S= Susceptible. The numbers following the location are the year of the experiment (2017 or 2018). Bars with the same letter are not significantly different from each other according to Tukey's HSD test at the significance level of 0.05. Anthracnose severity was evaluated as the visual estimate of percentage of leaf area with symptoms and signs of anthracnose.

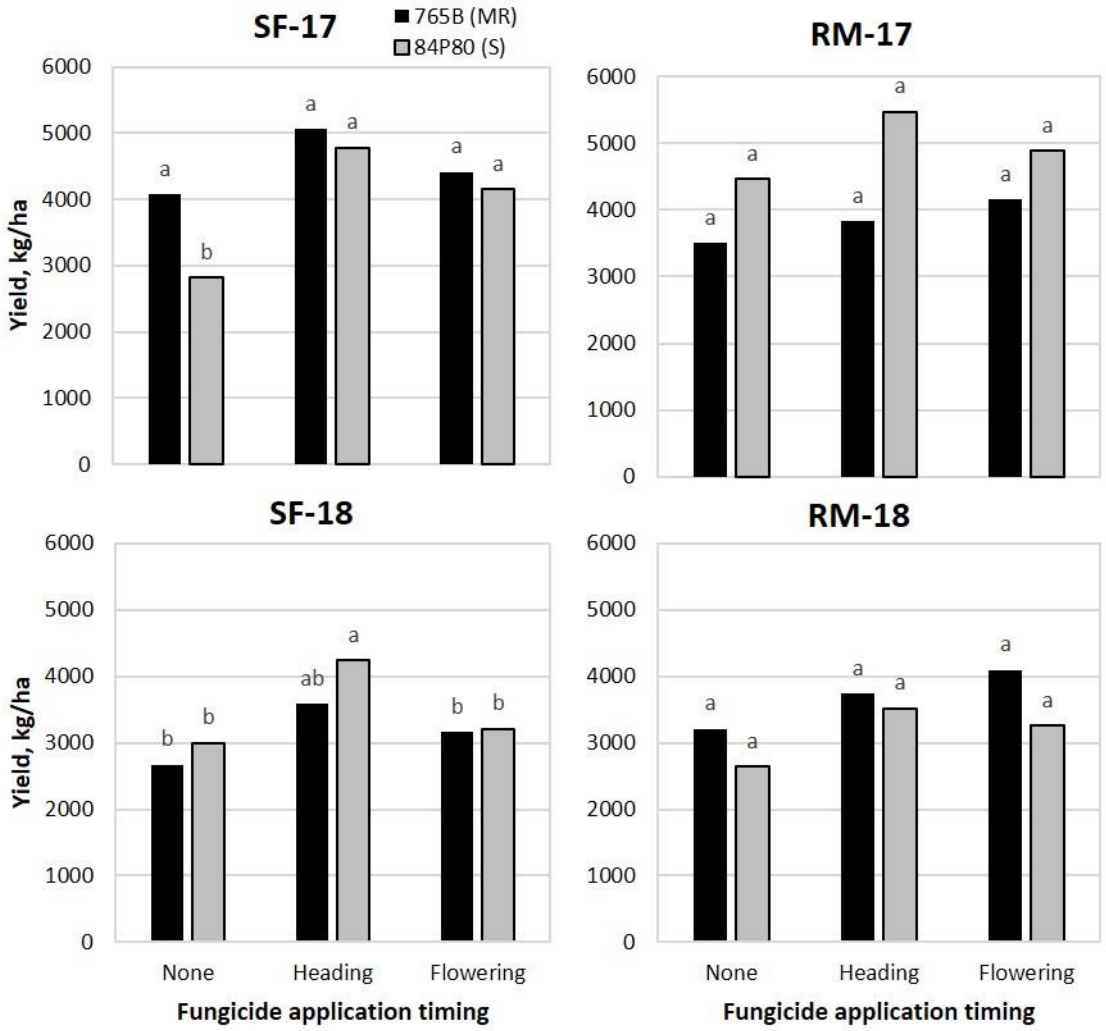

Fig 3. Variation in grain yield of two sorghum hybrids and three application timing of pyraclostrobin + fluxapyroxad (as 0.29 liter/ha of Priaxor) at four experimental locations. Experimental locations: SF=Suffolk, VA (Tidewater Agricultural Research and Extension Center) and RM= Rocky Mount, NC (Upper Coastal Plain Research Station). Two hybrids were compared and MR= Moderately Resistant and S= Susceptible. The numbers following the location are the year of the experiment (2017 or 2018). Bars with the same letter are not significantly different from each other according to Tukey's HSD test at the significance level of 0.05.

Chapter 5: *Fusarium* spp. associated with grain mold and mycotoxin contamination of sorghum in the mid-Atlantic U.S.

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B. Acharya extracted DNA, amplified and processed the sequences of the *Fusarium* isolates. D. G. Schmale and H. L. Mehl planned, coordinated and provided the oversight of the study. David Haak helped with the phylogenetic analysis of the sequences to build the consensus tree.

Keywords: Sorghum, Fusarium, grain mold, DON, fumonisin, phylogenetic analysis

Abstract

Numerous *Fusarium* species are associated with grain mold in sorghum production areas worldwide and the species associated vary by geographical locations and environmental conditions. Though associated mycotoxins have been detected from grain sorghum in the mid-Atlantic U.S., the diversity of *Fusarium* species associated with grain mold have not been characterized. Therefore, the objective of this study was to isolate and identify *Fusarium* spp. associated with grain mold in the mid-Atlantic U.S. Using grain samples that were contaminated with deoxynivalenol (DON) or fumonisin or both, a total of 79 *Fusarium* isolates were recovered from four locations. Partial sequences of three loci, translation elongation factor (TEF1α), DNA-directed RNA polymerase II largest subunit and second largest subunit (RPB1 and RPB2, respectively) of single spored *Fusarium* isolates were amplified, sequenced, aligned and a phylogenetic tree was generated. *F. graminearum, F. armeniacum, F. proliferatum, F. fujikuroi, F. verticillioides, F. thapsinum,* and *F. lacertarum* and additional six distinct *Fusarium* spp. in *Fusarium incarnatum-equiseti* species complex (FIESC) were identified. Of the total isolates, *Fusarium* in FIESC were most frequently recovered (48%). *Fusarium* spp. associated with sorghum grain mold in the mid-Atlantic have the potential to contaminate grain with mycotoxins including DON and fumonisins that can impact both human and animal health.

Introduction

Grain mold, a disease complex with more than 40 different genera of fungi, is a major constraint in sorghum production worldwide (Singh and Bandyopadhyay 2000). Yield losses range from 30 to100% depending on the cultivars, time of flowering, and maturity (Singh and Bandyopadhyay 2000). Species of *Fusarium, Curvularia, Alternaria, Phoma* and

Colletotrichum sublineola are of major concern in grain mold (Singh and Bandyopadhyay 2000) and the fungal species differ with locations (Bandyopadhyay et al. 1991b; Mpofu and McLaren 2014; Prom et al. 2015). In the U.S., majority of grain sorghum is produced in the semi-arid region of the country where environmental conditions are not favorable for the diseases but warm, humid conditions in the mid-Atlantic provide ideal conditions for disease development.

Mycotoxins are chemically diverse and harmful secondary metabolites produced by a diversity of fungi (Waliyar et al. 2008), and these toxins are sometimes involved in pathogenesis and antagonism (Mulè et al. 2005). There is potential for mycotoxin contamination of sorghum grain when mycotoxin-producing fungi are involved in the grain mold complex. The time for grain mold infection by *Fusarium* and other fungal species is from anthesis to maturity with milk and soft dough stages being the most susceptible (Funnell-Harris and Pedersen 2011; Melake-Berhan et al. 1996; Navi et al. 2005; Tarekegn et al. 2004). Chandrashekar and Satyanarayana (2006) reported that *Fusarium spp.* spread from hylar region on the pericarp surface into the endosperm of sorghum grain. In addition, insect infestation (ex: sorghum head-bugs, stinkbugs) also allow easy penetration of *Fusarium spp.* into the developing grain and worsens the condition either by opening up the avenues for the fungi through feeding or suppressing host defenses (Bluhm and Faske 2015; Ratnadass et al. 2003; Sharma et al. 2000). Hence, mycotoxin contamination can occur at pre-harvest during crop production and at postharvest during storage, processing, transportation or marketing (Waliyar et al. 2008). Trichothecenes, zearalenone, fumonisin, aflatoxins and alternaria toxins are some important mycotoxins produced by grain mold fungi, but fumonisin and aflatoxin are the two major problems (Chala et al. 2014; Osman et al. 2017; Silva et al. 2000; Waliyar et al. 2008) even though other mycotoxins such as deoxynivalenol (DON) and zearalenone (ZEA) have been

frequently detected (Chala et al. 2014; Hagler et al. 1987). Contamination of grain sorghum with mycotoxins produced by *Aspergillus* spp. and *Penicillium* spp. are of major concern in countries in Africa, South America and Asia (Apeh et al. 2016; Ayalew et al. 2006; Chala et al. 2014; González et al. 1997; Hussaini et al. 2009; Kange et al. 2015; Osman et al. 2017; Silva et al. 2000; Yassin et al. 2010) while this problem is minimal in North America where *Aspergillus* spp. and *Penicillium* spp. and associated mycotoxins are seldom recovered from grain sorghum (Bluhm and Faske 2015; Hagler et al. 1987; Prom et al. 2015). However, *Fusarium* spp. are commonly isolated from sorghum production areas but in varying frequencies ranging from most frequent genus in some locations (González et al. 1997) to the least frequent in the others (Prom et al. 2015).

Fusarium is a diverse genus, has more than 80 identified species (Leslie and Summerell 2006), and many of them are mycotoxin producers. A number of *Fusarium* species are reported to be associated with grain mold in sorghum from different sorghum production areas in the world and at least fourteen different species have been isolated from sorghum grains (Apeh et al. 2016; Bluhm and Faske 2015; Divakara et al. 2014; González et al. 1997; Hussaini et al. 2009; Leslie et al. 2005; Osman et al. 2017; Prom et al. 2015; Saubois et al. 1999; Sharma et al. 2011; Tarekegn et al. 2004, 2006; Yassin et al. 2010). In these studies, species in the *F. fujikuroi* species complex were consistently present in the grain mold complex. Members of *F. fujikuroi* species complex that cause grain mold were found in soil as well as in air samples above the sorghum fields in the U. S. (Funnell-Harris and Pedersen 2011). In addition, *F. graminearum, F. subglutinans* and several members of *F. incarnatum-equiseti* species complex were also recovered from the air samples above the sorghum fields in Nebraska (Funnell-Harris and Pedersen 2011).

DON, fumonisin, and zearalenone contamination in grain sorghum has been documented from molded sorghum grains in the mid-Atlantic U.S. with DON and fumonisin contamination in five sorghum hybrids ranging from below the limit of detection to 1.1 ppm and 0.8 ppm, respectively (Chapter 3; Hagler et al. 1987). The grain sorghum produced in the mid-Atlantic is used for animal feed, mainly swine and poultry (Balota et al. 2018) that are sensitive to these mycotoxins with swine being particularly sensitive to DON (advisory level of 5 ppm) (FDA, accessed Jan 6, 2019). Despite mycotoxin contamination reports, *Fusarium* species associated with sorghum and these mycotoxins have not been characterized. We hypothesized that at least two species of *Fusarium* are associated with grain mold in the mid-Atlantic. The objective of this study was to identify *Fusarium* species associated with grain mold to better understand the etiology of mycotoxin contamination of grain sorghum in the mid-Atlantic U.S.

Materials and methods

Collection of grain samples. Sorghum grain samples were collected at harvest from the Mid-Atlantic Official Variety Trials (Balota et al. 2017; Balota et al. 2016) at two locations in North Carolina and two locations in Virginia in 2015 and 2016 (Table 1). As part of another study (Chapter 3), grain was tested for mycotoxins using Veratox® kits (Neogen, Lansing, MI), and samples with at least 0.5 ppm of deoxynivalenol (DON) and/or 0.5 ppm fumonisin were selected for *Fusarium* isolation.

***Fusarium* isolations.** *Fusarium* was isolated and single-spore purified as previously described (Schmale et al. 2006) with modifications. For each sample, 20-25 grains were put in a 50 ml capped tube with 20-25 ml of distilled deionized water and shaken for 1 min. The water was decanted, grains were surface disinfested in freshly prepared 10% sodium hypochlorite for 1 min, and then grains were rinsed with water twice. After blotting dry on a sterile paper towel,

5-10 grains were transferred to 1/4 strength potato dextrose agar (PDA) (10 g PDA and 5 g agar l^{-1}) and incubated for 5-7 days at room temperature. *Fusarium* isolates were identified based on colony and spore morphology, and using a sterile toothpick, isolates were sub-cultured to 1/4 PDA and grown for 5-7 days at room temperature. Sterile coffee stick was used to scrap off spores from the plate, streaked once on 2% water agar with mycelial side on the plate. Then the direction of the coffee stick was changed and streaked 3-4 times perpendicular to the first streak followed by parallel streaks to distribute spores. After 12-24 h, single germinating spores were identified using a stereomicroscope with magnification of up to 100 x, and single-spores were transferred to ¼ PDA using a flame-sterilized minuten pin (www.bioquip.com , 0.15mm stainless steel pins, #1207SA) mounted on forceps. For storage, single-spored cultures were grown for 7-10 days on sterilized filter paper cut into halves and placed on 1/4 PDA. Colonized filter paper was aseptically transferred to an empty Petri dish and dried in the laminar flow hood for 24 h then cut into small pieces and stored in a sterile envelope at -20^{o}C until further use.

DNA isolation, PCR, and sequence analysis. For DNA isolation, *Fusarium* isolates were grown on ¼ PDA for 7-10 days, and 100-200 mg of mycelia were harvested using a sterile toothpick and placed into a 2 ml microcentrifuge tube containing 450 µL of Tris-EDTA SDS lysis buffer (30 mM Tris, 10 mM EDTA, 1% SDS, pH 8.0) and beads (BioSpec Products, Inc., OK). Fungal material was pulverized in a Mini-BeadBeater 24 (BioSpec Products, Inc., OK) at 1000 x g for 1 min then incubated in a thermal shaker at 60^{o}C (1,000 rpm, 30 min). Tubes were centrifuged at 14,000 x g for 30 min, and 370 µl of supernatant was transferred to a new tube and placed on ice. An equal volume of cold (4^{o}C) 4M ammonium acetate (NH_4OAc) was added to the supernatant and mixed by inverting. Ice-cold 100% ethanol (740 µL) was added, and tubes were mixed by inverting and placed at -20^{o}C for 30 min. Tubes were centrifuged at 14,000

x g for 5 min, and supernatant was discarded leaving the DNA pellet stuck to a side of the microcentrifuge tube. The pellet was washed twice with 500 µL of 100% ethanol, and open tubes were left upside down on paper towel to air. DNA pellets were re-suspended in 25 µL sterile purified water, and DNA concentration was measured using NanoVue Plus (GE Healthcare, Korea).

For molecular identification, translation elongation factor 1α (TEF1 α), DNA-directed RNA polymerase II largest subunit (RPB1) and second largest subunit (RPB2) loci were amplified using the primers in Table 2 (O'Donnell et al. 2010). PCR was conducted in 20 µL reaction mixture using AccuPower® HotStart PCR PreMix (Bioneer, Korea), 0.5 µL of each primer, 14 µL of ddH_2O and 5 µL of diluted DNA (5 ng/µL). PCR was performed using a T100™ Thermal Cycler (Bio-Rad, CA) with an initial denaturation step at 94°C for 5 min to activate *Taq* polymerase. For TEF1α, PCR consisted of 35 cycles of 94°C for 30 s, 53°C for 45 s, and 72 °C for 60 s. For RPB1 and RPB2, PCR consisted of 40 cycles of 94°C for 40 s, annealing for 60 s (RPB1 = 50°C, RPB2 = 59°C), and 72 °C for 90 s. All reactions included a final elongation step at 72 °C for 5 min. Amplicons were separated on a 1.5% agarose gel stained with GelRed® Nucleic Acid Gel Stain (Biotium Inc., CA) at 100 v for 60 min and visualized using a GelDoc ™ XR+ system (Bio-Rad, CA). Amplicons were enzymatically cleaned using Exo-SapIT (Thermo Fisher Scientific, MA) and sequenced in both directions using the primers in Table 2 at Eton Biosciences, NC. Sequences were assembled using pairwise/multiple alignment and de novo assemble in Geneious 8.0.5 with manual adjustments to obtain the consensus sequence. Consensus sequences for all isolates in this study and reference isolates representative of different *Fusarium* spp. (O'Donnell et al. 2013; O'Donnell et al. 2010) were aligned using MUSCLE in MEGA7 (Kumar et al. 2016). A maximum

likelihood tree was constructed using the combined partial sequences of TEF 1α, RPB1 and RPB2 for 79 isolates identified in this study, 27 *Fusarium* species reference isolates, and sequences from an outgroup species (*F. dimerum*) obtained from GenBank. TEF 1α, RPB1 and RPB2 sequences were then trimmed on both ends to retain 608 bp, 1392 bp and 1684 bp, respectively. The sequences of the three loci were concatenated for each isolate. Phylogenetic relationship among the *Fusarium* isolates were inferred from the combined three loci data using maximum likelihood (ML) in RAxML 7.2.8 plugin in Geneious 8.0.5 that used GTR CAT nucleotide model, rapid bootstrapping and search for best-scoring ML tree algorithm, 1000 bootstrap replications and partitioning for the three loci. GenBank accessions for isolates from this study and reference sequences are listed in Supplementary Table 1.

Results

A total of 79 isolates of *Fusarium* were recovered from eleven grain sorghum samples (Table 1, 3). Based on a multi-locus phylogenetic analysis, *Fusarium* isolated from sorghum grains were members of three species complexes: *Fusarium incarnatum-equiseti* species complex (FIESC), *Fusarium fujikuroi* species complex (FFSC), and *Fusarium sambucinum* species complex (FSAMSC). Overall, FIESC was the most frequently isolated from sorghum grains (48%, N=38 isolates, 11 samples) followed by FFSC (32%, N=25 isolates, 9 samples) and FSAMSC (20%, N=16 isolates, 6 samples). However, frequencies of isolates within each of these species complexes varied by location and grain sample (Table 1).

Grain samples varied in levels of mycotoxin contamination, and the sample with the second highest DON concentration (5 ppm) had the highest frequency of FSAMSC (86%), the species complex that includes the DON producing species *F. graminearum*. However, no

FSAMSC was recovered from the sample with 8 ppm DON. FFSC contains several species that produce fumonisin, and there was a correlation between fumonisin concentrations in the grain samples and frequencies of FFSC isolated from grains (r^2=0.37, P = 0.048). There were no significant relationships between fumonisin and FIESC or FSAMSC frequencies or between DON and frequencies of any of the species complexes (P>0.10).

In the multi-locus phylogenetic analysis, *Fusarium* spp. isolated in this study clustered with reference sequences within the FIESC, FFSC, and FSAMSC (Fig. 1), and isolates were assigned species names based on positions of isolates within monophyletic clades (Table S1, Table 3). There were 13 distinct phylogenetic species, seven within FIESC (FIESC 1, 4, 14, 16, 18, 25, and 26), four within FFSC (*F. fujikuroi*, *F. proliferatum*, *F. thapsinum*, and *F. verticillioides*), and two within FSAMSC (*F. armeniacum* and *F. graminearum*). Twenty-two isolates originating from all six locations (site-years) and 10 out of 11 grain samples formed a distinct clade within FIESC (99.6% bootstrap) and was a sister clade to FIESC 26 (Fig. 1). This was the most frequently isolated species in the current study (28% of all isolates), and though it was identified as FIESC 26 based on the identity of the most closely related reference isolate, these isolates likely comprise a distinct, uncharacterized species within the complex. The second most common species (20% of isolates) was *F. proliferatum* which was isolated from 5 of the 6 locations and approximately half of the samples (Table 3). *F. graminearum* (14%), FIESC 18 (8%), and *F. fujikuroi* (8%) were isolated from 5, 4, and 3 of the eleven grain samples, respectively. The remaining eight species were only isolated from one or two samples and at frequencies of less than 8%, indicating these *Fusarium* spp. are less commonly associated with sorghum grain mold in VA and NC (Table 3). The number of *Fusarium* spp. associated with the sorghum grain mold complex varied by sample and location (Table 3), but the number of

isolates examined in the study was low so it is unlikely the full diversity of *Fusarium* was isolated. However, all samples in this study were co-infected with multiple *Fusarium* species from at least two different species complexes.

Discussion

Sorghum grain mold, caused by a complex of many diverse fungal species, reduces grain yield and quality in sorghum producing areas where warm and humid weather conditions occur from flowering to maturity (Bandyopadhyay et al. 2000). Previous studies have indicated that grain mold and mycotoxin contamination are likely to occur when sorghum in grown in the mid-Atlantic region of the U.S. (Balota et al. 2014; Hagler et al. 1987), but the specific fungi involved have not been previously characterized. In the current study, 13 phylogenetically distinct *Fusarium* species in the FIESC, FFSC, and FSAMSC were isolated and identified from grain sorghum grown in VA and NC. Our study is in accordance with the previous studies that a complex of *Fusarium* species are associated with grain sorghum at a location (Apeh et al. 2016; Bluhm and Faske 2015; Divakara et al. 2014; González et al. 1997; Hussaini et al. 2009; Leslie et al. 2005; Osman et al. 2017; Prom et al. 2015; Saubois et al. 1999; Sharma et al. 2011; Tarekegn et al. 2004, 2006; Yassin et al. 2010).

