

ABSTRACT

BEACORN, JEAN AILEEN. Characterization and Management of Fusarium Head Blight in North Carolina Sorghum Fields. (Under the Direction of Dr. Lindsey D. Thiessen).

Sorghum (*Sorghum bicolor*) in North Carolina is primarily grown for food or animal feed. Growing sorghum in this region is challenged by environmental conditions conducive for infection and disease development by mycotoxigenic fungi, specifically for fungi associated with the Fusarium head blight (FHB) disease complex. Diversity, pathogenicity, and fungicide sensitivity of *Fusarium* species affecting North Carolina sorghum fields is not well characterized. Additionally, few fungicides are available for management of this disease in sorghum, and timing of fungicides is not well understood. In this project, *Fusarium* species that cause FHB were characterized using morphological and molecular techniques and examined for diversity, pathogenicity, and fungicide sensitivity of representative isolates from 10 North Carolina sorghum fields. *Fusarium graminearum*, *F. verticillioides*, *F. fujikuroi*, *F. proliferatum*, and members of the *F. incarnatum-equiseti* species complex (FIESC) were identified from FHB-infected sorghum. Pathogenicity assays showed no significant differences of isolate pathogenicity in relation to the effect of isolate type or the effect of variety; however, variability among isolate aggressiveness may be attributed to isolate diversity. Fungicide sensitivity assays revealed variability amongst isolate sensitivity to tebuconazole, prothioconazole, fluopyram, and trifloxystrobin. Isolates were less sensitive to trifloxystrobin. Efficacy of four fungicides (prothioconazole [Proline], prothioconazole + trifloxystrobin [Delaro], fluopyram + trifloxystrobin [Luna Sensation], and prothioconazole + tebuconazole [Prosaro]) at three physiological application timings (heading, anthesis, and soft-dough) were evaluated. Fungicide-timing experiments were conducted across three site-years and were examined for disease severity, AUDPC, and mycotoxin accumulation levels of deoxynivalenol (DON), fumonisin

(FUM), and zearalenone (ZEA) at harvest. Treatments in 2018 were not significantly different for final severity, AUDPC, DON, FUM, and ZEA ($P > 0.05$). Significant differences in final disease severity and AUDPC were detected for treatments in 2019 between locations ($P < 0.05$), but values were higher than the non-treated control. Significant differences among treatments were detected for DON between locations ($P < 0.05$). Lake Wheeler FUM samples were below the limit of detection (LOD) of the ELISA kits and were excluded from analysis. Rocky Mount treatments for FUM had no significant differences ($P > 0.05$). Field site data for ZEA was combined for statistical analysis, and significant differences were detected between Proline at anthesis and Prosaro at soft-dough ($P < 0.05$). These studies characterized *Fusarium* isolates associated with FHB in sorghum and tested potential chemical management strategies for FHB control.

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Characterization and Management of Fusarium Head Blight in North Carolina Sorghum Fields.

by
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DEDICATION

To my parents, Thomas and Frankie Beacorn.

BIOGRAPHY

Jean Beacorn was born January 28, 1995 in Raleigh, NC and grew up in Watkinsville, GA. She grew a passion for science in 7th grade. Through the eyes of determined and enthusiastic mentors, she nurtured her passion through grade school. Upon acceptance to the University of Georgia, she was catapulted into the world of agriculture and plant pathology. There she completed her bachelor's degree in Applied Biotechnology, worked in a mycology lab, and completed plant pathology internships with the USDA-ARS in Charleston, SC and Cornell University – all to continue feeding this passion that had grown into a full-blown fascination with microorganisms pathogenic to plants. These experiences led her to pursue a Master of Science degree in plant pathology at North Carolina State University under Dr. Lindsey Thiessen. Her research focuses on characterizing *Fusarium* spp. causing Fusarium head blight and evaluating fungicides for efficacy to this disease in North Carolina sorghum production.

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CHAPTER I. Literature Review

Sorghum Production

Sorghum, *Sorghum bicolor*, is a monocotyledonous plant in the family Poaceae (Kvas et al. 2009). Sorghum is a major component of poultry and livestock feed and human food (Shetty and Bhat 1997; Glenn 2007) and serves as an alternative bioenergy source (Zegada-Lizarazu and Monti 2012a; Erickson et al. 2012). The United States is a leading producer of the world's sorghum, having produced 1,219,919 kg/ha of sorghum in 2016 and 924,176 kg/ha of sorghum in 2017 (FAOSTAT 2017). In 2018, North Carolina growers had mean yields of 3,766 kg/ha (60 bushels/acre) of grain sorghum and produced 12,192,576 kg (480,000 bushels). On average, North Carolina yielded 22,401 kg/ha (22.4 metric tons/ha) of silage sorghum and produced 70,000,000,000 kg (70,000,000 metric tons) (USDA NASS 2018). Sorghum is grown in several counties in the Central Coastal, Northern Coastal, and the Southern Piedmont regions of North Carolina (USDA NASS 2008). Growing region adaptability supports a high production potential for sorghum, which may be related to the physiological potential of the plant.