Fusarium species in FIESC contains a complex of morphologically similar species that contains 28 distinct species within the complex that are pathogenic to plants, humans and animals (Castellá and Cabañes 2014; Chohan and Abid 2018; Donnell et al. 2009; Jacobs et al. 2018). *Fusarium* species in FIESC were reported to infect both the grains and stalk of sorghum and have been detected in air samples over the sorghum field as well as in leaf samples (Funnell-Harris et al. 2015; Funnell-Harris et al. 2017; Kelly et al. 2017; Pena et al. 2019).

Similar to our study, most of the *Fusarium* species associated with sorghum in Arkansas belonged to FIESC (Bluhm and Faske 2015). *Fusarium* species in FIESC are known producers of both trichothecenes A and B but not DON, and zearalenone (O'Donnell et al. 2018; Villani et al. 2016). Some of the phylogenetic species within FIESC used as a reference in this study are reported to produce mycotoxins. FIESC 25 is reported to produce diacetoxyscirpenol (DAS) and fusarenone x, FIESC 16 produces fusarenone x, FIESC 1 produces DAS and 4, 15-diacetylnivalenol (4, 15-diANIV), *F. laceratum* (FIESC 4) produce zearalenone and 4, 15-diANIV, and *F. equiseti* (FIESC 14) produces DAS and 4, 15-diANIV (O'Donnell et al. 2018; Villani et al. 2016). This indicates that a variety of mycotoxins including these produced by members of FIESC need to be tested in the future to understand the level of mycotoxin contamination in sorghum and its effect on the livestock in mid-Atlantic. Limited information was available about the association of *F. armeniacum* with grain sorghum but this species is reported to cause seed rot and root rot in soybean (Ellis et al. 2012), infect rice and grasses and produce trichothecene A mycotoxins, mainly T-2 and HT-2 with some isolates reported to produce zearalenone (Hong et al. 2015; Nichea et al. 2015). Isolates of *F. armeniacum* were recovered from one location (Lewiston-Woodville, NC) in our study where soybean is often rotated with grain sorghum (personal observation). This indicates that *F. armeniacum* could be a seedling pathogen of soybean in addition to that of grain sorghum in the mid-Atlantic, which requires further research for confirmation.

A number of studies have reported *Fusarium* species in the *Fusarium fujikuroi* species complex (FFSC) to be associated with grain mold in sorghum (Bluhm and Faske 2015; Divakara et al. 2014; Funnell-Harris and Pedersen 2011; González et al. 1997; Kelly et al. 2017; Leslie et al. 2005; Pena et al. 2019; Sharma et al. 2011; Tesso et al. 2010; Yassin et al.

2010) and *F. thapsinum* is reported to be the predominant or most frequently isolated species on sorghum grains in Australia (Kelly et al. 2017), India (Sharma et al. 2011), and Argentina (Pena et al. 2019). Funnell-Harris and Pederson (2011) also reported that the number of *Fusarium* species in FFSC increased in air samples above sorghum fields in Nebraska from anthesis to maturity, coinciding with the susceptible sorghum growth stages and were present in soil as well. In our study, *F. proliferatum, F. fujikuroi, F. verticillioides,* and *F. thapsinum* were found to be associated with grain sorghum and only one isolate of *F. thapsinum* was recovered from a location (Rocky Mount, NC) in contrast to some of the previous studies where it was a predominant or most frequently associated species. In other sorghum producing areas, *F. andiyazi, F. subglutinans, F. nygamai* and other species in FFSC were reported to be associated with grain sorghum (Funnell-Harris and Pedersen 2011; González et al. 1997; Kelly et al. 2017; Leslie et al. 2005; Osman et al. 2017; Sharma et al. 2011; Tesso et al. 2010; Yassin et al. 2010) but they were not recovered in our study. Majority of the *Fusarium* species in FFSC are known producers of fumonisin. However, *F. verticillioides* producing zearalenone and *F. nygamai* producing vomitoxin or Deoxynivalenol (DON) were reported from Saudi Arabia (Yassin et al. 2010).

Fusarium graminearum has been reported to be associated with grain sorghum in lesser frequency compared to some other species (Funnell-Harris and Pedersen 2011; González et al. 1997; Osman et al. 2017; Tarekegn et al. 2004) and are reported to colonize grain sorghum asymptomatically (Funnell-Harris and Pedersen 2011). In our study, *F. graminearum* was recovered from three locations in lower frequency with the highest frequency of 7 isolates from Lewiston-Woodville where it was the most frequently recovered *Fusarium* species. Cropping systems in mid-Atlantic include wheat, corn, and sorghum. *F. graminearum* is a well-

established pathogen of wheat and corn and their recovery from grain sorghum indicate a threat to the grain crops in mid-Atlantic. A molecular comparison of the isolates of *F. graminearum* from wheat, corn and sorghum might provide a better insight if the isolates from one crop are capable of infecting the others.

Fusarium species identified in this study were recovered from grain samples contaminated with deoxynivalenol, fumonisin, or both, but the mycotoxin-producing potential of the isolates was not examined. *Fusarium* species infecting grain sorghum are reported to cause stalk rot (Kelly et al. 2017; Petrovic et al. 2009; Tesso et al. 2010) and isolates previously recovered from stalks were reported to infect panicle and gains in Australia (Kelly et al. 2017). Similar studies in the mid-Atlantic will provide a better idea on the association of *Fusarium* species with sorghum and development of control strategies. In addition, conducting pathogenicity tests of the species identified in this study will help to differentiate a true pathogen and a saprophyte associated with grain sorghum.

Pathogens causing grain mold vary geographically and at particular location some pathogens might be predominant compared to the others (Little et al. 2012) making the deployment of pathogen-specific resistance genes less effective in all the problematic regions. Limited information is available on breeding for resistance to *Fusarium* in grain sorghum, which could be due to the association of diverse *Fusarium* species with grain mold. However, studies on corn and wheat indicate the presence of sources of resistance to more than one *Fusarium* species (Giomi et al. 2016; Mesterhazy 1982; Toth et al. 2008). Therefore, a future study to identify a source of resistance against different species of *Fusarium* associated with grain sorghum might lead to a development of *Fusarium* resistant grain sorghum hybrid suitable for mid-Atlantic.

Atotal of 79 *Fusarium* isolates were examined in this study so a full diversity of *Fusarium* species associated with grain sorghum in the mid-Atlantic may not be represented. However, 13 phylogenetically distinct *Fusarium* species belonging to FIESC, FFSC and FSAMSC were identified, and species within these groups have the potential to produce a diversity of mycotoxins. *Fusarium* species in FIESC were most frequently isolated followed by *F. proliferatum* and *F. graminearum.* DON and fumonisin contamination in sorghum can exceed the advisory limit in mid-Atlantic and requires attention to manage them during production and post harvest. A Majority of the *Fusarium* species identified in this study are known mycotoxin producers indicating the need for characterization of other mycotoxins that might be present in grains produced in the mid-Atlantic other than DON and fumonisin.

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Table 1. Sorghum grain sample origin, mycotoxin levels, and frequencies of different *Fusarium* spp. isolated.

Year	Location[a]	Grain sample ID	DON, ppm	Fumonisin, ppm	No. isolates	FIESC[b], %	FFSC[c], %	FSAMSC[d], %
2016	Lewiston-Woodville, NC	LW16-1	5	0.2	14	14	0	86
2016	Lewiston-Woodville, NC	LW16-4	1.3	4.4	4	50	50	0
2016	Rocky Mount, NC	RM16-1	1.1	0	7	86	0	14
2016	Rocky Mount, NC	RM16-2	0.3	4	9	78	11	11
2015	Suffolk, VA	SF15-1	0.6	3.1	7	57	43	0
2016	Suffolk, VA	SF16-1	0.4	9.9	8	25	75	0
2016	Suffolk, VA	SF16-3	0.5	3.4	5	60	40	0
2015	Windsor, VA	WS15-1	1	0.1	6	33	50	17
2015	Windsor, VA	WS15-2	8	1.2	5	80	20	0
2016	Windsor, VA	WS16-1	0.6	2.6	7	57	29	14
2016	Windsor, VA	WS16-2	0.4	2.4	7	29	71	0

[a]Locations refers to the field locations where grain sorghum samples were obtained for isolation.
[b]FIESC= Fusarium incarnatum-equiseti species complex.
[c]FFSC= *Fusarium fujikuroi* species complex.
[d]FSAMSC=Fusarium sambucinum species complex.

Table 2. Primers used for PCR and DNA sequencing in this study.

Locus	Gene product	Primer Designation	Primer Sequence (5'-3')	Use PCR	Use Sequencing
EF-1α	Translation elongation factor 1α			x	x
		EF1	ATGGGTAAGGARGACAAGAC		
		EF2	GGARGTACCAGTSATCATG	x	x
RPB1	RNA polymerase II largest subunit	Fa	CAYAARGARTCYATGATGGGWC	x	
		G2R	GTCATYTGDGTDGCDGGYTCDCC	x	
		F5	ATGGGTATYGTCCAGGAYTC		x
		F7	CRACACAGAAGAGTTTGAAGG		x
		F8	TTCTTCCACGCCATGGCTGGTCG		x
		R8	CAATGAGACCTTCTCGACCAGC		x
RPB2	RNA polymerase II second largest subunit	5f2	GGGGWGAYCAGAAGAAGGC	x	x
		11ar	GCRTGGATCTTRTCRTCSACC	x	x
		7cf	CCCATRGCTTGYTTRCCCAT		x
		7cr	ATGGGYAARCAAGCYATGGG		x

Table 3. Frequencies of *Fusarium* spp. isolated from sorghum grain in Virginia and North Carolina.

	Location and grain sample[b]												
	LW16		RM16		SF15	SF16		WS15		WS16		Total	No.
Species[a]	1	4	1	2	1	1	3	1	2	1	2	isolates	samples
FIESC 26	1	2	2	3	3	2	3	1	4	1	--	22	10
F. proliferatum	--	2	--	--	2	6	2	3	--	--	1	16	6
F. graminearum	7	--	1	1	--	--	--	1	--	1	--	11	5
FIESC 18	1	--	--	--	--	--	--	1	--	3	1	6	4
F. fujikuroi	--	--	--	--	1	--	--	--	--	1	4	6	3
F. armeniacum	5	--	--	--	--	--	--	--	--	--	--	5	1
FIESC 25	--	--	2	2	--	--	--	--	--	--	--	4	2
FIESC 1	--	--	--	2	--	--	--	--	--	--	--	2	1
FIESC 16	--	--	1	--	1	--	--	--	--	--	--	2	2
F. verticillioides	--	--	--	--	--	--	--	--	1	1	--	2	2
FIESC 4 (*F. lacertarum*)	--	--	--	--	--	--	--	--	--	--	1	1	1
FIESC 14 (*F. equiseti*)	--	--	1	--	--	--	--	--	--	--	--	1	1
F. thapsinum	--	--	--	1	--	--	--	--	--	--	--	1	1

[a]*Fusarium* species were identified based on multi-locus sequencing of the translation elongation factor 1-α and the RNA polymerase II largest and second largest subunit.
[b]LW= Lewiston-Woodville, NC; RM= Rocky Mount, NC; SF= Suffolk, VA; and WS=Windsor, VA. The numbers following the location are the year of the experiment and the numbers below the location indicate the grain sample.

Supplementary Table 1. Grain sample ID, isolate ID, *Fusarium* species, and GenBank accession numbers for isolates used in this study.

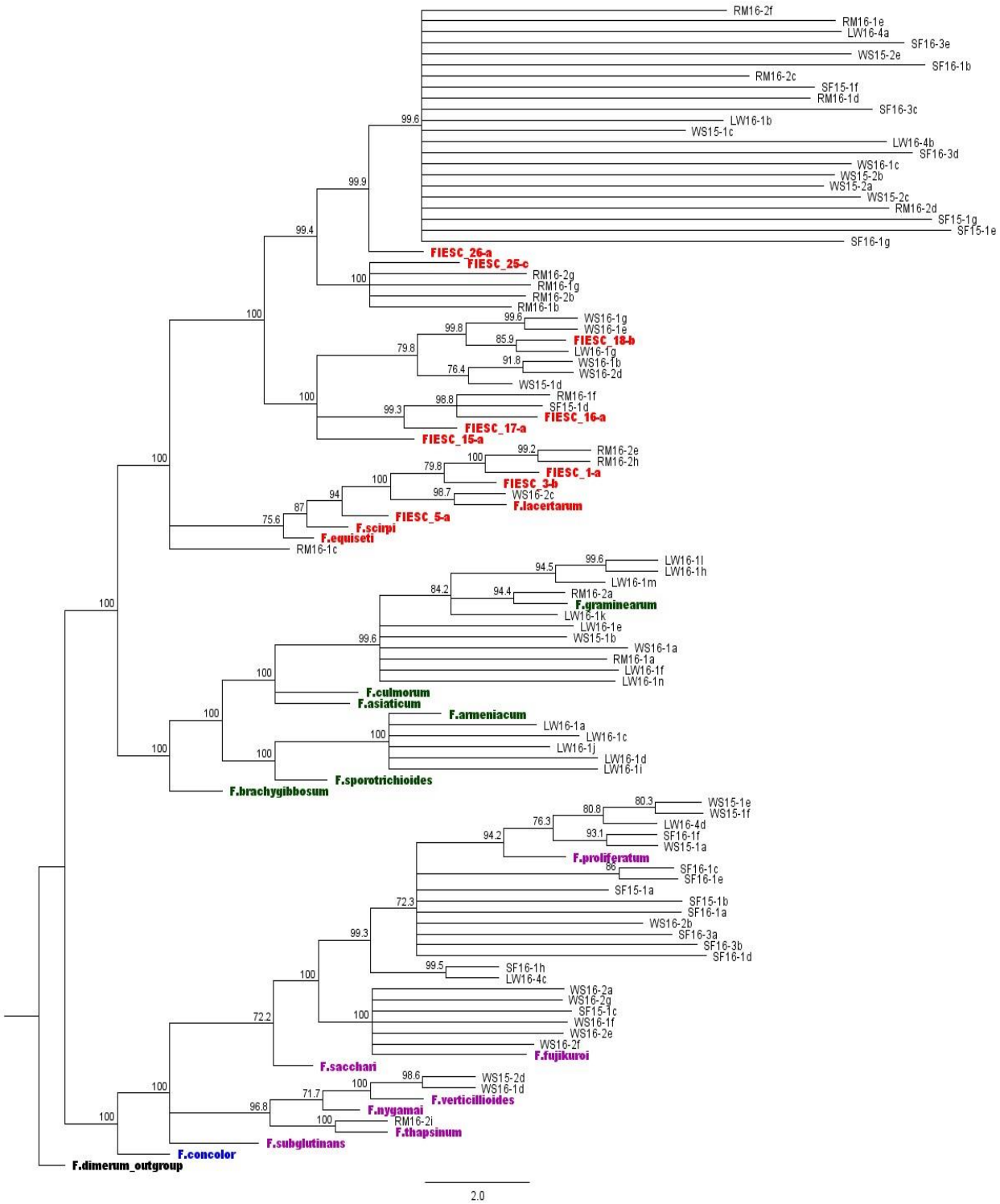
Grain Sample ID	Isolate ID	Species complex	Species	Accession no.		
				TEF	RPB1	RPB2
LW16-1	LW16-1a	FSAMSC	*F. armeniacum*	SAMN11306417	SAMN11306634	SAMN11307038
LW16-1	LW16-1b	FIESC	FIESC 26 (sister clade)	SAMN11306418	SAMN11306635	SAMN11307039
LW16-1	LW16-1c	FSAMSC	*F. armeniacum*	SAMN11306419	SAMN11306636	SAMN11307040
LW16-1	LW16-1d	FSAMSC	*F. armeniacum*	SAMN11306420	SAMN11306637	SAMN11307041
LW16-1	LW16-1e	FSAMSC	*F. graminearum*	SAMN11306421	SAMN11306638	SAMN11307042
LW16-1	LW16-1f	FSAMSC	*F. graminearum*	SAMN11306422	SAMN11306639	SAMN11307043
LW16-1	LW16-1g	FIESC	FIESC 18	SAMN11306423	SAMN11306640	SAMN11307044
LW16-1	LW16-1h	FSAMSC	*F. graminearum*	SAMN11306424	SAMN11306641	SAMN11307045
LW16-1	LW16-1i	FSAMSC	*F. armeniacum*	SAMN11306425	SAMN11306642	SAMN11307046
LW16-1	LW16-1j	FSAMSC	*F. armeniacum*	SAMN11306426	SAMN11306643	SAMN11307047
LW16-1	LW16-1k	FSAMSC	*F. graminearum*	SAMN11306427	SAMN11306644	SAMN11307048
LW16-1	LW16-1l	FSAMSC	*F. graminearum*	SAMN11306428	SAMN11306645	SAMN11307049
LW16-1	LW16-1m	FSAMSC	*F. graminearum*	SAMN11306429	SAMN11306646	SAMN11307050
LW16-1	LW16-1n	FSAMSC	*F. graminearum*	SAMN11306430	SAMN11306647	SAMN11307051
LW16-4	LW16-4a	FIESC	FIESC 26 (sister clade)	SAMN11306431	SAMN11306648	SAMN11307052
LW16-4	LW16-4b	FIESC	FIESC 26 (sister clade)	SAMN11306432	SAMN11306649	SAMN11307053
LW16-4	LW16-4c	FFSC	*F. proliferatum*	SAMN11306433	SAMN11306650	SAMN11307054
LW16-4	LW16-4d	FFSC	*F. proliferatum*	SAMN11306434	SAMN11306651	SAMN11307055
RM16-1	RM16-1a	FSAMSC	*F. graminearum*	SAMN11306444	SAMN11306661	SAMN11307065
RM16-1	RM16-1b	FIESC	FIESC 25	SAMN11306445	SAMN11306662	SAMN11307066

Grain Sample ID	Isolate ID	Species complex	Species	Accession no.		
				TEF	RPB1	RPB2
RM16-1	RM16-1c	FIESC	FIESC 14/ *F. equiseti* (sister clade)	SAMN11306446	SAMN11306663	SAMN11307067
RM16-1	RM16-1d	FIESC	FIESC 26 (sister clade)	SAMN11306447	SAMN11306664	SAMN11307068
RM16-1	RM16-1e	FIESC	FIESC 26 (sister clade)	SAMN11306448	SAMN11306665	SAMN11307069
RM16-1	RM16-1f	FIESC	FIESC 16	SAMN11306449	SAMN11306666	SAMN11307070
RM16-1	RM16-1g	FIESC	FIESC 25	SAMN11306450	SAMN11306667	SAMN11307071
RM16-2	RM16-2a	FSAMSC	*F. graminearum*	SAMN11306435	SAMN11306652	SAMN11307056
RM16-2	RM16-2b	FIESC	FIESC 25	SAMN11306436	SAMN11306653	SAMN11307057
RM16-2	RM16-2c	FIESC	FIESC 26 (sister clade)	SAMN11306437	SAMN11306654	SAMN11307058
RM16-2	RM16-2d	FIESC	FIESC 26 (sister clade)	SAMN11306438	SAMN11306655	SAMN11307059
RM16-2	RM16-2e	FIESC	FIESC 1	SAMN11306439	SAMN11306656	SAMN11307060
RM16-2	RM16-2f	FIESC	FIESC 26 (sister clade)	SAMN11306440	SAMN11306657	SAMN11307061
RM16-2	RM16-2g	FIESC	FIESC 25	SAMN11306441	SAMN11306658	SAMN11307062
RM16-2	RM16-2h	FIESC	FIESC 1	SAMN11306442	SAMN11306659	SAMN11307063
RM16-2	RM16-2i	FFSC	*F. thapsinum*	SAMN11306443	SAMN11306660	SAMN11307064
SF15-1	SF15-1a	FFSC	*F. proliferatum*	SAMN11306451	SAMN11306668	SAMN11307072
SF15-1	SF15-1b	FFSC	*F. proliferatum*	SAMN11306452	SAMN11306669	SAMN11307073
SF15-1	SF15-1c	FFSC	*F. fujikuroi*	SAMN11306453	SAMN11306670	SAMN11307074
SF15-1	SF15-1d	FIESC	FIESC 16	SAMN11306454	SAMN11306671	SAMN11307075
SF15-1	SF15-1e	FIESC	FIESC 26 (sister clade)	SAMN11306455	SAMN11306672	SAMN11307076
SF15-1	SF15-1f	FIESC	FIESC 26 (sister clade)	SAMN11306456	SAMN11306673	SAMN11307077
SF15-1	SF15-1g	FIESC	FIESC 26 (sister clade)	SAMN11306457	SAMN11306674	SAMN11307078
SF16-1	SF16-1a	FFSC	*F. proliferatum*	SAMN11306458	SAMN11306675	SAMN11307079
SF16-1	SF16-1b	FIESC	FIESC 26 (sister clade)	SAMN11306459	SAMN11306676	SAMN11307080

Grain Sample ID	Isolate ID	Species complex	Species	Accession no.		
				TEF	RPB1	RPB2
SF16-1	SF16-1c	FFSC	*F. proliferatum*	SAMN11306460	SAMN11306677	SAMN11307081
SF16-1	SF16-1d	FFSC	*F. proliferatum*	SAMN11306461	SAMN11306678	SAMN11307082
SF16-1	SF16-1e	FFSC	*F. proliferatum*	SAMN11306462	SAMN11306679	SAMN11307083
SF16-1	SF16-1f	FFSC	*F. proliferatum*	SAMN11306463	SAMN11306680	SAMN11307084
SF16-1	SF16-1g	FIESC	FIESC 26 (sister clade)	SAMN11306469	SAMN11306686	SAMN11307090
SF16-1	SF16-1h	FFSC	*F. proliferatum*	SAMN11306470	SAMN11306687	SAMN11307091
SF16-3	SF16-3a	FFSC	*F. proliferatum*	SAMN11306464	SAMN11306681	SAMN11307085
SF16-3	SF16-3b	FFSC	*F. proliferatum*	SAMN11306465	SAMN11306682	SAMN11307086
SF16-3	SF16-3c	FIESC	FIESC 26 (sister clade)	SAMN11306466	SAMN11306683	SAMN11307087
SF16-3	SF16-3d	FIESC	FIESC 26 (sister clade)	SAMN11306467	SAMN11306684	SAMN11307088
SF16-3	SF16-3e	FIESC	FIESC 26 (sister clade)	SAMN11306468	SAMN11306685	SAMN11307089
WS15-1	WS15-1a	FFSC	*F. proliferatum*	SAMN11306411	SAMN11306628	SAMN11307032
WS15-1	WS15-1b	FSAMSC	*F. graminearum*	SAMN11306412	SAMN11306629	SAMN11307033
WS15-1	WS15-1c	FIESC	FIESC 26 (sister clade)	SAMN11306413	SAMN11306630	SAMN11307034
WS15-1	WS15-1d	FIESC	FIESC 18	SAMN11306414	SAMN11306631	SAMN11307035
WS15-1	WS15-1e	FFSC	*F. proliferatum*	SAMN11306415	SAMN11306632	SAMN11307036
WS15-1	WS15-1f	FFSC	*F. proliferatum*	SAMN11306416	SAMN11306633	SAMN11307037
WS15-2	WS15-2a	FIESC	FIESC 26 (sister clade)	SAMN11306406	SAMN11306623	SAMN11307027
WS15-2	WS15-2b	FIESC	FIESC 26 (sister clade)	SAMN11306407	SAMN11306624	SAMN11307028
WS15-2	WS15-2c	FIESC	FIESC 26 (sister clade)	SAMN11306408	SAMN11306625	SAMN11307029
WS15-2	WS15-2d	FFSC	*F. verticillioides*	SAMN11306409	SAMN11306626	SAMN11307030
WS15-2	WS15-2e	FIESC	FIESC 26 (sister clade)	SAMN11306410	SAMN11306627	SAMN11307031
WS16-1	WS16-1a	FSAMSC	*F. graminearum*	SAMN11306399	SAMN11306616	SAMN11307020

Grain Sample ID	Isolate ID	Species complex	Species	Accession no.		
				TEF	RPB1	RPB2
WS16-1	WS16-1b	FIESC	FIESC 18	SAMN11306400	SAMN11306617	SAMN11307021
WS16-1	WS16-1c	FIESC	FIESC 26 (sister clade)	SAMN11306401	SAMN11306618	SAMN11307022
WS16-1	WS16-1d	FFSC	*F. verticillioides*	SAMN11306402	SAMN11306619	SAMN11307023
WS16-1	WS16-1e	FIESC	FIESC 18	SAMN11306403	SAMN11306620	SAMN11307024
WS16-1	WS16-1f	FFSC	*F. fujikuroi*	SAMN11306404	SAMN11306621	SAMN11307025
WS16-1	WS16-1g	FIESC	FIESC 18	SAMN11306405	SAMN11306622	SAMN11307026
WS16-2	WS16-2a	FFSC	*F. fujikuroi*	SAMN11306392	SAMN11306609	SAMN11307013
WS16-2	WS16-2b	FFSC	*F. proliferatum*	SAMN11306393	SAMN11306610	SAMN11307014
WS16-2	WS16-2c	FIESC	*F. lacertarum*	SAMN11306394	SAMN11306611	SAMN11307015
WS16-2	WS16-2d	FIESC	FIESC 18	SAMN11306395	SAMN11306612	SAMN11307016
WS16-2	WS16-2e	FFSC	*F. fujikuroi*	SAMN11306396	SAMN11306613	SAMN11307017
WS16-2	WS16-2f	FFSC	*F. fujikuroi*	SAMN11306397	SAMN11306614	SAMN11307018
WS16-2	WS16-2g	FFSC	*F. fujikuroi*	SAMN11306398	SAMN11306615	SAMN11307019
Reference	NRRL 26417	FIESC	FIESC 26-a	GQ505598.1	JX171522.1	JX171635.1
Reference	NRRL 32868	FIESC	FIESC 25c	GQ505617.1	HM347163.1	GQ505795.1
Reference	NRRL 32522	FIESC	FIESC 18b	GQ505612.1	HM347158.1	GQ505790.1
Reference	NRRL 34004	FIESC	FIESC 16a	GQ505628.1	HM347167.1	GQ505806.1
Reference	NRRL 34006	FIESC	FIESC 15a	GQ505630.1	HM347169.1	GQ505808.1
Reference	NRRL 32864	FIESC	FIESC 17a	GQ505613.1	HM347160.1	GQ505791.1
Reference	NRRL 43640	FIESC	FIESC 1a	GQ505667.1	HM347191.1	GQ505845.1
Reference	NRRL 28029	FIESC	FIESC 3b	GQ505602.1	HM347150.1	GQ505780.1
Reference	NRRL 34032	FIESC	FIESC 5a	GQ505635.1	HM347171.1	GQ505813.1
Reference	NRRL 20423	FIESC	*F. lacertarum*	GQ505593.1	JX171467.1	GQ505771.1

Grain Sample ID	Isolate ID	Species complex	Species	Accession no.		
				TEF	RPB1	RPB2
Reference	NRRL 43636	FIESC	*F. equiseti*	GQ505663.1	HM347189.1	GQ505841.1
Reference	NRRL 13402	FIESC	*F. scirpi*	GQ505592.1	JX171452.1	JX171566.1
Reference	NRRL 31084	FSAMSC	*F. graminearum*	HM744693.1	JX171531.1	HQ154481.1
Reference	NRRL 25475	FSAMSC	*F. culmorum*	KY873384.1	JX171515.1	JX171628.1
Reference	NRRL 13818	FSAMSC	*F. asiaticum*	AF212451.1	JX171459.1	JX171573.1
Reference	NRRL 43641	FSAMSC	*F. armeniacum*	GQ505430.1	HM347192.1	GQ505494.1
Reference	NRRL 43617	FFSC	*F. proliferatum*	HM347124.1	HM347185.1	EF470206.1
Reference	NRRL 43610	FFSC	*F. fujikuroi*	HM347123.1	HM347184.1	EF470199.1
Reference	NRRL 43608	FFSC	*F. verticillioides*	HM347122.1	HM347183.1	EF470197.1
Reference	NRRL 25229	FFSC	*F. thapsinum*	HM347115.1	HM347141.1	EF470123.1
Reference	NRRL 36140	FDSC	*F. dimerum*	HM347133.1	HM347203.1	HM347218.1
Reference	NRRL 44901	FFSC	*F. sacchari*	HM347125.1	HM347194.1	HM347212.1
Reference	NRRL 26421	FFSC	*F. nygamai*	HM347121.1	HM347147.1	EF470127.1
Reference	NRRL 54158	FFSC	*F. subglutinans*	HM347131.1	HM347201.1	HM347216.1
Reference	NRRL 25728	FCOSC	*F. concolor*	HM347119.1	HM347145.1	HM347211.1
Reference	NRRL 25479	FSAMSC	*F. sporotrichioides*	HM744652.1	HM347144.1	HQ154441.1
Reference	NRRL 34033	FSAMSC	*F. brachygibbosum*	GQ505418.1	HM347172.1	GQ505482.1


RM16-2f
RM16-1e
LW16-4a
SF16-3e
WS15-2e
SF16-1b
RM16-2c
SF15-1f
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SF16-3c
LW16-1b
WS15-1c
LW16-4b
SF16-3d
WS16-1c
WS15-2b
WS15-2a
WS15-2c
RM16-2d
SF15-1g
SF15-1e
SF16-1g
FIESC_26-a
FIESC_25-c
RM16-2g
RM16-1g
RM16-2b
RM16-1b
WS16-1g
WS16-1e
FIESC_18-b
LW16-1g
WS16-1b
WS16-2d
WS15-1d
RM16-1f
SF15-1d
FIESC_16-a
FIESC_17-a
FIESC_15-a
RM16-2e
RM16-2h
FIESC_1-a
FIESC_3-b
WS16-2c
F.lacertarum
FIESC_5-a
F.scirpi
F.equiseti
RM16-1c
LW16-1l
LW16-1h
LW16-1m
RM16-2a
F.graminearum
LW16-1k
LW16-1e
WS15-1b
WS16-1a
RM16-1a
LW16-1f
LW16-1n
F.culmorum
F.asiaticum
F.armeniacum
LW16-1a
LW16-1c
LW16-1j
LW16-1d
LW16-1i
F.sporotrichioides
F.brachygibbosum
WS15-1e
WS15-1f
LW16-4d
SF16-1f
WS15-1a
F.proliferatum
SF16-1c
SF16-1e
SF15-1a
SF15-1b
SF16-1a
WS16-2b
SF16-3a
SF16-3b
SF16-1d
SF16-1h
LW16-4c
WS16-2a
WS16-2g
SF15-1c
WS16-1f
WS16-2e
WS16-2f
F.fujikuroi
F.sacchari
WS15-2d
WS16-1d
F.verticillioides
F.nygamai
RM16-2i
F.thapsinum
F.subglutinans
F.concolor
F.dimerum_outgroup
2.0

Fig. 1. Best maximum likelihood (ML) phylogenetic tree obtained from the combined sequences of three loci (TEF 1α, RPB1 and RPB2) data for 97 *Fusarium* isolates, 79 isolates recovered in this study from grain sorghum and 27 known species as reference with sequences of the three loci deposited in GenBank are indicated by colored names. Boot strap support values ≥70% from 1000 replications are shown at the nodes. *F. dimerum* was selected as an outgroup and *F. concolor* was selected as a sister species to *Fusarium fujikuroi* species complex. The scale indicates the genetic distance between the species.

Chapter 6: Future directions

The work described here provides a basis for disease management in grain sorghum for the mid-Atlantic, but additional research on the biology of the pathogens and crop host are needed to further optimize fungicides and hybrids for the region. Some areas that need to be addressed to further this research are:

1. Evaluation of the genetic diversity and pathogenicity of *Colletotrichum sublineola* in the mid-Atlantic region.

2. Determination of the sensitivities of *C. sublineola* isolates to different fungicide chemistries and modes of action.

3. Characterization of mycotoxin producing potential and pathogenicity of *Fusarium* spp. associated with grain sorghum in the mid-Atlantic U.S.

4. Identification of sources of crop host resistance to *C. sublineoa* and grain mold fungi genotypes in the mid-Atlantic to facilitate breeding for regionally-adapted disease resistant grain sorghum hybrids.

Several studies have demonstrated that *C. sublineola* is a heterogeneous species composed of diverse pathotypes (Costa et al. 2011; Moore et al. 2010; Rosewich et al. 1998), and heterokaryosis has been observed within the species (Souza-Paccola et al. 2003). Designation of pathotypes within *C. sublineola* has previously been based on conidial morphology and culture characteristics which do not correspond to genetic diversity as the environmental conditions have a large influence on these morphological traits (Prom et al. 2012). In addition, a study from Brazil suggested that transposable elements activity resulted in genetic instability of *C. sublineola* strains (Favaro et al. 2007). More than 40 pathotypes of *C. sublineola*

have been reported from different parts of the world based on reactions of host differentials (Thakur et al. 2007), but a standard set of host differentials has not yet been developed. Due to the diversity in *C. sublineola* pathotypes, differences in levels of virulence as well as host genotype specificity have been reported (Prom et al. 2012; Were and Ochuodho 2012), and at a location, one pathotype can dominate the others in the population (Casela et al. 2001). The genetic diversity and pathogenicity *C. sublineola* populations in the mid-Atlantic have not been characterized, but differences in anthracnose severity among locations and hybrid by location interactions in our studies suggest site-specific variation in fungal populations may be contributing to observed levels of crop host disease resistance/susceptibility. We hypothesize that frequencies of different *C. sublineola* genotypes will vary across locations, and certain fungal genotypes will be associated with higher levels of virulence during crop infection. We have a collection of 60 *C. sublineola* isolates from 2 locations in Virginia and 3 locations in North Carolina collected over 3 years. Isolates should be genotyped using multi-locus sequence typing (MSLT) (Vanhee et al. 2010) and assessed for pathogenicity/virulence on sorghum host differentials (Prom et al. 2012). This will provide a basis for screening sorghum germplasm for resistance to *C. sublineola* genotypes/pathotypes that are dominant in the mid-Atlantic region.

Results of our study indicated that among three fungicide modes of action and five fungicide active ingredients labeled for use on grain sorghum, only a single QoI active ingredient (pyraclostrobin) provided an acceptable level of disease control (Appendix). However, QoI resistance has been reported for many fungal pathogens (FRAC, 2012) and two species of *Colletotrichum, C. graminicola* and *C. gloeosporioides* isolated from annual blue grass/bent garss and strawberry, respectively were resistant to QoI fungicides (FRAC, 2012). Hence, there is a high risk of resistance development in *C. sublineola* and it is important to document current

levels of sensitivity to different active ingredients including newer fungicides that are not yet labeled for sorghum. The sensitivity of the anthracnose isolates to the fungicide chemistries used in this study has not been tested and the confirmation of *C. sublineola* as a heterogenous species warrants sensitivity testing. We hypothesize that isolates of *C. sublineola* collected from different locations in mid-Atlantic will differ in their sensitivity to different fungicide chemistries and some isolates may already be resistant to some of the chemistries. Isolates should be tested using radial growth assay and/or germination assay (Appendix). This will provide an idea about the frequency of resistance development to different fungicides at different locations and required techniques to manage the pathogen in the mid-Atlantic.

In our relatively small survey of *Fusarium* associated with grain sorghum in the mid-Atlantic, thirteen phylogenetically distinct species were identified. All the *Fusarium* species identified in this study, with an exception of FIESC 26 and FIESC 18, are potential mycotoxin producers (O'Donnell et al. 2018; Villani et al. 2016). However, the pathogenicity of the different isolates/species and their potential to infect and produce mycotoxins in sorghum was not determined, and this information is critical for understanding risk of sorghum grain mycotoxin contamination in the mid-Atlantic region. We hypothesize that the isolates of different *Fusarium* species identified in this study are mycotoxin producers and are pathogenic to sorghum. A preliminary greenhouse study was conducted to test the pathogenicity of 14 isolates of *Fusarium* species identified in this study by inoculating the plants at full flowering stage. However, it was not possible to re-isolate *Fusarium* spp. from the grain and complete Koch's postulates due to a lack of grain development by inoculated plants and the check. However, the inoculated *Fusarium* species were recovered from the florets that were aborted post inoculation (Appendix). Hence, the pathogenicity of these *Fusarium* isolates needs to be examined

preferably at the milk or soft dough stage, a preferred stage for grain mold infection (Funnell-Harris and Pedersen 2011; Melake-Berhan et al. 1996; Navi et al. 2005; Tarekegn et al. 2004), to identify the true grain mold pathogens from that of the secondary invaders or saprophytes. In addition, examining the mycotoxin producing ability of these isolates will provide a better understanding on the seriousness of the presence of these species in the mid-Atlantic grain sorghum for use as animal feed.

Numerous studies have been conducted with the aim of identifying resistance genes to anthracnose and grain mold in sorghum (Buiate et al. 2010; Chala and Tronsmo 2012; Cuevas et al. 2014; Erpelding 2008; Marley et al. 2005; Moore et al. 2009; Prom and Erpelding 2009; Prom et al. 2014; Prom et al. 2012; Thakur and Mathur 2000). A number of putative resistance genes and loci have been identified conferring resistance to specific *C. sublineola* genotypes (Biruma et al. 2012; Costa et al. 2011; Erpelding and Prom 2004; Mehta et al. 2005; Murali Mohan et al. 2010; Perumal et al. 2009; Ramasamy et al. 2008). However, the host resistance approach has not succeeded due to the diversity in the pathogen population that can adapt rapidly to the changes in the resistant sorghum lines deployed. In addition, resistant lines/ hybrids effective in one geographical area may be susceptible in another area (Ali and Warren 1987) and the breakdown of resistance in a very short period of time under different environmental conditions have also been reported (Wharton et al. 2001). Therefore, to identify resistant sorghum genotype for a location, Prom et al. (2012) suggested screening of sorghum lines with a mix of local *C. sublineola* isolates in the target environment as a practical way of coping with this problem. Hence, the dominant *C. sublineola* genotypes that will be identified from the first objective can be utilized. Similar to the pathotype diversity within *C. sublineola*, pathogens causing grain mold vary from location to location and at particular location some pathogens

might be predominant compared to the other making the deployment of the pathogen- specific resistance genes less effective in all the problematic regions (Little et al. 2012). Sorghum accessions have been evaluated under mid-Atlantic conditions in a preliminary study aimed at identifying potential sources of resistance to anthracnose and grain mold. Four hundred and seven sorghum accessions were screened in Virginia for three years to identify resistant germplasm and a sub-sample of 98 accessions were also tested for deoxynivalenol (DON) contamination. The accessions varied in their susceptibility to both the diseases as well as for DON contamination (Appendix). Seventeen accessions with low disease severity to both the diseases and DON contamination lower than 1 ppm were identified (Appendix). Hence, we hypothesize that multi-location screening of resistant or moderately resistant sorghum accessions identified in the preliminary study will help identify potential sources of resistance for the region. This will lead to the identification of accessions with stable resistance that can be used to breed and develop hybrids that are well-adapted to the region.

Addressing the first three objectives will provide a better understanding of the pathogens' biology that will be critical for optimizing disease management strategies for the region. With the identification of sorghum accessions with resistance, an additional tool to manage diseases will be provided as the development and use of disease resistant hybrids will reduce the need for fungicide use, improve yield and quality of grains, and increase farm profitability.

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APPENDIX

I. Protocols

A. Single spore isolation of sorghum anthracnose isolates

1. Place a section of leaf sample with anthracnose in moist chamber for 1-2 days at room temperature.
2. Observe the leaf sample under the stereomicroscope, setae and spore mass should be visible.
3. Flame sterile a needle or use sterile toothpick to pick spores from the leaf surface and transfer into 1% water agar plate.
4. Streak it in water agar plate with sterile coffee stick or tooth pick.
5. Let the spores germinate for 24 hrs. in an incubator at 28^{o}C.
6. Transfer 2-3 single germinating spore of each isolate under stereomicroscope with magnification of up to 100x, use a flame sterilized minuten pin (www.bioquip.com , 0.15mm stainless steel pins, #1207SA) mounted on a forceps to cut the agar around the spore.
7. Let it grow for 5-7 days in an incubator at 28^{o}C.
8. Pick one single spore pure culture and transfer it to the center of a 1/4th PDA with sterile Whitman No. 1 filter paper halves.
9. Let it grow for 7-10 days on the filter paper.
10. Pick the filter paper with sterile forceps and transfer it to an empty sterile Petri Dish for drying for 12-24 hrs.
11. Save the isolate, after cutting the filter paper into smaller pieces, in a zip lock bag in a sterile envelope at -20^{o}C until further use.

B. Protocol for isolation of *Fusarium spp.* from sorghum grains

1. **Preparing sorghum grains for plating in 1/4th PDA**
   a. Put 15-20 grains in a 50ml capped tube with about 20-25 ml of distilled water
   b. Shake it with cap on for about 1 minute
   c. Drain out the water
   d. Prepare fresh 10% bleach in one cup (50ml) and two cups of distilled deionized water
   e. Now, put grains in 10% bleach and stir or swirl for 1 min
   f. Transfer grains to first distilled water and swirl for 1 min
   g. Transfer grains to second distilled water and swirl for 1 min
   h. Now, put the grains on a sterile paper towel and let it dry
   i. Finally transfer the grains in 1/4th PDA (5-10 grains per plate) and label the plates with the name of the isolate, date and your initials, and let it grow for 5-7days
2. **Isolation of Fusarium isolates**
   a. Check the plates with sorghum grains for the growth
   b. Identify Fusarium based on morphology and color. If needed use microscope to verify.
   c. Based on the growth from the grains either transfer to another plate and/or
   d. Streak on water agar using sterile coffee stick.
      Use coffee stick to scrap off some mycelia from the plate, streak once on water agar with mycelial side on the plate. Then change the direction of the coffee stick and streak 3-4 times perpendicular to the first streak followed by parallel streaks. This will allow distribution of spores sparsely so that it is easier to pick.
   e. After 12-24 hr of streaking, observe the plates under compound microscope at 4x-10x to locate germinating single spores that can be picked easily and mark with a marker. (This saves time to search single spore under the microscope.)
   f. Now, using a stereomicroscope with magnification of up to 100x, find the marker mark and find the spore using the combination of focus and magnification.
   g. Once a spore that is by itself is located, use a flame sterilized minuten pin (www.bioquip.com , 0.15mm stainless steel pins, #1207SA) mounted on a forceps to cut the agar around the spore.
   h. Then pick the spore with agar and transfer it to a new 1/4th PDA. Transfer 3-4 single spores to make sure to get a single spore of one Fusarium species. Using marker, mark the spot where the spore is placed on the back of the plate.
   i. Let the spores grow for another 5-7 days.
3. **Storing of single spore isolate using sterile filter paper**
   a. After 5-7 days, check for the growth of a single colony from the spore.

b. Cut filter paper into halves and wrap it in aluminum foil and sterilize by autoclaving.
c. Place two halves of a filter paper on 1/4th PDA with a small gap in between.
d. Now, transfer a piece of agar from one of the single spore colony in between filter paper halves and label the plate with the name of the isolate, date and your initials.
e. Let it grow for another 7-10 days or until the filter paper is covered by the mycelia.
f. Pick the filter paper with sterile forceps and put in an empty Petri-dish and keep it under the hood for 24hrs for drying.
g. Then using ethanol sterilized scissors and flame sterilized forceps cut the filter paper into small squares and put it in a zip lock bag then in a sterile envelope. Label the envelope and store it at -20^{o}C for further use.