The physiological form of sorghum may influence its high yield potential for North Carolina producers, such as continually producing tillers, arid and wet climate tolerance, producing a genetically fixed number of leaves, and insensitivity to photoperiod (Arkin et al. 1976). Sorghum originated in tropical regions and is more heat and drought tolerant than C3 crops, like wheat, that originated in more temperate regions (Machado and Paulsen 2001). Sorghum uses C4 photosynthetic mechanisms that promote more efficient CO₂, solar radiation, water, and nitrogen utilization compared to C3 crops (Prasad et al. 2006). Unlike the C4 photosynthetic pathway, the C3 photosynthetic pathway fixes atmospheric carbon dioxide forming the 3-carbon intermediate, 3-phosphoglycerate, instead of a 4-carbon intermediate. Heat

and drought stresses affect the ability of plants to make osmotic adjustments by increasing the rate of evapotranspiration and by interfering in biochemical processes that produce and utilize solutes, namely glucose, involved in osmotic adjustments (Machado and Paulsen 2001). C4 photosynthetic adaptation allows for more efficient carbon utilization, especially during sub-optimal growing conditions when leaf water potential is low. This adaptation is especially important for maintaining sorghum nutrient uptake and utilization during physiological and grain developmental stages.

Sorghum has identifiable growth stages important for production and for marking disease occurrence. Growth stages zero through one occur right after planting and include the beginning of imbibition, radical emergence from the caryopsis, coleoptile emergence from caryopsis, leaf at coleoptile tip, and emergence. In stages two, three, and four, respectively, first leaf, third leaf sheath, and fifth leaf sheath are observed. Tillering occurs at the fifth stage, and the sixth stage constitutes stem elongation. Flag leaf is seen at the seventh stage. The eighth stage is referred to as booting, and by stage nine the panicle can be seen. Stage ten encompasses anthesis (Vanderlip and Reeves 1972). Depending on environmental conditions, panicle size, and sorghum variety, anthesis occurs for approximately 6-9 days (Frederiksen and Odvody 2000). From anthesis until grain maturity, sorghum panicles are susceptible to grain mold and head blight pathogens. At stage eleven, grains mature. The sequence for grain maturation begins with grains at milk stage, followed by the soft dough stage, hard dough stage, and black layer formation, resulting in physiologically mature grains (Frederiksen and Odvody 2000). Black layer formation occurs 25-55 days after flowering, depending on the hybrid and environment, and seeds are typically harvested 10-20 days after black layer formation (Frederiksen and Odvody 2000). Growth stages of grain maturation constitute phases during which the plant is susceptible to invasion and

colonization by plant pathogens, especially during warm and humid environmental conditions conducive for disease development.

Despite its high yield potential, maximal sorghum production has several limitations, especially diseases. Grain molds and head blight pathogens, including *Aspergillus*, *Penicillium*, and *Fusarium*, reduce grain yield and quality as well as produce mycotoxins that can cause illness and death in livestock and humans that consume contaminated grain. *Fusarium* spp. are of particular concern to sorghum production in the Southeastern United States because the hot, humid environment, a characteristic of the region, favors disease development. In cereal crops, Fusarium head blight (FHB) is caused by species from *F. fujikuroi* species complex (FFSC), *F. graminearum* species complex (FGSC), *F. oxysporum* species complex (FOSC), *F. solani* species complex (FSSC), and *F. incarnatum-equiseti* species complex (FIESC), many of which are capable of producing mycotoxins (Little and Magill 2009; Walter et al. 2010). Outside of these species complexes, *F. avenaceum* and *F. poae* can also contribute to head blight infections (Moretti 2017). In the 1990's, FHB epidemics cost wheat and barley producers in the United States approximately \$3 billion from grain rejection due to deoxynivalenol contamination (Moretti 2017). Additionally, fumonisin contamination in maize costs the swine industry around \$18 million annually (Moretti 2017). Furthermore, approximately 25% of global feed and food output is contaminated by these mycotoxins (Moretti 2017). Production and accumulation of mycotoxins in sorghum used for food and feed is harmful to people and livestock upon consumption due to potential adverse health effects (Moretti 2017; Desjardins and Proctor 2007). Despite regulatory limits to minimize mycotoxin incidence in marketable grain, there are few FHB management strategies that result in mycotoxin-free grain. Current sorghum varieties do not have known FHB resistance. Field experiments in North Carolina assessed sorghum variety

susceptibility to FHB for Funk's 'G-522DR', DeKalb 'DK-61', Pioneer 8311, Northrup King '2799', Coker 7681 BR, Warner 744DR, NK 2660, Pioneer 8333, Pioneer 'B815', NK 'Savannah 5', Pioneer '8515' (Hagler et al. 1986). Mycotoxin analysis of these varieties revealed zearalenone at high prevalence in sorghum hybrids in North Carolina (Hagler et al. 1986). The limited number of varieties available to combat FHB and mycotoxin production is a significant hurdle for producers to reduce mycotoxin-contaminated grain.