C. DNA extraction protocol

I. Prepare 4 M ammonium acetate, 80 ml
   1. Dissolve 24.67g NH4Ac in 80 ml distilled deionized water
   2. Filter into a 100 ml sterile bottle (Do not autoclave with ammonium acetate, autoclaving breaks it down)
   3. Store at 4^{o}C until use.

II. DNA extraction
   1. Transfer 100-200 mg of fungal mycelia from 1/4th PDA plate (cultured for 7-10 days) to a "Mikro-Schraubrechre" 2 ml microtube using a sterile toothpick.
   2. Add 450 µL of lysis buffer to each tube (Can stop here if needed)
   3. Place the tubes in mini bead beater for 1 min.
   4. Place tubes in thermal shaker at 60^{o}C and 1000 rpm for 30 mins.
   5. Centrifuge tubes at 14,000 x g for 30 mins.
   6. Transfer 370 µL supernatant to new sterile and labelled tubes. Do not disturb the pellet. If disturbed, centrifuge again. (Tubes with pellet can be autoclaved and discarded.
   7. Put tubes containing supernatant on ice. (Can stop here if needed)
   8. Add 370 µL 4M refrigerated Ammonium Acetate (NH_4OAc) (4^{o}C stored).
   9. Mix tubes by inverting several times. While upside down, may need to flick the end of the tube for proper mixing.
   10. Add 740 µL ice-cold 100% ethanol (-20^{o}C stored). Mix tubes by inverting.
   11. Place tubes at -20^{o}C for 30 mins.
   12. Spin tubes at 14,000 x g for 5 mins.
   13. Pour off supernatant. Pellet may or may not be visible at this step but should remain stuck to a side of the micro centrifuge tube.
   14. Use pipette tip to remove as much of ethanol as possible without disturbing pellet.
   15. Leave open tubes upside down on paper towel to air dry sample. Can leave for 1-12hr at room temperature or can keep under the laminar flow hood for 1.5hr-2hr.
   16. Add 25 µL sterile ddH2O and dissolve the DNA pellet.
   17. Test the DNA concentration using NanoDrop.

D. ExoSAP-IT PCR clean-up (manufacturer) protocol

The PCR product needs to be cleaned to get a high quality double stranded DNA for sequencing. After PCR, unconsumed dNTPs and primers remaining in the PCR product interferes with the sequencing, hence, ExoSAP-IT removes these unwanted dNTPs and primers using two hydrolytic enzymes: Exonuclease I degrades residual single-stranded primers and any extraneous single-stranded DNA produced by PCR. While Shrimp Alkaline Phosphatase hydrolyzes remaining dNTPs from the PCR product.

1. Add 2 µL of ExoSAP-IT to 5 µL of PCR product. If more PCR product, add Exo SAP-IT proportionally.
2. Mix and incubate at 37^{o}C for 15 min followed by heating to 80^{o}C for 15 min to inactivate EXoSAP-IT.
3. The DNA is now ready for further use.

E. Protocol for Polymerase Chain Reaction (PCR) amplification of Translation Elongation Factor 1α (TEF-1α) locus from *Fusarium spp.*

1. Extract DNA from a monoconidial pure culture
2. Quantify the DNA concentration
3. Dilute DNA in sterile double distilled water to a working concentration of 1-20ng/ul
4. Reaction mix for TEF 1α amplification (20 ul vol.)

   For PCR, pre-mix (AccuPower® HotStart PCR PreMix) was available so add only the following in each reaction.

Ingredients	Name	1 reaction
a. Forward primer	EF1	0.5 µl
b. Reverse primer	EF2	0.5 µl
c. DNA	Sample DNA	5.0 µl
d. Water	ddH_2O	14.0 µl

5. Make a master mix of primers and water first for total number of samples and add 15ul of mix to each PCR tube and finally add 5µl of sample DNA.
6. TEF1α amplification thermocycler program.
   1) 94ºC for 5 min instead of 1min
      (to activate *Taq* in the premix which is room temperature stable)
   2) 94ºC for 30 sec. (for denaturation)
   3) 53ºC for 45 sec. (for annealing)
   4) 72ºC for 1 min. (for elongation)
   5) Go to step 2, repeat step 2-4, 34 times
   6) 72ºC for 5 min.
   7) 4ºC until turnoff

After amplification, run sample in gel electrophoresis to check the presence of TEF1α amplicon of about 700bp.

F. Protocol for Polymerase Chain Reaction (PCR) amplification of DNA-directed RNA polymerase II largest subunit (RPB1) from *Fusarium spp*.

1. Extract DNA from a monoconidial pure culture
2. Quantify the DNA concentration
3. Dilute DNA in sterile double distilled water to a working concentration of 1-20ng/µl
4. Reaction mix for RPB1 amplification (20 µl vol.)

   For PCR, pre-mix (AccuPower® HotStart PCR PreMix) was available so add only the following in each reaction.

Ingredients	Name	1 reaction
Forward primer	Fa	0.5µl
Reverse primer	G2R	0.5µl
DNA	Sample DNA	5.0µl
Water	ddH_2O	14.0µl

5. Make a master mix of primers and water first for total number of samples and add 15ul of mix to each PCR pre-mix tube and finally add 5µl of sample DNA.
6. Vortex the mix and centrifuge.
7. RPB1 amplification thermocycler program.
   1) 94ºC for 5 min instead of 1 min.
      (To activate *Taq* in the premix which is room temperature stable)
   2) 94ºC for 40 sec. (for denaturation)
   3) 50ºC for 1 min. (for annealing)
   4) 72ºC for 1min 30 sec. (for elongation)
   5) Go to step 2, repeat step II-IV, 40 times
   6) 72ºC for 5 min.
   7) 4ºC until turnoff

After amplification, run sample in gel electrophoresis to check the presence of RPB1 amplicon of about 1.5kb.

G. Protocol for Polymerase Chain Reaction (PCR) amplification of DNA-directed RNA polymerase II second largest subunit (RPB2) from *Fusarium spp.*

1. Extract DNA from a monoconidial pure culture
2. Quantify the DNA concentration
3. Dilute DNA in sterile double distilled water to a working concentration of 1-20ng/µl
4. Reaction mix for RPB2 amplification (20 µl vol.)

   For PCR, pre-mix (AccuPower® HotStart PCR PreMix) was available so add only the following in each reaction.

Ingredients	Name	1 reaction
Forward primer	5f2	0.5µl
Reverse primer	11ar	0.5µl
DNA	Sample DNA	5.0µl
Water	ddH_2O	14.0µl

5. Make a master mix of primers and water first for total number of samples and add 15ul of mix to each PCR pre-mix tube and finally add 5µl of sample DNA.
6. Vortex the mix and centrifuge.
7. RPB2 amplification thermocycler program.
   1) 94^{o}C for 5 min instead of 1 min.
      (To activate *Taq* in the premix which is room temperature stable)
   2) 94^{o}C for 40 sec. (for denaturation)
   3) 58.7^{o}C for 1 min. (for annealing)
   4) 72^{o}C for 1min 30 sec. (for extention)
   5) Go to step 2, repeat step II-IV, 40 times
   6) 72^{o}C for 5 min.
   7) 4^{o}C until turnoff

After amplification, run sample in gel electrophoresis to check the presence of RPB2 amplicon of about 1.8kb.

II. Fungicide sensitivity assay

Objective: To test the fungicide sensitivity of sorghum anthracnose isolates.

Protocol for fungicide sensitivity assay (Radial growth assay)

A total of about 60 isolates of *C. sublineolum* collected from fungicide trials 2015-2017 and from seeds of Official Variety Trial will be used.

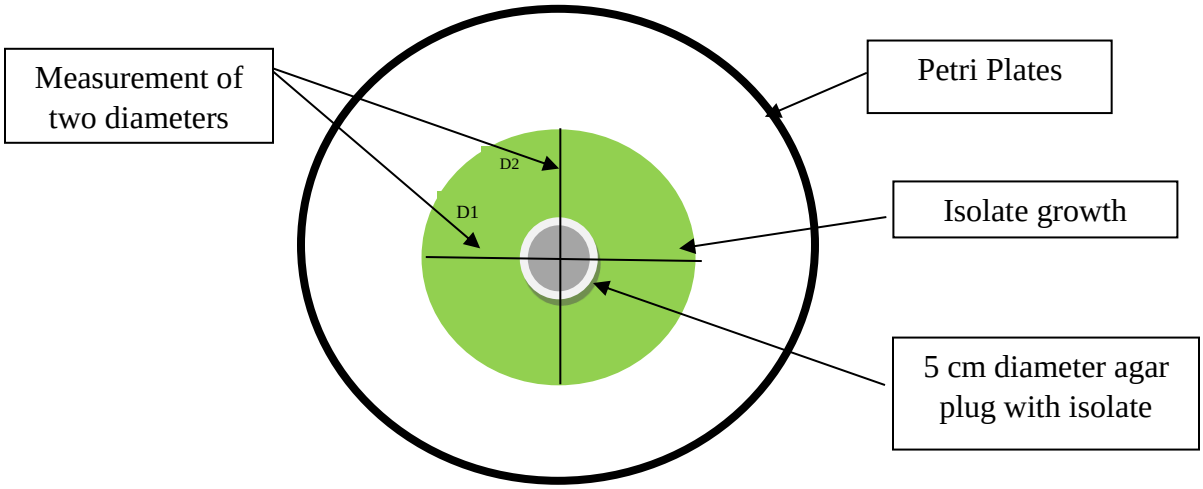
1. Five fungicide chemistries will be used including a non-treated control to test each isolate.

a. Pyraclostrobin
b. Fluxapyroxad
c. Azoxystrobin
d. Picoxystrobin
e. Propiconazole

2. Media preparation:

a. First PDA (Potato Dextrose Agar) will be prepared by adding 40g of PDA to 1L of deionized distilled water.
b. Autoclave at 121^{o}C and 15psi for 30 min. Take media out of autoclave and place in a stirrer to allow it to cool to about 50^{o}C.
c. Each fungicide will be added to molten PDA (at 50^{o}C) at the following rates: 0.1ppm, 1 ppm, 10ppm and 100 ppm. In order to inhibit alternative respiration in treated samples, salicylhydroxamic acid (SHAM) dissolved in acetone:methanol (1:1) will be added to the molten PDA at a final concentration of 100 µg ml^{-1} . Unamended PDA and PDA with only SHAM plates will serve as controls.

3. Agar plugs (0.5 cm diameter) containing actively growing mycelium of 10-day old isolates will be placed on the center of PDA plates. Each isolate will have 3 replications for each rate of a fungicide.
4. The plates will be incubated at room temperature for 10 days in a pre-sterilized plastic box.
5. Diameter of mycelial growth will be measured after 10 days of inoculation. Two measurements at right angle to one another will be taken and the mean of these measurements will represent the colony diameter of a replicate.
6. Relative mycelial growth will be calculated based on the mean of three replicates of each isolate tested (colony diameter on fungicide amended media /colony diameter on unamended media x 100).



7. The EC_{50} concentration for each fungicide will be determined based on the concentration that reduce the radial growth by 50%.

Or,

Protocol for fungicide sensitivity assay (Germination assay)

A total of about 60 isolates of *C. sublineolum* collected from fungicide trials 2015-2017 and from seeds of Official Variety Trial will be used.

1. Five fungicide chemistries will be used including a non-treated control to test each isolate.

a. Pyraclostrobin
b. Fluxapyroxad
c. Azoxystrobin
d. Picoxystrobin
e. Propiconazole

2. Media preparation:

a. First PDA (Potato Dextrose Agar) will be prepared by adding 40g of PDA to 1L of deionized distilled water.
b. Autoclave at 121^{o}C and 15psi for 30 min. Take media out of autoclave and place in a stirrer to allow it to cool to about 50^{o}C.
c. Each fungicide will be added to molten PDA (at 50^{o}C) at the following rates: 0.1ppm, 1 ppm, 10ppm and 100 ppm. In order to inhibit alternative respiration in treated samples, salicylhydroxamic acid (SHAM) dissolved in acetone:methanol (1:1) will be added to the molten PDA at a final concentration of 100 µg ml^{-1} . Unamended PDA and PDA with SHAM plates will serve as controls.

3. Each isolate will be grown in ¼ PDA for 10 days under fluorescent light to induce spore production.
4. Spores will be harvested and a final suspension of 10^4 spores ml^{-1} will be obtained using a hemocytometer and 75 µl of the suspension will be placed in the amended plate and spread with a sterile glass-rod.

5. The plates will be incubated at room temperature for 24 hrs in a pre-sterilized plastic box.
6. Germination of 100 random spores will be assessed and the number of spores that germinated and did not germinate will be noted
7. The EC_{50} concentration for each fungicide will be determined based on the concentration that reduced the germination by 50%.

Table 1. Preliminary data from five *C. sublineola* isolates with three replications.

Isolate	Fungicide	Conc	Rep	D1	D1-0.5	D2	D2-0.5	Mean Gth
CsFL01-16-101.1	Fluxapyroxad	100	1	3.8	3.3	3.6	3.1	3.2
CsFL01-16-101.1	Fluxapyroxad	10	1	3.8	3.3	3.9	3.4	3.35
CsFL01-16-101.1	Fluxapyroxad	1	1	4.5	4	4.5	4	4
CsFL01-16-101.1	Fluxapyroxad	0.1	1	4.2	3.7	4.2	3.7	3.7
CsFL01-16-101.1	Pyraclostribin	100	1	0	0	0	0	0
CsFL01-16-101.1	Pyraclostribin	10	1	0	0	0	0	0
CsFL01-16-101.1	Pyraclostribin	1	1	0.8	0.3	0.7	0.2	0.25
CsFL01-16-101.1	Pyraclostribin	0.1	1	1	0.5	1.1	0.6	0.55
CsFL01-16-101.1	Propiconazole	100	1	0	0	0	0	0
CsFL01-16-101.1	Propiconazole	10	1	1.3	0.8	1.2	0.7	0.75
CsFL01-16-101.1	Propiconazole	1	1	2.5	2	2.5	2	2
CsFL01-16-101.1	Propiconazole	0.1	1	3.5	3	3.5	3	3
CsFL01-16-101.1	Azoxystrobin	100	1	0.6	0.1	0.6	0.1	0.1
CsFL01-16-101.1	Azoxystrobin	10	1	0.9	0.4	0.8	0.3	0.35
CsFL01-16-101.1	Azoxystrobin	1	1	1.2	0.7	1.1	0.6	0.65
CsFL01-16-101.1	Azoxystrobin	0.1	1	2.5	2	2.3	1.8	1.9
CsFL01-16-101.1	Picoxystrobin	100	1	1	0.5	1	0.5	0.5
CsFL01-16-101.1	Picoxystrobin	10	1	1	0.5	1	0.5	0.5
CsFL01-16-101.1	Picoxystrobin	1	1	1.9	1.4	1.9	1.4	1.4
CsFL01-16-101.1	Picoxystrobin	0.1	1	3.1	2.6	3.2	2.7	2.65
CsFL01-16-101.1	PDA+SHAM	0	1	4.3	3.8	4.4	3.9	3.85
CsFL01-16-101.1	PDA only	0	1	4.7	4.2	4.6	4.1	4.15
CsFL01-16-102.1	Fluxapyroxad	100	1	3.8	3.3	3.9	3.4	3.35
CsFL01-16-102.1	Fluxapyroxad	10	1	3.8	3.3	3.5	3	3.15
CsFL01-16-102.1	Fluxapyroxad	1	1	4.3	3.8	4.4	3.9	3.85
CsFL01-16-102.1	Fluxapyroxad	0.1	1	4	3.5	3.8	3.3	3.4
CsFL01-16-102.1	Pyraclostribin	100	1	0	0	0	0	0
CsFL01-16-102.1	Pyraclostribin	10	1	0	0	0	0	0
CsFL01-16-102.1	Pyraclostribin	1	1	0	0	0	0	0
CsFL01-16-102.1	Pyraclostribin	0.1	1	1.1	0.6	1	0.5	0.55
CsFL01-16-102.1	Propiconazole	100	1	0	0	0	0	0
CsFL01-16-102.1	Propiconazole	10	1	1.5	1	1.3	0.8	0.9
CsFL01-16-102.1	Propiconazole	1	1	2.6	2.1	2.5	2	2.05
CsFL01-16-102.1	Propiconazole	0.1	1	3.5	3	3.4	2.9	2.95
CsFL01-16-102.1	Azoxystrobin	100	1	1	0.5	1	0.5	0.5
CsFL01-16-102.1	Azoxystrobin	10	1	1.2	0.7	1.2	0.7	0.7
CsFL01-16-102.1	Azoxystrobin	1	1	1.5	1	1.7	1.2	1.1
CsFL01-16-102.1	Azoxystrobin	0.1	1	2.4	1.9	2.4	1.9	1.9
CsFL01-16-102.1	Picoxystrobin	100	1	0.8	0.3	0.8	0.3	0.3

Isolate	Fungicide	Conc	Rep	D1	D1-0.5	D2	D2-0.5	Mean Gth
CsFL01-16-102.1	Picoxystrobin	10	1	1	0.5	1	0.5	0.5
CsFL01-16-102.1	Picoxystrobin	1	1	1.8	1.3	1.8	1.3	1.3
CsFL01-16-102.1	Picoxystrobin	0.1	1	3.3	2.8	3.1	2.6	2.7
CsFL01-16-102.1	PDA+SHAM	0	1	4.2	3.7	4.5	4	3.85
CsFL01-16-102.1	PDA only	0	1	4.7	4.2	4.5	4	4.1
CsFL01-16-104.2	Fluxapyroxad	100	1	5.8	5.3	5.7	5.2	5.25
CsFL01-16-104.2	Fluxapyroxad	10	1	5.7	5.2	5.8	5.3	5.25
CsFL01-16-104.2	Fluxapyroxad	1	1	6.6	6.1	6.5	6	6.05
CsFL01-16-104.2	Fluxapyroxad	0.1	1	6.8	6.3	6.8	6.3	6.3
CsFL01-16-104.2	Pyraclostribin	100	1	0.9	0.4	0.8	0.3	0.35
CsFL01-16-104.2	Pyraclostribin	10	1	0.9	0.4	0.9	0.4	0.4
CsFL01-16-104.2	Pyraclostribin	1	1	1.1	0.6	1.1	0.6	0.6
CsFL01-16-104.2	Pyraclostribin	0.1	1	1.7	1.2	1.8	1.3	1.25
CsFL01-16-104.2	Propiconazole	100	1	NA	NA	NA	NA	NA
CsFL01-16-104.2	Propiconazole	10	1	1.7	1.2	1.8	1.3	1.25
CsFL01-16-104.2	Propiconazole	1	1	3.3	2.8	3.4	2.9	2.85
CsFL01-16-104.2	Propiconazole	0.1	1	4.8	4.3	5	4.5	4.4
CsFL01-16-104.2	Azoxystrobin	100	1	1.3	0.8	1.2	0.7	0.75
CsFL01-16-104.2	Azoxystrobin	10	1	1.6	1.1	1.6	1.1	1.1
CsFL01-16-104.2	Azoxystrobin	1	1	1.8	1.3	1.7	1.2	1.25
CsFL01-16-104.2	Azoxystrobin	0.1	1	3.1	2.6	3.1	2.6	2.6
CsFL01-16-104.2	Picoxystrobin	100	1	1.7	1.2	1.5	1	1.1
CsFL01-16-104.2	Picoxystrobin	10	1	1.6	1.1	1.7	1.2	1.15
CsFL01-16-104.2	Picoxystrobin	1	1	2.5	2	2.4	1.9	1.95
CsFL01-16-104.2	Picoxystrobin	0.1	1	5	4.5	5	4.5	4.5
CsFL01-16-104.2	PDA+SHAM	0	1	6.9	6.4	7	6.5	6.45
CsFL01-16-104.2	PDA only	0	1	7.2	6.7	7.1	6.6	6.65
CsFL01-16-115.1	Fluxapyroxad	100	1	4	3.5	3.2	2.7	3.1
CsFL01-16-115.2	Fluxapyroxad	10	1	4	3.5	3.7	3.2	3.35
CsFL01-16-115.3	Fluxapyroxad	1	1	4.5	4	4.6	4.1	4.05
CsFL01-16-115.4	Fluxapyroxad	0.1	1	4.1	3.6	3.5	3	3.3
CsFL01-16-115.5	Pyraclostribin	100	1	0	0	0	0	0
CsFL01-16-115.6	Pyraclostribin	10	1	0	0	0	0	0
CsFL01-16-115.7	Pyraclostribin	1	1	1	0.5	0.9	0.4	0.45
CsFL01-16-115.8	Pyraclostribin	0.1	1	1.3	0.8	1.3	0.8	0.8
CsFL01-16-115.9	Propiconazole	100	1	0	0	0	0	0
CsFL01-16-115.10	Propiconazole	10	1	1.3	0.8	1.3	0.8	0.8
CsFL01-16-115.11	Propiconazole	1	1	2.5	2	2.5	2	2
CsFL01-16-115.12	Propiconazole	0.1	1	3.3	2.8	3.5	3	2.9
CsFL01-16-115.13	Azoxystrobin	100	1	0.6	0.1	0.6	0.1	0.1

Isolate	Fungicide	Conc	Rep	D1	D1-0.5	D2	D2-0.5	Mean Gth
CsFL01-16-115.14	Azoxystrobin	10	1	0.8	0.3	0.7	0.2	0.25
CsFL01-16-115.15	Azoxystrobin	1	1	0.9	0.4	0.7	0.2	0.3
CsFL01-16-115.16	Azoxystrobin	0.1	1	2.7	2.2	2.5	2	2.1
CsFL01-16-115.17	Picoxystrobin	100	1	1	0.5	0.8	0.3	0.4
CsFL01-16-115.18	Picoxystrobin	10	1	0.9	0.4	0.8	0.3	0.35
CsFL01-16-115.19	Picoxystrobin	1	1	1.5	1	1.3	0.8	0.9
CsFL01-16-115.20	Picoxystrobin	0.1	1	3.3	2.8	3.3	2.8	2.8
CsFL01-16-115.21	PDA+SHAM	0	1	4.6	4.1	4.4	3.9	4
CsFL01-16-115.22	PDA only	0	1	4.9	4.4	4.5	4	4.2
CsFL01-16-123.1	Fluxapyroxad	100	1	3.8	3.3	3.5	3	3.15
CsFL01-16-123.1	Fluxapyroxad	10	1	3.8	3.3	3.5	3	3.15
CsFL01-16-123.1	Fluxapyroxad	1	1	4.2	3.7	4.2	3.7	3.7
CsFL01-16-123.1	Fluxapyroxad	0.1	1	4.1	3.6	4	3.5	3.55
CsFL01-16-123.1	Pyraclostribin	100	1	0	0	0	0	0
CsFL01-16-123.1	Pyraclostribin	10	1	0	0	0	0	0
CsFL01-16-123.1	Pyraclostribin	1	1	0	0	0	0	0
CsFL01-16-123.1	Pyraclostribin	0.1	1	0.9	0.4	0.8	0.3	0.35
CsFL01-16-123.1	Propiconazole	100	1	0	0	0	0	0
CsFL01-16-123.1	Propiconazole	10	1	1.3	0.8	1.3	0.8	0.8
CsFL01-16-123.1	Propiconazole	1	1	2.5	2	2.5	2	2
CsFL01-16-123.1	Propiconazole	0.1	1	3.5	3	3.5	3	3
CsFL01-16-123.1	Azoxystrobin	100	1	0.9	0.4	0.9	0.4	0.4
CsFL01-16-123.1	Azoxystrobin	10	1	1.1	0.6	1.1	0.6	0.6
CsFL01-16-123.1	Azoxystrobin	1	1	1.4	0.9	1.4	0.9	0.9
CsFL01-16-123.1	Azoxystrobin	0.1	1	2.7	2.2	2.5	2	2.1
CsFL01-16-123.1	Picoxystrobin	100	1	1	0.5	0.8	0.3	0.4
CsFL01-16-123.1	Picoxystrobin	10	1	1	0.5	0.9	0.4	0.45
CsFL01-16-123.1	Picoxystrobin	1	1	2	1.5	1.8	1.3	1.4
CsFL01-16-123.1	Picoxystrobin	0.1	1	3.3	2.8	3.1	2.6	2.7
CsFL01-16-123.1	PDA+SHAM	0	1	4.5	4	4.2	3.7	3.85
CsFL01-16-123.1	PDA only	0	1	4.7	4.2	4.7	4.2	4.2
CsFL01-16-101.1	Fluxapyroxad	100	2	5.7	5.2	5.3	4.8	5
CsFL01-16-101.1	Fluxapyroxad	10	2	5.7	5.2	5.1	4.6	4.9
CsFL01-16-101.1	Fluxapyroxad	1	2	6.1	5.6	6.1	5.6	5.6
CsFL01-16-101.1	Fluxapyroxad	0.1	2	6.6	6.1	6.6	6.1	6.1
CsFL01-16-101.1	Pyraclostribin	100	2	1.1	0.6	1	0.5	0.55
CsFL01-16-101.1	Pyraclostribin	10	2	1.1	0.6	1	0.5	0.55
CsFL01-16-101.1	Pyraclostribin	1	2	1.3	0.8	1.2	0.7	0.75
CsFL01-16-101.1	Pyraclostribin	0.1	2	1.8	1.3	1.9	1.4	1.35
CsFL01-16-101.1	Propiconazole	100	2	NA	NA	NA	NA	NA

Isolate	Fungicide	Conc	Rep	D1	D1-0.5	D2	D2-0.5	Mean Gth
CsFL01-16-101.1	Propiconazole	10	2	1.9	1.4	1.8	1.3	1.35
CsFL01-16-101.1	Propiconazole	1	2	3.4	2.9	3.6	3.1	3
CsFL01-16-101.1	Propiconazole	0.1	2	5.2	4.7	5	4.5	4.6
CsFL01-16-101.1	Azoxystrobin	100	2	1.2	0.7	1.4	0.9	0.8
CsFL01-16-101.1	Azoxystrobin	10	2	1.4	0.9	1.5	1	0.95
CsFL01-16-101.1	Azoxystrobin	1	2	2	1.5	2	1.5	1.5
CsFL01-16-101.1	Azoxystrobin	0.1	2	3.3	2.8	3.2	2.7	2.75
CsFL01-16-101.1	Picoxystrobin	100	2	1.5	1	1.8	1.3	1.15
CsFL01-16-101.1	Picoxystrobin	10	2	1.7	1.2	1.7	1.2	1.2
CsFL01-16-101.1	Picoxystrobin	1	2	2.7	2.2	2.9	2.4	2.3
CsFL01-16-101.1	Picoxystrobin	0.1	2	4.8	4.3	4.6	4.1	4.2
CsFL01-16-101.1	PDA+SHAM	0	2	6.7	6.2	6.5	6	6.1
CsFL01-16-101.1	PDA only	0	2	7.5	7	7.5	7	7
CsFL01-16-102.1	Fluxapyroxad	100	2	5.4	4.9	5.4	4.9	4.9
CsFL01-16-102.1	Fluxapyroxad	10	2	5.5	5	5.5	5	5
CsFL01-16-102.1	Fluxapyroxad	1	2	6.6	6.1	6.5	6	6.05
CsFL01-16-102.1	Fluxapyroxad	0.1	2	6.1	5.6	6.2	5.7	5.65
CsFL01-16-102.1	Pyraclostribin	100	2	0.9	0.4	0.9	0.4	0.4
CsFL01-16-102.1	Pyraclostribin	10	2	0.8	0.3	0.7	0.2	0.25
CsFL01-16-102.1	Pyraclostribin	1	2	1	0.5	1	0.5	0.5
CsFL01-16-102.1	Pyraclostribin	0.1	2	1.3	0.8	1.4	0.9	0.85
CsFL01-16-102.1	Propiconazole	100	2	NA	NA	NA	NA	NA
CsFL01-16-102.1	Propiconazole	10	2	1.6	1.1	1.6	1.1	1.1
CsFL01-16-102.1	Propiconazole	1	2	3.3	2.8	3.3	2.8	2.8
CsFL01-16-102.1	Propiconazole	0.1	2	4.9	4.4	5	4.5	4.45
CsFL01-16-102.1	Azoxystrobin	100	2	1.3	0.8	1.2	0.7	0.75
CsFL01-16-102.1	Azoxystrobin	10	2	1.7	1.2	1.6	1.1	1.15
CsFL01-16-102.1	Azoxystrobin	1	2	2	1.5	1.8	1.3	1.4
CsFL01-16-102.1	Azoxystrobin	0.1	2	3.4	2.9	3.4	2.9	2.9
CsFL01-16-102.1	Picoxystrobin	100	2	1.5	1	1.5	1	1
CsFL01-16-102.1	Picoxystrobin	10	2	1.8	1.3	1.7	1.2	1.25
CsFL01-16-102.1	Picoxystrobin	1	2	2.4	1.9	2.4	1.9	1.9
CsFL01-16-102.1	Picoxystrobin	0.1	2	4.7	4.2	4.6	4.1	4.15
CsFL01-16-102.1	PDA+SHAM	0	2	6.6	6.1	6.6	6.1	6.1
CsFL01-16-102.1	PDA only	0	2	7.5	7	7.5	7	7
CsFL01-16-104.2	Fluxapyroxad	100	2	5.7	5.2	6.1	5.6	5.4
CsFL01-16-104.2	Fluxapyroxad	10	2	5.6	5.1	5.7	5.2	5.15
CsFL01-16-104.2	Fluxapyroxad	1	2	NA	NA	NA	NA	NA
CsFL01-16-104.2	Fluxapyroxad	0.1	2	NA	NA	NA	NA	NA
CsFL01-16-104.2	Pyraclostribin	100	2	0.9	0.4	0.8	0.3	0.35

Isolate	Fungicide	Conc	Rep	D1	D1-0.5	D2	D2-0.5	Mean Gth
CsFL01-16-104.2	Pyraclostribin	10	2	0.9	0.4	0.8	0.3	0.35
CsFL01-16-104.2	Pyraclostribin	1	2	1.2	0.7	1.1	0.6	0.65
CsFL01-16-104.2	Pyraclostribin	0.1	2	1.7	1.2	1.6	1.1	1.15
CsFL01-16-104.2	Propiconazole	100	2	NA	NA	NA	NA	NA
CsFL01-16-104.2	Propiconazole	10	2	1.7	1.2	1.8	1.3	1.25
CsFL01-16-104.2	Propiconazole	1	2	3.4	2.9	3.4	2.9	2.9
CsFL01-16-104.2	Propiconazole	0.1	2	4.8	4.3	4.7	4.2	4.25
CsFL01-16-104.2	Azoxystrobin	100	2	1.5	1	1.3	0.8	0.9
CsFL01-16-104.2	Azoxystrobin	10	2	1.6	1.1	1.7	1.2	1.15
CsFL01-16-104.2	Azoxystrobin	1	2	2.1	1.6	2.1	1.6	1.6
CsFL01-16-104.2	Azoxystrobin	0.1	2	3.2	2.7	3.3	2.8	2.75
CsFL01-16-104.2	Picoxystrobin	100	2	NA	NA	NA	NA	NA
CsFL01-16-104.2	Picoxystrobin	10	2	1.8	1.3	1.8	1.3	1.3
CsFL01-16-104.2	Picoxystrobin	1	2	2.5	2	2.6	2.1	2.05
CsFL01-16-104.2	Picoxystrobin	0.1	2	4.7	4.2	4.8	4.3	4.25
CsFL01-16-104.2	PDA+SHAM	0	2	6	5.5	5.6	5.1	5.3
CsFL01-16-104.2	PDA only	0	2	7.2	6.7	7.1	6.6	6.65
CsFL01-16-115.23	Fluxapyroxad	100	2	5.4	4.9	5.3	4.8	4.85
CsFL01-16-115.24	Fluxapyroxad	10	2	5.8	5.3	5.7	5.2	5.25
CsFL01-16-115.25	Fluxapyroxad	1	2	6.4	5.9	6.2	5.7	5.8
CsFL01-16-115.26	Fluxapyroxad	0.1	2	6.2	5.7	6.2	5.7	5.7
CsFL01-16-115.27	Pyraclostribin	100	2	1.2	0.7	1.1	0.6	0.65
CsFL01-16-115.28	Pyraclostribin	10	2	1	0.5	1.1	0.6	0.55
CsFL01-16-115.29	Pyraclostribin	1	2	1.2	0.7	1.2	0.7	0.7
CsFL01-16-115.30	Pyraclostribin	0.1	2	1.6	1.1	1.7	1.2	1.15
CsFL01-16-115.31	Propiconazole	100	2	NA	NA	NA	NA	NA
CsFL01-16-115.32	Propiconazole	10	2	1.8	1.3	1.8	1.3	1.3
CsFL01-16-115.33	Propiconazole	1	2	3.5	3	3.5	3	3
CsFL01-16-115.34	Propiconazole	0.1	2	5.1	4.6	5.1	4.6	4.6
CsFL01-16-115.35	Azoxystrobin	100	2	1.2	0.7	1.3	0.8	0.75
CsFL01-16-115.36	Azoxystrobin	10	2	1.5	1	1.6	1.1	1.05
CsFL01-16-115.37	Azoxystrobin	1	2	1.9	1.4	2	1.5	1.45
CsFL01-16-115.38	Azoxystrobin	0.1	2	3	2.5	3.3	2.8	2.65
CsFL01-16-115.39	Picoxystrobin	100	2	1.5	1	1.6	1.1	1.05
CsFL01-16-115.40	Picoxystrobin	10	2	1.5	1	1.6	1.1	1.05
CsFL01-16-115.41	Picoxystrobin	1	2	2.4	1.9	2.4	1.9	1.9
CsFL01-16-115.42	Picoxystrobin	0.1	2	4.6	4.1	4.6	4.1	4.1
CsFL01-16-115.43	PDA+SHAM	0	2	6.1	5.6	6.1	5.6	5.6
CsFL01-16-115.44	PDA only	0	2	7.3	6.8	7.2	6.7	6.75
CsFL01-16-123.1	Fluxapyroxad	100	2	5.7	5.2	5.7	5.2	5.2

Isolate	Fungicide	Conc	Rep	D1	D1-0.5	D2	D2-0.5	Mean Gth
CsFL01-16-123.1	Fluxapyroxad	10	2	5.6	5.1	5.8	5.3	5.2
CsFL01-16-123.1	Fluxapyroxad	1	2	NA	NA	NA	NA	NA
CsFL01-16-123.1	Fluxapyroxad	0.1	2	6.4	5.9	6.6	6.1	6
CsFL01-16-123.1	Pyraclostribin	100	2	1	0.5	1	0.5	0.5
CsFL01-16-123.1	Pyraclostribin	10	2	1	0.5	1	0.5	0.5
CsFL01-16-123.1	Pyraclostribin	1	2	1.2	0.7	1.2	0.7	0.7
CsFL01-16-123.1	Pyraclostribin	0.1	2	1.8	1.3	1.8	1.3	1.3
CsFL01-16-123.1	Propiconazole	100	2	NA	NA	NA	NA	NA
CsFL01-16-123.1	Propiconazole	10	2	1.8	1.3	1.7	1.2	1.25
CsFL01-16-123.1	Propiconazole	1	2	3.8	3.3	3.6	3.1	3.2
CsFL01-16-123.1	Propiconazole	0.1	2	5	4.5	5.1	4.6	4.55
CsFL01-16-123.1	Azoxystrobin	100	2	1.6	1.1	1.4	0.9	1
CsFL01-16-123.1	Azoxystrobin	10	2	1.4	0.9	1.4	0.9	0.9
CsFL01-16-123.1	Azoxystrobin	1	2	2.1	1.6	2.1	1.6	1.6
CsFL01-16-123.1	Azoxystrobin	0.1	2	3.2	2.7	3.3	2.8	2.75
CsFL01-16-123.1	Picoxystrobin	100	2	1.5	1	1.6	1.1	1.05
CsFL01-16-123.1	Picoxystrobin	10	2	2	1.5	1.8	1.3	1.4
CsFL01-16-123.1	Picoxystrobin	1	2	2.4	1.9	2.6	2.1	2
CsFL01-16-123.1	Picoxystrobin	0.1	2	5	4.5	5	4.5	4.5
CsFL01-16-123.1	PDA+SHAM	0	2	6.4	5.9	6.5	6	5.95
CsFL01-16-123.1	PDA only	0	2	7.3	6.8	7.2	6.7	6.75
CsFL01-16-101.1	Fluxapyroxad	100	3	5.4	4.9	5.4	4.9	4.9
CsFL01-16-101.1	Fluxapyroxad	10	3	5	4.5	5.1	4.6	4.55
CsFL01-16-101.1	Fluxapyroxad	1	3	5.6	5.1	5.6	5.1	5.1
CsFL01-16-101.1	Fluxapyroxad	0.1	3	5.5	5	5.5	5	5
CsFL01-16-101.1	Pyraclostribin	100	3	0.9	0.4	0.9	0.4	0.4
CsFL01-16-101.1	Pyraclostribin	10	3	0.8	0.3	0.7	0.2	0.25
CsFL01-16-101.1	Pyraclostribin	1	3	1.1	0.6	1.1	0.6	0.6
CsFL01-16-101.1	Pyraclostribin	0.1	3	1.6	1.1	1.9	1.4	1.25
CsFL01-16-101.1	Propiconazole	100	3	1.1	0.6	0.9	0.4	0.5
CsFL01-16-101.1	Propiconazole	10	3	1.2	0.7	1.3	0.8	0.75
CsFL01-16-101.1	Propiconazole	1	3	2.4	1.9	2.4	1.9	1.9
CsFL01-16-101.1	Propiconazole	0.1	3	4.3	3.8	4.3	3.8	3.8
CsFL01-16-101.1	Azoxystrobin	100	3	0.6	0.1	0.5	0	0.05
CsFL01-16-101.1	Azoxystrobin	10	3	0.7	0.2	0.7	0.2	0.2
CsFL01-16-101.1	Azoxystrobin	1	3	1.2	0.7	0.8	0.3	0.5
CsFL01-16-101.1	Azoxystrobin	0.1	3	3	2.5	3	2.5	2.5
CsFL01-16-101.1	Picoxystrobin	100	3	1.3	0.8	1.2	0.7	0.75
CsFL01-16-101.1	Picoxystrobin	10	3	1.5	1	1.3	0.8	0.9
CsFL01-16-101.1	Picoxystrobin	1	3	2.4	1.9	2.4	1.9	1.9

Isolate	Fungicide	Conc	Rep	D1	D1-0.5	D2	D2-0.5	Mean Gth
CsFL01-16-101.1	Picoxystrobin	0.1	3	4.3	3.8	4.3	3.8	3.8
CsFL01-16-101.1	PDA+SHAM	0	3	5.8	5.3	6	5.5	5.4
CsFL01-16-101.1	PDA only	0	3	5.7	5.2	5.8	5.3	5.25
CsFL01-16-102.1	Fluxapyroxad	100	3	5	4.5	5.1	4.6	4.55
CsFL01-16-102.1	Fluxapyroxad	10	3	5.5	5	5.3	4.8	4.9
CsFL01-16-102.1	Fluxapyroxad	1	3	6.5	6	6.5	6	6
CsFL01-16-102.1	Fluxapyroxad	0.1	3	7	6.5	7	6.5	6.5
CsFL01-16-102.1	Pyraclostribin	100	3	0.7	0.2	0.7	0.2	0.2
CsFL01-16-102.1	Pyraclostribin	10	3	0.7	0.2	0.6	0.1	0.15
CsFL01-16-102.1	Pyraclostribin	1	3	0.9	0.4	0.8	0.3	0.35
CsFL01-16-102.1	Pyraclostribin	0.1	3	1.5	1	1.5	1	1
CsFL01-16-102.1	Propiconazole	100	3	0	0	0	0	0
CsFL01-16-102.1	Propiconazole	10	3	2	1.5	1.7	1.2	1.35
CsFL01-16-102.1	Propiconazole	1	3	2.5	2	2.4	1.9	1.95
CsFL01-16-102.1	Propiconazole	0.1	3	4.5	4	4.7	4.2	4.1
CsFL01-16-102.1	Azoxystrobin	100	3	0.6	0.1	0.5	0	0.05
CsFL01-16-102.1	Azoxystrobin	10	3	0.6	0.1	0.6	0.1	0.1
CsFL01-16-102.1	Azoxystrobin	1	3	1.3	0.8	1.2	0.7	0.75
CsFL01-16-102.1	Azoxystrobin	0.1	3	4	3.5	4.1	3.6	3.55
CsFL01-16-102.1	Picoxystrobin	100	3	1.7	1.2	1.8	1.3	1.25
CsFL01-16-102.1	Picoxystrobin	10	3	1.9	1.4	1.8	1.3	1.35
CsFL01-16-102.1	Picoxystrobin	1	3	2.9	2.4	2.9	2.4	2.4
CsFL01-16-102.1	Picoxystrobin	0.1	3	5.6	5.1	5.5	5	5.05
CsFL01-16-102.1	PDA+SHAM	0	3	6	5.5	6	5.5	5.5
CsFL01-16-102.1	PDA only	0	3	6.2	5.7	6.2	5.7	5.7
CsFL01-16-104.2	Fluxapyroxad	100	3	5.7	5.2	5.6	5.1	5.15
CsFL01-16-104.2	Fluxapyroxad	10	3	5.4	4.9	5.5	5	4.95
CsFL01-16-104.2	Fluxapyroxad	1	3	6.3	5.8	6.5	6	5.9
CsFL01-16-104.2	Fluxapyroxad	0.1	3	6.5	6	6.3	5.8	5.9
CsFL01-16-104.2	Pyraclostribin	100	3	1.1	0.6	0.9	0.4	0.5
CsFL01-16-104.2	Pyraclostribin	10	3	0.8	0.3	0.8	0.3	0.3
CsFL01-16-104.2	Pyraclostribin	1	3	1.6	1.1	1.5	1	1.05
CsFL01-16-104.2	Pyraclostribin	0.1	3	1.4	0.9	1.4	0.9	0.9
CsFL01-16-104.2	Propiconazole	100	3	0	0	0	0	0
CsFL01-16-104.2	Propiconazole	10	3	1.5	1	1.5	1	1
CsFL01-16-104.2	Propiconazole	1	3	2.6	2.1	2.6	2.1	2.1
CsFL01-16-104.2	Propiconazole	0.1	3	4.7	4.2	4.6	4.1	4.15
CsFL01-16-104.2	Azoxystrobin	100	3	1.3	0.8	1.1	0.6	0.7
CsFL01-16-104.2	Azoxystrobin	10	3	1.1	0.6	1.1	0.6	0.6
CsFL01-16-104.2	Azoxystrobin	1	3	2.2	1.7	2.2	1.7	1.7

Isolate	Fungicide	Conc	Rep	D1	D1-0.5	D2	D2-0.5	Mean Gth
CsFL01-16-104.2	Azoxystrobin	0.1	3	3.8	3.3	4	3.5	3.4
CsFL01-16-104.2	Picoxystrobin	100	3	1.6	1.1	1.6	1.1	1.1
CsFL01-16-104.2	Picoxystrobin	10	3	1.8	1.3	1.8	1.3	1.3
CsFL01-16-104.2	Picoxystrobin	1	3	3	2.5	3	2.5	2.5
CsFL01-16-104.2	Picoxystrobin	0.1	3	5.1	4.6	5.1	4.6	4.6
CsFL01-16-104.2	PDA+SHAM	0	3	6.5	6	6.5	6	6
CsFL01-16-104.2	PDA only	0	3	7.1	6.6	6.9	6.4	6.5
CsFL01-16-115.1	Fluxapyroxad	100	3	5.7	5.2	5.5	5	5.1
CsFL01-16-115.2	Fluxapyroxad	10	3	5.2	4.7	5.1	4.6	4.65
CsFL01-16-115.3	Fluxapyroxad	1	3	5.7	5.2	5.5	5	5.1
CsFL01-16-115.4	Fluxapyroxad	0.1	3	5.6	5.1	5.5	5	5.05
CsFL01-16-115.5	Pyraclostribin	100	3	1.1	0.6	1.1	0.6	0.6
CsFL01-16-115.6	Pyraclostribin	10	3	0.8	0.3	0.8	0.3	0.3
CsFL01-16-115.7	Pyraclostribin	1	3	1	0.5	0.9	0.4	0.45
CsFL01-16-115.8	Pyraclostribin	0.1	3	1.1	0.6	1.1	0.6	0.6
CsFL01-16-115.9	Propiconazole	100	3	0.8	0.3	0.7	0.2	0.25
CsFL01-16-115.10	Propiconazole	10	3	1.3	0.8	1.3	0.8	0.8
CsFL01-16-115.11	Propiconazole	1	3	2.5	2	2.5	2	2
CsFL01-16-115.12	Propiconazole	0.1	3	4.5	4	4.5	4	4
CsFL01-16-115.13	Azoxystrobin	100	3	0.8	0.3	0.7	0.2	0.25
CsFL01-16-115.14	Azoxystrobin	10	3	1.1	0.6	1	0.5	0.55
CsFL01-16-115.15	Azoxystrobin	1	3	1.8	1.3	1.7	1.2	1.25
CsFL01-16-115.16	Azoxystrobin	0.1	3	3.4	2.9	3.4	2.9	2.9
CsFL01-16-115.17	Picoxystrobin	100	3	1.2	0.7	1.1	0.6	0.65
CsFL01-16-115.18	Picoxystrobin	10	3	1.3	0.8	1.3	0.8	0.8
CsFL01-16-115.19	Picoxystrobin	1	3	2.7	2.2	2.6	2.1	2.15
CsFL01-16-115.20	Picoxystrobin	0.1	3	4.6	4.1	4.6	4.1	4.1
CsFL01-16-115.21	PDA+SHAM	0	3	6	5.5	5.9	5.4	5.45
CsFL01-16-115.22	PDA only	0	3	6.5	6	6.5	6	6
CsFL01-16-123.1	Fluxapyroxad	100	3	5.4	4.9	5.4	4.9	4.9
CsFL01-16-123.1	Fluxapyroxad	10	3	5	4.5	5.1	4.6	4.55
CsFL01-16-123.1	Fluxapyroxad	1	3	5.8	5.3	6	5.5	5.4
CsFL01-16-123.1	Fluxapyroxad	0.1	3	5.8	5.3	5.6	5.1	5.2
CsFL01-16-123.1	Pyraclostribin	100	3	0	0	0	0	0
CsFL01-16-123.1	Pyraclostribin	10	3	0.9	0.4	1	0.5	0.45
CsFL01-16-123.1	Pyraclostribin	1	3	1.1	0.6	1	0.5	0.55
CsFL01-16-123.1	Pyraclostribin	0.1	3	1.4	0.9	1.6	1.1	1
CsFL01-16-123.1	Propiconazole	100	3	0.7	0.2	0.6	0.1	0.15
CsFL01-16-123.1	Propiconazole	10	3	1.2	0.7	1.2	0.7	0.7
CsFL01-16-123.1	Propiconazole	1	3	2.3	1.8	2.3	1.8	1.8

Isolate	Fungicide	Conc	Rep	D1	D1-0.5	D2	D2-0.5	Mean Gth
CsFL01-16-123.1	Propiconazole	0.1	3	4.5	4	4.3	3.8	3.9
CsFL01-16-123.1	Azoxystrobin	100	3	0.8	0.3	0.7	0.2	0.25
CsFL01-16-123.1	Azoxystrobin	10	3	1	0.5	0.9	0.4	0.45
CsFL01-16-123.1	Azoxystrobin	1	3	1.5	1	1.3	0.8	0.9
CsFL01-16-123.1	Azoxystrobin	0.1	3	3.2	2.7	3.1	2.6	2.65
CsFL01-16-123.1	Picoxystrobin	100	3	1	0.5	1.2	0.7	0.6
CsFL01-16-123.1	Picoxystrobin	10	3	1.4	0.9	1.2	0.7	0.8
CsFL01-16-123.1	Picoxystrobin	1	3	2.4	1.9	2.3	1.8	1.85
CsFL01-16-123.1	Picoxystrobin	0.1	3	4.5	4	4.5	4	4
CsFL01-16-123.1	PDA+SHAM	0	3	6.2	5.7	6.2	5.7	5.7
CsFL01-16-123.1	PDA only	0	3	6.2	5.7	6.1	5.6	5.65

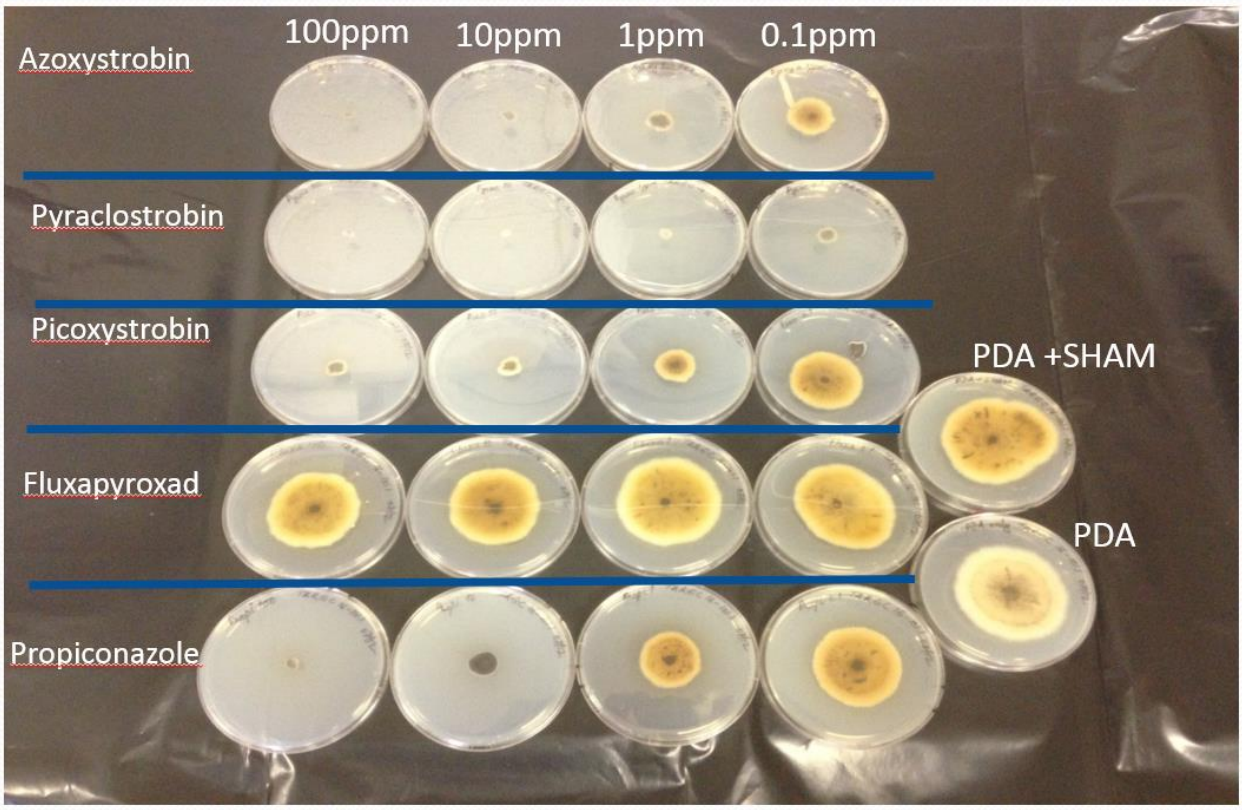


Fig. 1. Example of *Colletotrichum sublineola* isolate radial growth assay on PDA plates with different fungicide active ingredients at varying concentrations and controls.

III. Greenhouse Koch's postulates on *Fusarium* species

Objective: To perform Koch's postulate in the greenhouse on the *Fusarium species* isolated from sorghum grain.

Protocol

1. A sorghum hybrid '84P80' will be grown in a three-gallon plastic pots with a mix of sterile field soil and top soil in the ratio of 1:1 in a greenhouse.
2. Three seeds will be plated in a pot and will be thinned to a single plant per pot after a week of emergence.
3. The plants will be watered twice daily with an automated irrigation system in the greenhouse.
4. The plants will be allowed to grow until the entire head is at flowering.
5. Two isolates of a Fusarium species (single spore isolated and identified to the species) will be grown in ¼ PDA for 5 days.
6. The spores from each isolate will be harvested by adding 10 ml of sterile distilled water to the plate with the isolate and dislodged with a sterile glass rod. Then, the suspension is prepared by passing it through 4-layer of sterile cheese cloth.
7. The final concentration is calculated using hemocytometer and adjusted to 10^5 spores ml^{-1} for each isolate.
8. Using the spray bottle, approximately 5 ml of the suspension will be sprayed (single spray is equivalent to 1 ml) on the flowering head outside the greenhouse and placed in a moist chamber prepared in the green house for 3 days.
9. There will be two isolates of each Fusarium species with two replicates for each isolate and a control sprayed with sterile distilled water.
10. The plants will be allowed to grow until physiological maturity and the grains will be harvested.
11. The harvested grain will be cultured in ¼ PDA and the recovery of the species will be compared morphologically to that of the original isolates used to inoculate the plants.

IV. Sorghum germplasm screening

Objective: To screen sorghum germplasm for resistance to anthracnose, grain mold and DON contamination.

Method: A total of 407 sorghum accessions obtained from Plant Genetic Resources Conservation Unit, GA were evaluated in 5" x 2" plots with 2-3 replications over time from 2014 to 2016. The disease severity for anthracnose and grain mold were rated at the hard dough stage. Anthracnose severity was rated as the percentage of the leaf area with symptoms and signs of anthracnose and grain mold severity was rated as the percentage of the sorghum grains with symptoms and signs of mold in the head. The DON contamination was evaluated in a sub-sample of 98 accessions using staple isotope dilution assay (SIDA) with two replications of grain samples collected from 2014 and 2015.

Table 2. Raw data from the field screening of sorghum accessions.

Sorghum Accession	Grain Color	Country	HM% 2014	HM% 2015	HM% 2016	Anth % 2014	Anth % 2016	DON (ppm), 2014	DON (ppm), 2015	Mean HM%	Mean Anth %	Mean DON (ppm)
PI566819	dark red	United States, Virginia	0	35	1	4	0	0.12	0.08	12	2	0.1
PI595718	red	United States, Texas	25	85	50	2	1	0.16	0.08	53	2	0.12
PI533965	brown	Uganda	2	10	2	1	1	0.2	0.12	5	1	0.16
PI576366	brown	India	5	90	15	1	2	0.24	0.08	37	2	0.16
PI641824	brown	NA	1	0	1	7	10	0.32	0	1	9	0.16
PI17548	brown	Australia, New South Wales	1	0	1	5	35	0.32	0	1	20	0.16
PI653616	red	United States, Mississippi	1	15	.	4	0	0.12	0.24	8	2	0.18
PI656105	brown	NA	5	2	5	8	0	0.32	0.04	4	4	0.18
PI641836	red	NA	1	75	1	2	30	0.04	0.36	26	16	0.2
PI597966	brown	United States, Texas	10	45	20	1	1	0.32	0.12	25	1	0.22
PI533752	dark red	NA	1	10	2	2	3	0.28	0.16	4	3	0.22
PI534009	brown	India	5	10	.	1	35	0.32	0.16	8	18	0.24
PI641849	brown	NA	1	40	1	2	.	0.28	0.24	14	2	0.26
PI656104	brown	NA	1	1	3	3	10	0.48	0.04	2	7	0.26
PI533912	dark red	Sudan	20	55	25	1	35	0.36	0.16	33	18	0.26
PI533901	red	Japan	5	90	20	1	40	0.48	0.12	38	21	0.3
PI533814	yellow	India	10	85	15	2	50	0.36	0.24	37	26	0.3
PI533913	white	Sudan	7	90	5	2	1	0.44	0.2	34	2	0.32
PI533855	white	India	10	90	25	7	25	0.48	0.16	42	16	0.32
PI595740	brown	United States, Texas	10	1	3	1	35	0.6	0.04	5	18	0.32
PI533800	red	NA	5	30	10	3	55	0.44	0.2	15	29	0.32
PI533766	white	NA	15	95	30	1	10	0.44	0.24	47	6	0.34
PI656121	red	NA	1	10	5	2	25	0.12	0.6	5	14	0.36
PI533876	red	Nigeria	5	40	20	1	0	0.4	0.36	22	1	0.38
PI533991	yellow	Sudan	20	10	7	3	0	0.68	0.12	12	2	0.4

Sorghum Accession	Grain Color	Country	HM% 2014	HM% 2015	HM% 2016	Anth % 2014	Anth % 2016	DON (ppm), 2014	DON (ppm), 2015	Mean HM%	Mean Anth %	Mean DON (ppm)
PI534144	dark red	Uganda	5	95	7	4	0	0.76	0.04	36	2	0.4
PI656103	brown	NA	5	35	2	8	25	0.52	0.28	14	17	0.4
PI576390	yellow	India	10	75	25	1	25	0.52	0.36	37	13	0.44
PI533986	yellow	Sudan	5	40	3	1	1	0.48	0.44	16	1	0.46
PI534037	yellow	Chad	5	20	5	1	35	0.84	0.08	10	18	0.46
PI533788	dark red	NA	20	65	20	1	1	0.76	0.2	35	1	0.48
PI533956	brown	Congo	2	0	1	3	0	0.84	0.12	1	2	0.48
PI576350	red	United States	1	55	1	4	.	0.28	0.72	19	4	0.5
PI533943	white	India	15	70	20	1	30	0.84	0.16	35	16	0.5
PI533754	brown	NA	5	10	2	2	30	0.84	0.16	6	16	0.5
PI576332	dark red	China	5	60	2	1	40	0.8	0.24	22	21	0.52
PI656118	dark red	NA	3	5	35	8	10	0.72	0.36	14	9	0.54
PI533833	dark red	Uganda	5	55	2	2	35	1.08	0.12	21	19	0.6
PI597968	brown	United States, Texas	5	25	3	1	0	0.84	0.4	11	1	0.62
PI534112	brown	India	5	25	7	2	0	1.16	0.12	12	1	0.64
PI533877	yellow	Nigeria	15	90	55	1	3	1.16	0.12	53	2	0.64
PI533785	red	United States, Texas	20	55	15	0	1	0.68	0.6	30	1	0.64
PI534054	white	Kenya	20	65	35	1	40	0.88	0.4	40	21	0.64
PI533919	brown	Ethiopia	25	55	3	1	0	1.12	0.2	28	1	0.66
PI656074	white	NA	5	5	15	5	20	1.32	0.04	8	13	0.68
PI533996	yellow	Sudan	10	40	15	1	25	1.04	0.32	22	13	0.68
PI597965	yellow	United States, Texas	10	60	35	1	35	1.04	0.32	35	18	0.68
PI533821	dark red	Tanzania	10	30	2	3	0	1.04	0.36	14	2	0.7
PI534028	white	India	15	15	25	1	3	1	0.4	18	2	0.7
PI576381	yellow	Ethiopia	20	90	55	2	7	0.8	0.64	55	5	0.72
PI656110	brown	NA	5	20	20	3	0	1.36	0.16	15	2	0.76
PI533762	white	NA	30	70	20	1	25	1.4	0.16	40	13	0.78

Sorghum Accession	Grain Color	Country	HM% 2014	HM% 2015	HM% 2016	Anth % 2014	Anth % 2016	DON (ppm), 2014	DON (ppm), 2015	Mean HM%	Mean Anth %	Mean DON (ppm)
PI534155	dark red	Ethiopia	70	90	30	1	0	1.28	0.32	63	1	0.8
PI576385	white	Nigeria	20	100	65	1	1	1.56	0.12	62	1	0.84
PI533967	white	Uganda	15	85	15	2	25	1.52	0.2	38	14	0.86
PI656075	yellow	NA	10	55	20	5	25	1.64	0.12	28	15	0.88
PI533824	dark red	Nigeria	15	30	20	5	5	1.72	0.08	22	5	0.9
PI552856	red	United States, Texas	15	30	20	2	25	1.36	0.48	22	14	0.92
PI656095	yellow	NA	20	90	40	5	0	1.84	0.16	50	3	1
PI656056	white	United States, Indiana	10	25	25	2	25	1.8	0.2	20	14	1
PI533911	white	Sudan	10	90	10	2	25	0.44	1.64	37	14	1.04
PI329440	red	Ethiopia	15	20	10	1	15	1.72	0.4	15	8	1.06
PI533948	white	United States	10	40	25	1	35	2	0.24	25	18	1.12
PI656054	white	Mali	1	4	.	8	0	1.12	1.16	3	4	1.14
PI534115	white	Ethiopia	10	4	7	1	35	2.12	0.16	7	18	1.14
PI534105	yellow	Uganda	20	15	20	1	35	2.16	0.12	18	18	1.14
PI576428	yellow	Ethiopia	15	85	15	4	45	0.76	1.52	38	25	1.14
PI533758	yellow	NA	40	80	5	3	5	1.24	1.2	42	4	1.22
PI533863	white	Chad	15	75	35	1	1	2.32	0.28	42	1	1.3
PI534167	brown	Unknown	10	85	45	1	3	2.44	0.16	47	2	1.3
PI576340	red	South Africa	15	75	10	1	35	1.88	1	33	18	1.44
PI656114	red	NA	20	75	3	5	1	2.84	0.16	33	3	1.5
PI533938	red	Zaire	25	20	3	2	5	2.56	0.64	16	4	1.6
PI534117	dark red	Uganda	5	2	1	2	0	3.36	0.04	3	1	1.7
PI329435	white	Ethiopia	15	50	15	1	25	3.16	0.36	27	13	1.76
PI24969	red	China	40	60	20	5	25	1.68	1.88	40	15	1.78
PI585295	yellow	United States, Texas	15	40	15	3	10	3.2	0.4	23	7	1.8
PI595714	white	United States, Texas	40	85	20	2	40	2.04	1.56	48	21	1.8

Sorghum Accession	Grain Color	Country	HM% 2014	HM% 2015	HM% 2016	Anth % 2014	Anth % 2016	DON (ppm), 2014	DON (ppm), 2015	Mean HM%	Mean Anth %	Mean DON (ppm)
PI656066	yellow	NA	1	15	.	4	40	1.8	1.84	8	22	1.82
PI641874	red	NA	70	55	65	2	.	3.48	0.2	63	2	1.84
PI533822	white	Tanzania	40	65	40	4	1	3.88	0.28	48	3	2.08
PI533776	yellow	NA	25	50	40	2	1	3.12	1.08	38	2	2.1
PI656006	white	United States, Texas	10	85	35	4	60	3.72	0.48	43	32	2.1
PI534133	yellow	Ethiopia	40	60	80	1	25	3.96	0.32	60	13	2.14
PI656089	red	NA	70	70	1	2	5	4.52	0	47	4	2.26
PI533792	red	NA	5	60	7	3	30	3.56	1.04	24	17	2.3
PI597972	white	United States, Texas	40	90	75	2	1	4.48	0.2	68	2	2.34
PI656063	brown	NA	8	75	.	11	25	5	0.44	42	18	2.72
PI597945	yellow	United States, Texas	70	90	85	2	1	5.68	0.2	82	2	2.94
PI576426	red	Ethiopia	60	70	60	2	1	4.76	1.32	63	2	3.04
PI576375	yellow	Ethiopia	40	80	25	3	0	5.48	0.68	48	2	3.08
PI655997	red	United States, Texas	20	100	20	1	20	6.44	0.12	47	11	3.28
PI533831	white	Sudan	40	80	20	3	20	6	1.04	47	12	3.52
PI656004	yellow	United States, Texas	25	75	35	8	55	5.76	1.36	45	32	3.56
PI597960	white	United States, Texas	40	55	75	1	35	5.84	2.04	57	18	3.94
PI533980	white	China	40	85	35	1	25	5.72	2.44	53	13	4.08
PI533882	red	Nigeria	15	70	35	1	1	7	1.32	40	1	4.16
PI561072	white	United States, Texas	15	15	20	4	7	10.52	0.16	17	6	5.34
PI533924	brown	Ethiopia	5	35	1	0	0	NA	NA	14	0	NA
PI534123	brown	Ethiopia	0	1	1	1	0	NA	NA	1	1	NA
PI576349	brown	United States	0	.	2	1	0	NA	NA	1	1	NA
PI533927	brown	Ethiopia	2	1	2	1	0	NA	NA	2	1	NA
PI576391	brown	India	5	3	2	1	0	NA	NA	3	1	NA
PI597964	brown	United States, Texas	2	10	2	1	0	NA	NA	5	1	NA
PI534124	brown	NA	2	20	1	1	0	NA	NA	8	1	NA

Sorghum Accession	Grain Color	Country	HM% 2014	HM% 2015	HM% 2016	Anth % 2014	Anth % 2016	DON (ppm), 2014	DON (ppm), 2015	Mean HM%	Mean Anth %	Mean DON (ppm)
PI533962	brown	Sudan	5	30	1	1	0	NA	NA	12	1	NA
PI533939	brown	Ethiopia	25	.	1	1	0	NA	NA	13	1	NA
PI533902	brown	Ethiopia	5	40	5	1	0	NA	NA	17	1	NA
PI533871	red	Nigeria	3	45	15	1	0	NA	NA	21	1	NA
PI656069	white	NA	3	55	5	1	0	NA	NA	21	1	NA
PI534079	white	Nigeria	15	35	25	1	0	NA	NA	25	1	NA
PI656007	white	NA	10	50	15	1	0	NA	NA	25	1	NA
PI533689	red	Tanzania	20	55	2	1	0	NA	NA	26	1	NA
PI597976	white	United States, Texas	15	50	20	1	0	NA	NA	28	1	NA
PI576337	brown	Uganda	2	90	1	1	0	NA	NA	31	1	NA
PI534163	white	United States	5	95	2	1	0	NA	NA	34	1	NA
PI534075	yellow	Nigeria	5	65	40	1	0	NA	NA	37	1	NA
PI533949	white	Sudan	15	100	45	1	0	NA	NA	53	1	NA
PI659695	brown	NA	70	90	1	1	0	NA	NA	54	1	NA
PI533760	white	NA	75	85	35	0	1	NA	NA	65	1	NA
PI576348	red	United States	0	3	2	1	1	NA	NA	2	1	NA
PI534138	brown	Sudan	7	0	1	2	0	NA	NA	3	1	NA
PI597820	brown	United States, Texas	2	2	5	1	.	NA	NA	3	1	NA
PI656093	white	NA	7	15	2	2	0	NA	NA	8	1	NA
PI534101	yellow	Japan	10	.	10	1	1	NA	NA	10	1	NA
PI595739	white	United States, Texas	15	15	5	1	1	NA	NA	12	1	NA
PI656002	white	United States, Oklahoma	15	4	30	1	.	NA	NA	16	1	NA
PI533961	brown	South Africa	5	50	7	2	0	NA	NA	21	1	NA
PI534070	red	Nigeria	5	35	25	2	0	NA	NA	22	1	NA
PI576399	white	Sudan	15	55	15	2	0	NA	NA	28	1	NA
PI533964	white	Sudan	10	75	5	1	1	NA	NA	30	1	NA

Sorghum Accession	Grain Color	Country	HM% 2014	HM% 2015	HM% 2016	Anth % 2014	Anth % 2016	DON (ppm), 2014	DON (ppm), 2015	Mean HM%	Mean Anth %	Mean DON (ppm)
PI576411	white	South Africa	15	55	20	1	1	NA	NA	30	1	NA
PI653617	brown	United States, Mississippi	2	90	1	2	0	NA	NA	31	1	NA
PI533807	white	NA	20	70	5	1	1	NA	NA	32	1	NA
PI576387	white	Sudan	10	80	10	2	0	NA	NA	33	1	NA
PI533750	brown	NA	10	70	35	1	1	NA	NA	38	1	NA
PI655990	red	United States, Texas	40	55	30	1	.	NA	NA	42	1	NA
PI656100	white	NA	30	90	10	2	0	NA	NA	43	1	NA
PI597952	white	United States, Texas	15	90	40	2	0	NA	NA	48	1	NA
PI655976	red	United States, Kansas	15	90	40	1	.	NA	NA	48	1	NA
PI533915	white	Kenya	15	95	40	2	0	NA	NA	50	1	NA
PI597980	white	United States, Texas	30	90	30	2	0	NA	NA	50	1	NA
PI533954	red	South Africa	40	70	50	1	1	NA	NA	53	1	NA
PI595744	white	United States, Texas	60	65	45	1	.	NA	NA	57	1	NA
PI597950	white	United States, Texas	20	60	90	1	.	NA	NA	57	1	NA
PI655977	red	United States, Texas	10	95	70	1	.	NA	NA	58	1	NA
PI655971	white	NA	60	60	60	1	.	NA	NA	60	1	NA
PI651496	white	NA	1	1	.	3	0	NA	NA	1	2	NA
PI656081	red	NA	3	5	1	3	0	NA	NA	3	2	NA
PI533985	brown	Sudan	7	10	1	2	1	NA	NA	6	2	NA
PI533972	yellow	Uganda	10	5	5	3	0	NA	NA	7	2	NA
PI656061	brown	India	2	15	15	2	1	NA	NA	11	2	NA
PI533987	brown	Sudan	7	40	5	3	0	NA	NA	17	2	NA
PI534063	white	Nigeria	5	15	60	3	0	NA	NA	27	2	NA
PI533940	white	Tanzania	7	80	15	1	2	NA	NA	34	2	NA
PI534139	red	Sudan	5	5	1	4	0	NA	NA	4	2	NA

Sorghum Accession	Grain Color	Country	HM% 2014	HM% 2015	HM% 2016	Anth % 2014	Anth % 2016	DON (ppm), 2014	DON (ppm), 2015	Mean HM%	Mean Anth %	Mean DON (ppm)
PI533976	brown	South Africa	5	10	1	4	0	NA	NA	5	2	NA
PI656068	white	NA	10	20	.	2	.	NA	NA	15	2	NA
PI656000	white	United States, Texas	20	25	20	2	.	NA	NA	22	2	NA
PI534092	yellow	Nigeria	15	4	50	1	3	NA	NA	23	2	NA
PI576339	white	Zimbabwe	25	15	35	1	3	NA	NA	25	2	NA
PI656071	white	NA	10	50	25	3	1	NA	NA	28	2	NA
PI533759	yellow	NA	10	70	15	2	2	NA	NA	32	2	NA
PI533878	yellow	Nigeria	20	50	30	2	2	NA	NA	33	2	NA
PI595741	white	United States, Texas	5	95	20	2	.	NA	NA	40	2	NA
PI655975	red	United States	30	80	35	2	.	NA	NA	48	2	NA
PI609456	white	Mali	5	1	.	5	0	NA	NA	3	3	NA
PI656106	brown	NA	20	10	2	5	0	NA	NA	11	3	NA
PI561472	white	Honduras	5	25	5	2	3	NA	NA	12	3	NA
PI656027	yellow	NA	15	15	5	5	0	NA	NA	12	3	NA
PI656117	white	NA	30	40	1	2	3	NA	NA	24	3	NA
PI534145	white	Rhodesia	10	100	85	2	3	NA	NA	65	3	NA
PI576347	red	United States	5	2	20	3	.	NA	NA	9	3	NA
PI595743	brown	United States, Texas	7	15	10	1	5	NA	NA	11	3	NA
PI576425	brown	Ethiopia	10	15	15	1	5	NA	NA	13	3	NA
PI552961	white	United States, Texas	15	30	20	3	3	NA	NA	22	3	NA
PI656055	white	United States, Indiana	12	35	70	3	.	NA	NA	39	3	NA
PI659753	red	NA	15	65	.	3	.	NA	NA	40	3	NA
PI534046	white	Sudan	15	95	25	1	5	NA	NA	45	3	NA
PI656019	red	United States, Texas	15	85	35	3	.	NA	NA	45	3	NA
PI656065	white	NA	15	90	.	5	1	NA	NA	53	3	NA
PI655973	white	United States, Texas	30	90	45	3	.	NA	NA	55	3	NA

Sorghum Accession	Grain Color	Country	HM% 2014	HM% 2015	HM% 2016	Anth % 2014	Anth % 2016	DON (ppm), 2014	DON (ppm), 2015	Mean HM%	Mean Anth %	Mean DON (ppm)
PI659692	brown	United States, Texas	0	1	3	4	3	NA	NA	1	4	NA
PI576130	white	India, Andhra Pradesh	1	15	.	2	5	NA	NA	8	4	NA
PI656023	yellow	NA	7	.	20	4	3	NA	NA	14	4	NA
PI574455	red	United States, Texas	15	15	25	2	5	NA	NA	18	4	NA
PI656077	brown	NA	8	45	45	2	5	NA	NA	33	4	NA
PI534088	yellow	Nigeria	30	70	80	2	5	NA	NA	60	4	NA
PI597946	brown	United States, Texas	2	5	3	4	.	NA	NA	3	4	NA
PI533789	yellow	NA	5	10	3	1	7	NA	NA	6	4	NA
PI656025	red	China	5	25	2	4	.	NA	NA	11	4	NA
PI656070	yellow	NA	7	15	15	3	5	NA	NA	12	4	NA
PI655983	red	NA	2	90	1	4	.	NA	NA	31	4	NA
PI656111	white	NA	35	30	65	4	.	NA	NA	43	4	NA
PI656030	red	United States	30	85	75	4	.	NA	NA	63	4	NA
PI533841	white	Nigeria	15	100	40	2	7	NA	NA	52	5	NA
PI642998	red	NA	1	5	1	5	.	NA	NA	2	5	NA
PI655981	white	Mali	5	0	2	10	0	NA	NA	2	5	NA
PI656090	white	NA	10	15	.	5	5	NA	NA	13	5	NA
PI534132	red	Ethiopia	60	25	50	5	.	NA	NA	45	5	NA
PI597975	red	United States, Texas	15	90	50	3	7	NA	NA	52	5	NA
PI576418	red	Nigeria	5	.	7	1	10	NA	NA	6	6	NA
PI656082	brown	NA	5	.	20	1	10	NA	NA	13	6	NA
PI533903	brown	Ethiopia	3	15	35	1	10	NA	NA	18	6	NA
PI533839	white	Nigeria	5	60	15	1	10	NA	NA	27	6	NA
PI534114	brown	Pakistan	7	75	10	1	10	NA	NA	31	6	NA
PI597967	white	United States, Texas	40	80	25	1	10	NA	NA	48	6	NA
PI152651	white	Sudan	30	95	20	2	10	NA	NA	48	6	NA

Sorghum Accession	Grain Color	Country	HM% 2014	HM% 2015	HM% 2016	Anth % 2014	Anth % 2016	DON (ppm), 2014	DON (ppm), 2015	Mean HM%	Mean Anth %	Mean DON (ppm)
PI656115	white	NA	5	10	7	3	10	NA	NA	7	7	NA
PI656079	white	NA	15	15	2	8	5	NA	NA	11	7	NA
PI656116	brown	NA	5	35	10	3	10	NA	NA	17	7	NA
PI533997	white	NA	15	75	20	4	10	NA	NA	37	7	NA
PI534116	brown	Ethiopia	0	5	1	1	15	NA	NA	2	8	NA
PI534047	red	Sudan	10	20	20	1	15	NA	NA	17	8	NA
PI656003	red	United States, Arizona	2	60	1	1	15	NA	NA	21	8	NA
PI656040	yellow	NA	5	45	15	1	15	NA	NA	22	8	NA
PI656060	brown	NA	10	40	15	1	15	NA	NA	22	8	NA
PI597958	white	United States, Texas	25	45	25	1	15	NA	NA	32	8	NA
PI595745	brown	United States, Texas	5	60	35	1	15	NA	NA	33	8	NA
PI576380	white	Ethiopia	30	65	7	1	15	NA	NA	34	8	NA
PI655985	red	United States, Texas	20	55	30	1	15	NA	NA	35	8	NA
PI576345	yellow	South Africa	10	95	40	1	15	NA	NA	48	8	NA
PI595720	white	United States, Texas	20	85	45	1	15	NA	NA	50	8	NA
PI656046	brown	China	3	10	1	2	15	NA	NA	5	9	NA
PI534108	yellow	Uganda	15	15	1	2	15	NA	NA	10	9	NA
PI533761	red	NA	3	45	7	2	15	NA	NA	18	9	NA
PI533989	white	Sudan	10	60	5	2	15	NA	NA	25	9	NA
PI548797	white	United States, Texas	15	40	20	2	15	NA	NA	25	9	NA
PI656017	white	United States	10	40	.	2	15	NA	NA	25	9	NA
PI659691	yellow	United States, Texas	70	90	80	2	15	NA	NA	80	9	NA
PI656057	white	United States, Indiana	10	.	5	3	15	NA	NA	8	9	NA
PI533839	white	Nigeria	5	70	20	3	15	NA	NA	32	9	NA
PI656015	white	Sudan	25	40	45	3	15	NA	NA	37	9	NA

Sorghum Accession	Grain Color	Country	HM% 2014	HM% 2015	HM% 2016	Anth % 2014	Anth % 2016	DON (ppm), 2014	DON (ppm), 2015	Mean HM%	Mean Anth %	Mean DON (ppm)
PI34911	white	United States, Texas	15	40	2	4	15	NA	NA	19	10	NA
PI6561073	white	United States, Texas	10	55	10	4	15	NA	NA	25	10	NA
PI656101	red	NA	15	70	1	4	15	NA	NA	29	10	NA
PI533769	white	NA	15	95	25	4	15	NA	NA	45	10	NA
PI656038	yellow	NA	1	1	20	10	.	NA	NA	7	10	NA
PI656035	white	Niger	10	55	10	5	15	NA	NA	25	10	NA
PI656097	white	NA	20	90	15	10	.	NA	NA	42	10	NA
PI656109	red	NA	15	95	15	10	.	NA	NA	42	10	NA
PI656112	white	NA	70	95	40	5	15	NA	NA	68	10	NA
PI597971	brown	United States, Texas	2	25	5	1	20	NA	NA	11	11	NA
PI48770	white	South Africa, Cape Province	10	.	20	1	20	NA	NA	15	11	NA
PI534021	white	India	7	40	2	1	20	NA	NA	16	11	NA
PI655970	white	United States, Kansas	10	35	10	1	20	NA	NA	18	11	NA
PI540816	yellow	United States, Texas	10	1	50	1	20	NA	NA	20	11	NA
PI534127	dark red	NA	5	55	7	1	20	NA	NA	22	11	NA
PI607931	red	United States, Texas	5	65	15	1	20	NA	NA	28	11	NA
PI656078	white	NA	30	80	5	1	20	NA	NA	38	11	NA
PI533910	white	Sudan	10	95	20	1	20	NA	NA	42	11	NA
PI656119	white	NA	50	90	20	1	20	NA	NA	53	11	NA
PI597951	white	United States, Texas	20	95	65	1	20	NA	NA	60	11	NA
PI534104	brown	Uganda	10	55	3	2	20	NA	NA	23	11	NA
PI533842	white	India	30	35	7	2	20	NA	NA	24	11	NA
PI576376	white	Ethiopia	30	90	25	2	20	NA	NA	48	11	NA
PI659693	white	NA	85	100	20	2	20	NA	NA	68	11	NA
PI613536	yellow	United States, Kansas	80	85	65	2	20	NA	NA	77	11	NA

Sorghum Accession	Grain Color	Country	HM% 2014	HM% 2015	HM% 2016	Anth % 2014	Anth % 2016	DON (ppm), 2014	DON (ppm), 2015	Mean HM%	Mean Anth %	Mean DON (ppm)
PI576359	white	India	5	10	35	3	20	NA	NA	17	12	NA
PI533856	white	India	5	50	10	3	20	NA	NA	22	12	NA
PI656073	white	NA	15	80	2	4	20	NA	NA	32	12	NA
PI533757	white	NA	30	80	3	4	20	NA	NA	38	12	NA
PI276837	white	Ethiopia	0	10	.	5	20	NA	NA	5	13	NA
PI656036	white	NA	5	15	15	10	15	NA	NA	12	13	NA
PI656113	white	NA	35	90	55	5	20	NA	NA	60	13	NA
PI35038	dark red	United States, Texas	0	1	1	1	25	NA	NA	1	13	NA
PI533937	dark red	Rhodesia	15	25	10	1	25	NA	NA	17	13	NA
PI656018	red	United States, Texas	5	80	7	1	25	NA	NA	31	13	NA
PI655986	red	United States, Texas	15	50	35	1	25	NA	NA	33	13	NA
PI655984	white	United States, Texas	25	70	20	1	25	NA	NA	38	13	NA
PI534053	white	Uganda	30	65	25	1	25	NA	NA	40	13	NA
PI656108	brown	NA	40	50	30	1	25	NA	NA	40	13	NA
PI533970	white	Uganda	30	80	35	1	25	NA	NA	48	13	NA
PI533866	white	Sudan	30	95	65	1	25	NA	NA	63	13	NA
PI656013	red	China	1	5	1	2	25	NA	NA	2	14	NA
PI576386	brown	Uganda	5	50	5	2	25	NA	NA	20	14	NA
PI533838	white	Nigeria	60	100	80	2	25	NA	NA	80	14	NA
PI656032	yellow	Senegal	5	3	5	3	25	NA	NA	4	14	NA
PI656080	red	NA	10	.	2	3	25	NA	NA	6	14	NA
PI656067	white	NA	1	10	10	3	25	NA	NA	7	14	NA
PI656044	white	South Africa	15	20	3	8	20	NA	NA	13	14	NA
PI642992	white	NA	15	1	25	3	25	NA	NA	14	14	NA
PI655980	brown	United States, Texas	2	80	1	3	25	NA	NA	28	14	NA
PI561071	white	United States, Texas	10	70	40	3	25	NA	NA	40	14	NA
PI656096	yellow	NA	25	95	25	3	25	NA	NA	48	14	NA

Sorghum Accession	Grain Color	Country	HM% 2014	HM% 2015	HM% 2016	Anth % 2014	Anth % 2016	DON (ppm), 2014	DON (ppm), 2015	Mean HM%	Mean Anth %	Mean DON (ppm)
PI576434	white	Nigeria	5	35	15	4	25	NA	NA	18	15	NA
PI656026	white	NA	5	20	15	5	25	NA	NA	13	15	NA
PI655995	red	United States, Illinois	1	50	2	10	20	NA	NA	18	15	NA
PI656059	white	Nicaragua	10	40	10	5	25	NA	NA	20	15	NA
PI656086	red	NA	15	15	30	5	25	NA	NA	20	15	NA
PI534096	brown	Mali	0	75	2	0	30	NA	NA	26	15	NA
PI656011	white	United States, Texas	5	70	5	10	20	NA	NA	27	15	NA
PI656072	brown	NA	10	40	45	5	25	NA	NA	32	15	NA
PI533810	white	India	10	75	35	10	20	NA	NA	40	15	NA
PI576364	red	India	2	5	2	1	30	NA	NA	3	16	NA
PI595702	yellow	United States, Texas	20	2	10	1	30	NA	NA	11	16	NA
PI656053	white	United States, Nebraska	15	25	30	1	30	NA	NA	23	16	NA
PI533845	white	Nepal	5	90	20	1	30	NA	NA	38	16	NA
PI597961	white	United States, Texas	15	80	20	1	30	NA	NA	38	16	NA
PI656052	white	United States, Nebraska	25	80	35	1	30	NA	NA	47	16	NA
PI655994	red	United States, Maryland	1	.	1	2	30	NA	NA	1	16	NA
PI656012	white	United States	30	3	25	2	30	NA	NA	19	16	NA
PI656014	red	NA	2	60	1	2	30	NA	NA	21	16	NA
PI656102	brown	NA	8	90	2	2	30	NA	NA	33	16	NA
PI656042	white	NA	40	55	50	2	30	NA	NA	48	16	NA
PI576352	white	Botswana	15	95	45	2	30	NA	NA	52	16	NA
PI565121	white	Zimbabwe	10	1	10	3	30	NA	NA	7	17	NA
PI533830	red	Sudan	50	70	35	3	30	NA	NA	52	17	NA
PI534135	white	Ethiopia	20	95	.	3	30	NA	NA	58	17	NA

Sorghum Accession	Grain Color	Country	HM% 2014	HM% 2015	HM% 2016	Anth % 2014	Anth % 2016	DON (ppm), 2014	DON (ppm), 2015	Mean HM%	Mean Anth %	Mean DON (ppm)
PI598870	brown	United States, Texas	3	10	2	4	30	NA	NA	5	17	NA
PI656091	yellow	NA	10	.	70	4	30	NA	NA	40	17	NA
PI656020	red	United States, Texas	15	95	35	4	30	NA	NA	48	17	NA
PI656062	white	NA	8	20	10	10	25	NA	NA	13	18	NA
PI656037	white	South Africa	1	35	10	5	30	NA	NA	15	18	NA
PI656120	white	NA	40	90	2	5	30	NA	NA	44	18	NA
PI656029	yellow	United States, Texas	20	80	50	5	30	NA	NA	50	18	NA
PI533936	brown	Tanzania	5	10	1	1	35	NA	NA	5	18	NA
PI542406	brown	United States, Arkansas	2	20	1	1	35	NA	NA	8	18	NA
PI534157	white	Ethiopia	7	.	20	1	35	NA	NA	14	18	NA
PI655993	red	United States, Texas	20	4	30	1	35	NA	NA	18	18	NA
PI576393	brown	Ethiopia	7	70	10	1	35	NA	NA	29	18	NA
PI655987	red	United States, Texas	15	50	25	1	35	NA	NA	30	18	NA
PI642791	red	United States, Texas	15	60	20	1	35	NA	NA	32	18	NA
PI533794	white	NA	10	80	25	1	35	NA	NA	38	18	NA
PI656001	red	United States, Texas	5	80	35	1	35	NA	NA	40	18	NA
PI655989	red	United States, Texas	30	70	40	1	35	NA	NA	47	18	NA
PI533799	white	NA	20	100	40	1	35	NA	NA	53	18	NA
PI656098	yellow	NA	5	35	10	7	30	NA	NA	17	19	NA
PI585291	red	United States, Texas	15	.	25	2	35	NA	NA	20	19	NA
PI655972	white	United States, Kansas	15	45	7	2	35	NA	NA	22	19	NA
PI576396	brown	Uganda	7	85	15	2	35	NA	NA	36	19	NA
PI655979	yellow	United States, Texas	15	60	40	2	35	NA	NA	38	19	NA
PI534097	white	Japan	10	85	30	2	35	NA	NA	42	19	NA
PI656083	white	NA	7	40	35	3	35	NA	NA	27	19	NA

Sorghum Accession	Grain Color	Country	HM% 2014	HM% 2015	HM% 2016	Anth % 2014	Anth % 2016	DON (ppm), 2014	DON (ppm), 2015	Mean HM%	Mean Anth %	Mean DON (ppm)
PI655982	red	Australia	15	90	35	3	35	NA	NA	47	19	NA
PI533755	white	NA	80	90	75	3	35	NA	NA	82	19	NA
PI533852	white	India	90	95	60	3	35	NA	NA	82	19	NA
PI601816	white	Mali	7	30	20	4	35	NA	NA	19	20	NA
PI656024	white	NA	3	70	7	4	35	NA	NA	27	20	NA
PI656092	red	NA	15	75	7	4	35	NA	NA	32	20	NA
PI656022	white	United States, Texas	12	30	25	5	35	NA	NA	22	20	NA
PI659694	white	India, Andhra Pradesh	75	25	75	5	35	NA	NA	58	20	NA
PI642793	red	United States, Texas	10	1	25	6	35	NA	NA	12	21	NA
PI534099	white	Japan	30	10	15	1	40	NA	NA	18	21	NA
PI655978	yellow	United States, Texas	10	1	50	1	40	NA	NA	20	21	NA
PI656010	yellow	United States, Texas	15	25	35	1	40	NA	NA	25	21	NA
PI655991	red	United States, Texas	10	50	20	1	40	NA	NA	27	21	NA
PI655992	white	United States, Texas	15	60	20	1	40	NA	NA	32	21	NA
PI655988	white	United States, Texas	10	75	20	1	40	NA	NA	35	21	NA
PI656016	white	United States, Kansas	15	75	25	1	40	NA	NA	38	21	NA
PI534128	brown	NA	25	65	35	1	40	NA	NA	42	21	NA
PI576394	white	Sudan	15	70	45	1	40	NA	NA	43	21	NA
PI629059	white	United States, Texas	20	2	30	2	40	NA	NA	17	21	NA
PI542718	red	China, Shaanxi	2	65	2	2	40	NA	NA	23	21	NA
PI533979	white	South Africa	10	90	35	2	40	NA	NA	45	21	NA
PI533921	red	Ethiopia	80	95	15	2	40	NA	NA	63	21	NA
PI656039	white	NA	5	.	15	8	35	NA	NA	10	22	NA
PI656034	white	NA	10	20	15	3	40	NA	NA	15	22	NA
PI656031	white	NA	10	70	15	8	35	NA	NA	32	22	NA

Sorghum Accession	Grain Color	Country	HM% 2014	HM% 2015	HM% 2016	Anth % 2014	Anth % 2016	DON (ppm), 2014	DON (ppm), 2015	Mean HM%	Mean Anth %	Mean DON (ppm)
PI656050	white	NA	10	60	30	8	35	NA	NA	33	22	NA
PI629034	yellow	United States, Texas	15	45	50	3	40	NA	NA	37	22	NA
PI656099	white	NA	15	55	40	8	35	NA	NA	37	22	NA
PI651492	white	United States, Texas	1	10	.	5	40	NA	NA	6	23	NA
PI655999	brown	United States, Texas	2	35	1	5	40	NA	NA	13	23	NA
PI656051	white	Niger	7	35	15	10	35	NA	NA	19	23	NA
PI655996	yellow	United States, Texas	10	50	40	0	45	NA	NA	33	23	NA
PI598069	brown	United States, Texas	3	1	2	1	45	NA	NA	2	23	NA
PI533955	red	South Africa	5	3	10	1	45	NA	NA	6	23	NA
PI533998	brown	NA	15	15	5	1	45	NA	NA	12	23	NA
PI656009	white	United States, Texas	20	30	25	1	45	NA	NA	25	23	NA
PI564163	white	United States	15	50	20	1	45	NA	NA	28	23	NA
PI655974	white	United States, Texas	20	80	25	1	45	NA	NA	42	23	NA
PI595699	white	United States, Texas	40	55	75	1	45	NA	NA	57	23	NA
PI656107	brown	NA	5	15	25	2	45	NA	NA	15	24	NA
PI659696	red	NA	7	5	20	3	45	NA	NA	11	24	NA
PI656043	white	NA	5	30	.	3	45	NA	NA	18	24	NA
PI656008	white	El Salvador	5	80	7	8	40	NA	NA	31	24	NA
PI656047	white	India	7	65	20	8	40	NA	NA	31	24	NA
PI656085	yellow	NA	30	85	15	8	40	NA	NA	43	24	NA
PI656064	white	NA	1	20	35	4	45	NA	NA	19	25	NA
PI533843	white	India	10	90	25	4	45	NA	NA	42	25	NA
PI656094	white	NA	15	100	30	4	45	NA	NA	48	25	NA
PI656005	red	United States, Kansas	7	40	5	10	40	NA	NA	17	25	NA
PI576333	white	United States	10	1	10	1	50	NA	NA	7	26	NA
PI597949	white	United States, Texas	5	20	25	1	50	NA	NA	17	26	NA

Sorghum Accession	Grain Color	Country	HM% 2014	HM% 2015	HM% 2016	Anth % 2014	Anth % 2016	DON (ppm), 2014	DON (ppm), 2015	Mean HM%	Mean Anth %	Mean DON (ppm)
PI564165	red	United States, Texas	20	60	50	1	50	NA	NA	43	26	NA
PI576373	white	Japan	20	75	35	1	50	NA	NA	43	26	NA
PI597973	white	United States, Texas	40	85	50	1	50	NA	NA	58	26	NA
PI534148	brown	Ethiopia	60	85	75	1	50	NA	NA	73	26	NA
PI534137	white	Sudan	15	.	40	2	50	NA	NA	28	26	NA
PI629040	red	United States, Texas	10	80	20	2	50	NA	NA	37	26	NA
PI656041	yellow	United States	30	75	30	2	50	NA	NA	45	26	NA
PI656021	red	NA	40	.	60	2	50	NA	NA	50	26	NA
PI656087	white	NA	30	80	50	7	45	NA	NA	53	26	NA
PI656033	red	United States	80	85	35	2	50	NA	NA	67	26	NA
PI656049	white	Botswana	10	.	20	3	50	NA	NA	15	27	NA
PI656088	red	NA	90	90	60	3	50	NA	NA	80	27	NA
PI656048	white	Mali	20	40	20	4	50	NA	NA	27	27	NA
PI656076	white	NA	20	60	30	4	50	NA	NA	37	27	NA
PI533957	brown	Unknown	2	20	2	1	55	NA	NA	8	28	NA
PI655998	yellow	United States, Nebraska	30	20	50	1	55	NA	NA	33	28	NA
PI576401	white	India	60	75	70	1	55	NA	NA	68	28	NA
PI564164	red	United States, Texas	30	60	50	2	55	NA	NA	47	29	NA
PI656058	yellow	United States, Indiana	15	65	20	10	50	NA	NA	33	30	NA
PI576437	white	Brazil	10	60	20	2	60	NA	NA	30	31	NA
PI576435	white	Uganda	10	70	20	3	60	NA	NA	33	32	NA
PI656028	red	Botswana	2	15	15	5	60	NA	NA	11	33	NA

V. List of sorghum anthracnose (*Colletotrichum sublineola*) isolates.

Isolate	Location	Source	Year
CsFL01-15-403.1	Suffolk, VA	Leaf	2015
CsFL01-15-411.1	Suffolk, VA	Leaf	2015
CsFL01-15-413.1	Suffolk, VA	Leaf	2015
CsFL01-15-417.1	Suffolk, VA	Leaf	2015
CsFL01-15-420.1	Suffolk, VA	Leaf	2015
CsFL01-15-407.3	Suffolk, VA	Leaf	2015
CsFL01-16-101.1	Suffolk, VA	Leaf	2016
CsFL01-16-102.1	Suffolk, VA	Leaf	2016
CsFL01-16-104.2	Suffolk, VA	Leaf	2016
CsFL01-16-115.1	Suffolk, VA	Leaf	2016
CsFL01-16-119.1	Suffolk, VA	Leaf	2016
CsFL01-16-123.1	Suffolk, VA	Leaf	2016
CsFL01-16-124.1	Suffolk, VA	Leaf	2016
CsFL01-17-208.1	Suffolk, VA	Leaf	2017
CsFL01-17-209.1	Suffolk, VA	Leaf	2017
CsFL01-17-210.1	Suffolk, VA	Leaf	2017
CsFL01-17-217.1	Suffolk, VA	Leaf	2017
CsFL02-15-201.1	Rocky Mount, NC	Leaf	2015
CsFL02-15-205.1	Rocky Mount, NC	Leaf	2015
CsFL02-15-207.1	Rocky Mount, NC	Leaf	2015
CsFL02-15-213.1	Rocky Mount, NC	Leaf	2015
CsFL02-15-215.1	Rocky Mount, NC	Leaf	2015
CsFL02-15-222.1	Rocky Mount, NC	Leaf	2015
CsFL02-16-203.1	Rocky Mount, NC	Leaf	2016
CsFL02-16-207.1	Rocky Mount, NC	Leaf	2016
CsFL02-16-213.1	Rocky Mount, NC	Leaf	2016
CsFL02-16-215.1	Rocky Mount, NC	Leaf	2016
CsFL02-16-213.2	Rocky Mount, NC	Leaf	2016
CsFL02-16-215.2	Rocky Mount, NC	Leaf	2016
CsFL03-16-201.1	Lewiston-Woodville, NC	Leaf	2016
CsFL03-16-205.1	Lewiston-Woodville, NC	Leaf	2016
CsFL03-16-207.1	Lewiston-Woodville, NC	Leaf	2016
CsFL03-16-213.1	Lewiston-Woodville, NC	Leaf	2016
CsFL03-16-215.1	Lewiston-Woodville, NC	Leaf	2016
CsFL03-16-222.1	Lewiston-Woodville, NC	Leaf	2016
CsFL04-16-303.1	Kinston, NC	Leaf	2016
CsFL04-16-305.1	Kinston, NC	Leaf	2016
CsFL04-16-307.1	Kinston, NC	Leaf	2016
CsFL04-16-311.1	Kinston, NC	Leaf	2016

Isolate	Location	Source	Year
CsFL04-16-312.1	Kinston, NC	Leaf	2016
CsFL04-16-313.1	Kinston, NC	Leaf	2016
CsFL04-16-315.1	Kinston, NC	Leaf	2016
CsFL04-16-316.1	Kinston, NC	Leaf	2016
CsFL04-16-319.1	Kinston, NC	Leaf	2016
CsFL04-16-320.1	Kinston, NC	Leaf	2016
CsFL04-16-321.1	Kinston, NC	Leaf	2016
CsFL04-17-411.1	Kinston, NC	Leaf	2017
CsFL04-17-412.1	Kinston, NC	Leaf	2017
CsFL04-17-415.1	Kinston, NC	Leaf	2017
CsFL04-17-422.1	Kinston, NC	Leaf	2017
SFFF16-SR.1	Suffolk, VA	Stalk, rot	2016
SFFF16-SR.2	Suffolk, VA	Stalk, rot	2016
SFFF16-SR.3	Suffolk, VA	Stalk, rot	2016
SFFF16-SR.4	Suffolk, VA	Stalk, rot	2016
KSDC16-210.4S	Kinston, NC	Grain	2016
KSDC16-410.2S	Kinston, NC	Grain	2016
KSDC16-503.2S	Kinston, NC	Grain	2016
KSDC16-702.2S	Kinston, NC	Grain	2016
KSDC16-702.5S	Kinston, NC	Grain	2016
KSDC16-210.4S	Kinston, NC	Grain	2016
KSFS16-119.2S	Kinston, NC	Grain	2016
KSFS16-119.4S	Kinston, NC	Grain	2016
LWDC16-210.2S	Lewiston-Woodville, NC	Grain	2016
LWDC16-210.3S	Lewiston-Woodville, NC	Grain	2016
LWDC16-410.1S	Lewiston-Woodville, NC	Grain	2016
LWDC16-410.2S	Lewiston-Woodville, NC	Grain	2016
LWDC16-410.4S	Lewiston-Woodville, NC	Grain	2016
IOWDC16-105.2S	Isle of Wight, VA	Grain	2016
IOWFS15-106.1S	Isle of Wight, VA	Grain	2015
IOWFS15-106.5S	Isle of Wight, VA	Grain	2015
SFDC15-108.3S	Suffolk, VA	Grain	2015
SFFS16-112.2S	Suffolk, VA	Grain	2016
SFFS16-112.3S	Suffolk, VA	Grain	2016
SFFS16-119.1S	Suffolk, VA	Grain	2016

VI. List of *Fusarium* isolates collected from sorghum grain.

Fusarium Isolate ID	Location	Year	Crop
SFDC15-108.1	Suffolk, VA	2015	Double crop
SFDC15-108.2	Suffolk, VA	2015	Double crop
SFDC15-108.3	Suffolk, VA	2015	Double crop
SFDC15-108.4	Suffolk, VA	2015	Double crop
SFDC15-108.5	Suffolk, VA	2015	Double crop
SFDC15-108.6	Suffolk, VA	2015	Double crop
SFDC15-108.7	Suffolk, VA	2015	Double crop
SFDC15-108.8	Suffolk, VA	2015	Double crop
SFDC15-108.9	Suffolk, VA	2015	Double crop
SFDC15-108.10	Suffolk, VA	2015	Double crop
IOWDC15-107.1	Isle of Wight, VA	2015	Double crop
IOWDC15-107.2	Isle of Wight, VA	2015	Double crop
IOWDC15-107.3	Isle of Wight, VA	2015	Double crop
IOWDC15-107.4	Isle of Wight, VA	2015	Double crop
IOWDC15-107.5	Isle of Wight, VA	2015	Double crop
IOWDC15-107.6	Isle of Wight, VA	2015	Double crop
IOWDC15-107.7	Isle of Wight, VA	2015	Double crop
IOWDC15-107.8	Isle of Wight, VA	2015	Double crop
IOWDC15-107.9	Isle of Wight, VA	2015	Double crop
LWDC16-109.1	Lewiston-Woodville, NC	2016	Double crop
LWDC16-109.10	Lewiston-Woodville, NC	2016	Double crop
LWDC16-109.11	Lewiston-Woodville, NC	2016	Double crop
LWDC16-109.12	Lewiston-Woodville, NC	2016	Double crop
LWDC16-109.13	Lewiston-Woodville, NC	2016	Double crop
LWDC16-109.14	Lewiston-Woodville, NC	2016	Double crop
LWDC16-109.15	Lewiston-Woodville, NC	2016	Double crop
LWDC16-109.16	Lewiston-Woodville, NC	2016	Double crop
LWDC16-109.17	Lewiston-Woodville, NC	2016	Double crop
LWDC16-109.18	Lewiston-Woodville, NC	2016	Double crop
LWDC16-109.2	Lewiston-Woodville, NC	2016	Double crop
LWDC16-109.3.1	Lewiston-Woodville, NC	2016	Double crop
LWDC16-109.3.2	Lewiston-Woodville, NC	2016	Double crop
LWDC16-109.4	Lewiston-Woodville, NC	2016	Double crop
LWDC16-109.5	Lewiston-Woodville, NC	2016	Double crop
LWDC16-109.6	Lewiston-Woodville, NC	2016	Double crop
LWDC16-109.7	Lewiston-Woodville, NC	2016	Double crop
LWDC16-109.8	Lewiston-Woodville, NC	2016	Double crop
LWDC16-104.2	Lewiston-Woodville, NC	2016	Double crop
LWDC16-503.1	Lewiston-Woodville, NC	2016	Double crop

Fusarium Isolate ID	Location	Year	Crop
SFDC16-101.1	Suffolk, VA	2016	Double crop
SFDC16-101.2	Suffolk, VA	2016	Double crop
SFDC16-101.3	Suffolk, VA	2016	Double crop
SFDC16-101.4	Suffolk, VA	2016	Double crop
SFDC16-101.5	Suffolk, VA	2016	Double crop
SFDC16-101.6	Suffolk, VA	2016	Double crop
SFDC16-101.7	Suffolk, VA	2016	Double crop
SFDC16-101.8	Suffolk, VA	2016	Double crop
SFDC16-101.9	Suffolk, VA	2016	Double crop
SFDC16-101.10	Suffolk, VA	2016	Double crop
SFDC16-101.11	Suffolk, VA	2016	Double crop
SFDC16-101.12	Suffolk, VA	2016	Double crop
SFDC16-307.5	Suffolk, VA	2016	Double crop
RMDC16-109.1.1	Rocky Mount, NC	2016	Double crop
RMDC16-109.1.2	Rocky Mount, NC	2016	Double crop
RMDC16-109.10.1	Rocky Mount, NC	2016	Double crop
RMDC16-109.10.2	Rocky Mount, NC	2016	Double crop
RMDC16-109.11	Rocky Mount, NC	2016	Double crop
RMDC16-109.2.1	Rocky Mount, NC	2016	Double crop
RMDC16-109.2.2	Rocky Mount, NC	2016	Double crop
RMDC16-109.5	Rocky Mount, NC	2016	Double crop
RMDC16-109.6	Rocky Mount, NC	2016	Double crop
RMDC16-109.7	Rocky Mount, NC	2016	Double crop
RMDC16-109.8	Rocky Mount, NC	2016	Double crop
RMDC16-109.9	Rocky Mount, NC	2016	Double crop
IOWDC16-104.1	Isle of Wight, VA	2016	Double crop
IOWDC16-104.2	Isle of Wight, VA	2016	Double crop
IOWDC16-104.3	Isle of Wight, VA	2016	Double crop
IOWDC16-104.4	Isle of Wight, VA	2016	Double crop
IOWDC16-104.5	Isle of Wight, VA	2016	Double crop
IOWDC16-104.6	Isle of Wight, VA	2016	Double crop
IOWDC16-104.7	Isle of Wight, VA	2016	Double crop
IOWDC16-104.8	Isle of Wight, VA	2016	Double crop
IOWDC16-104.9.1	Isle of Wight, VA	2016	Double crop
IOWDC16-104.9.2	Isle of Wight, VA	2016	Double crop
SFFS15-109.1	Suffolk, VA	2015	Full season
IOWFS15-106.1	Isle of Wight, VA	2015	Full season
IOWFS15-106.2	Isle of Wight, VA	2015	Full season
IOWFS15-106.3	Isle of Wight, VA	2015	Full season
IOWFS15-106.4	Isle of Wight, VA	2015	Full season

Fusarium Isolate ID	Location	Year	Crop
IOWFS15-106.5	Isle of Wight, VA	2015	Full season
IOWFS15-106.6	Isle of Wight, VA	2015	Full season
LWFS16-101.1	Lewiston-Woodville, NC	2016	Full season
LWFS16-101.10	Lewiston-Woodville, NC	2016	Full season
LWFS16-101.15	Lewiston-Woodville, NC	2016	Full season
LWFS16-101.2.1	Lewiston-Woodville, NC	2016	Full season
LWFS16-101.2.2	Lewiston-Woodville, NC	2016	Full season
LWFS16-101.9	Lewiston-Woodville, NC	2016	Full season
KSFS16-119.1	Kinston, NC	2016	Full season
KSFS16-119.2	Kinston, NC	2016	Full season
KSFS16-119.3	Kinston, NC	2016	Full season
KSFS16-119.4	Kinston, NC	2016	Full season
KSFS16-119.5	Kinston, NC	2016	Full season
KSFS16-119.6	Kinston, NC	2016	Full season
KSFS16-119.7	Kinston, NC	2016	Full season
KSFS16-119.8	Kinston, NC	2016	Full season
KSFS16-101.1	Kinston, NC	2016	Full season
KSFS16-101.2	Kinston, NC	2016	Full season
KSFS16-101.3	Kinston, NC	2016	Full season
SFFS16-112.1	Suffolk, VA	2016	Full season
SFFS16-112.2	Suffolk, VA	2016	Full season
SFFS16-112.3	Suffolk, VA	2016	Full season
SFFS16-112.4	Suffolk, VA	2016	Full season
SFFS16-112.5	Suffolk, VA	2016	Full season
SFFS16-112.6	Suffolk, VA	2016	Full season
SFFS16-112.7	Suffolk, VA	2016	Full season
SFFS16-112.8	Suffolk, VA	2016	Full season
RMFS16-116.1	Rocky Mount, NC	2016	Full season
RMFS16-116.2	Rocky Mount, NC	2016	Full season
RMFS16-116.3	Rocky Mount, NC	2016	Full season
RMFS16-116.4	Rocky Mount, NC	2016	Full season
RMFS16-116.5	Rocky Mount, NC	2016	Full season
RMFS16-116.6	Rocky Mount, NC	2016	Full season
RMFS16-116.7	Rocky Mount, NC	2016	Full season
RMFS16-116.8	Rocky Mount, NC	2016	Full season
RMFS16-116.9	Rocky Mount, NC	2016	Full season
RMFS16-116.10	Rocky Mount, NC	2016	Full season
RMFS16-116.11	Rocky Mount, NC	2016	Full season
RMFS16-116.12	Rocky Mount, NC	2016	Full season
RMFS16-116.13	Rocky Mount, NC	2016	Full season

Fusarium Isolate ID	Location	Year	Crop
RMFS16-116.14	Rocky Mount, NC	2016	Full season
IOWFS16-110.1	Isle of Wight, VA	2016	Full season
IOWFS16-110.2	Isle of Wight, VA	2016	Full season
IOWFS16-110.3	Isle of Wight, VA	2016	Full season
IOWFS16-110.4	Isle of Wight, VA	2016	Full season
IOWFS16-110.5	Isle of Wight, VA	2016	Full season
IOWFS16-110.6	Isle of Wight, VA	2016	Full season
IOWFS16-110.7	Isle of Wight, VA	2016	Full season
IOWFS16-110.8	Isle of Wight, VA	2016	Full season
IOWFS16-110.9	Isle of Wight, VA	2016	Full season