Causal Agents of Fusarium Head Blight

Fusarium spp. are ascomycete fungi capable of causing disease on several hosts, including cereal crops, vegetables, fruits and trees (Nguyen et al. 2017). Many of these crops are produced in rotation, which contributes to inoculum accumulation across subsequent growing seasons. *Fusarium* spp. colonize the plant as a biotroph but will switch to a necrotrophic lifestyle after successful colonization and invasion (Dweba et al. 2017). *Fusarium* spp. are associated with both symptomatic and asymptomatic crop plants, complicating disease detection and management (Leslie et al. 1990). Plant symptoms associated with FHB-infected sorghum include premature bleaching and necrosis of panicles, reddening and necrosis of peduncle, rachis, rachis branches, pith tissues, and drooping of rachis branches when harvest is postponed (Figure 1.2) (Walter et al. 2010; Castor and Frederiksen 1980). Symptoms of FHB may be accompanied by white or pink fluffy mycelia (grain mold) on infected grains and panicles (Walter et al. 2010), but signs may not be externally visible on all infected grains. It is important to distinguish between grain mold and FHB because disease manifestation ultimately determines which management strategies are most effective. Grain mold refers to molding of sorghum panicles and grains during wet weather or after a delayed harvest. Head blight is an infection that occurs at

flowering but does not manifest itself as a visible mold (Hagler et al. 1986; Williams and Rao 1981). Lodging is an additional problem, occasionally indicative of high inoculum pressure, where the base of the panicle or stalk breaks due to degradation of pith tissue and senescence of stalk pith cells (Tesso et al. 2004; Bramel-Cox and Claflin 1989). Stem lodging may also be induced by environmental factors such as excess precipitation and heat stress (Bramel-Cox and Claflin 1989). Genetics can also contribute to lodging; for example, bioenergy sorghum grows taller and has a larger biomass than grain sorghum, which may lead to a higher incidence of stem lodging (Gomez et al. 2017).

FHB is polycyclic and has both sexual and asexual life cycles (Dweba et al. 2017). The sexual cycle is primarily important for management of FHB because infection by *Fusarium* occurs during grain anthesis through later stages of grain development by ascospores (Prandini et al. 2009; McMullen et al. 2012; Osborne and Stein 2007). Primary inoculum (ascospores) are windblown or water-splashed from perithecia to susceptible panicles (McMullen et al. 2012; L. Shah et al. 2018a; Leplat et al. 2013). Perithecia are formed from overwintering mycelium and chlamydospores in crop residues, primarily stems/stalks and grains (Osborne and Stein 2007). The florets and developing caryopses are colonized before black layer formation (Little and Magill 2009). Successful grain infection and colonization depend on climatic factors, including temperature and relative humidity or moisture (Wagacha and Muthomi 2007; Osborne and Stein 2007; Bandyopadhyay et al. 2000; L. Shah et al. 2018a; Cowger and Sutton 2005). Anthesis is the critical infection stage, especially in warm and humid environments when *Fusarium* spores release, germinate, invade, and then colonize susceptible panicles (Wegulo et al. 2013). The asexual lifecycle is defined by production of macroconidia and microconidia after grain colonization or from alternative grass hosts (Leslie 2014). Mycelia produce conidiophores that

produce microconidia, and sporodochia that produce macroconidia; chlamydospores are formed on or within hyphae and macroconidia and function as an overwintering structure (Dweba et al. 2017; L. Shah et al. 2018a). Macroconidia and microconidia are windblown or water splashed and are responsible for initiating secondary infection sites that can further contribute to mycotoxin contamination of grain (Dweba et al. 2017; L. Shah et al. 2018a). In small grains, FHB infection at anthesis is monocyclic (Wegulo et al. 2015); however, sorghum varieties may produce additional tillers after the primary tiller on a single plant, and plants may not develop uniformly across a field. Development at different time-points during the growing season allows secondary infections on subsequent tillers of the same plant, indicative of a polycyclic disease.

Fusarium species from several FHB species complexes can cause disease in sorghum, most commonly FFSC and FGSC (Moretti 2017). Species from FOOSC, FSSC, and FIESC also infect sorghum (Moretti 2017; Funnell-Harris and Pedersen 2011). This is important because within each species complex, individual species may require different environmental and biological conditions for optimal growth and development, posing problems for management options.

Differences in life cycle may influence the degree to which management practices are effective. The optimum temperature and humidity required for mycelial growth, sporulation (conidia), conidia germination, infection frequency by conidia, perithecial maturation, ascospore release, ascospore germination, infection by ascospores, and seedling inhibition may differ for *F. graminearum*, *F. culmorum*, *F. poae*, and *F. avenaceum* (Osborne and Stein 2007; Leplat et al. 2013). For example, *F. graminearum* and *F. culmorum* optimally produce conidia at 32°C while *F. avenaceum* optimally produces conidia at 28°C (Osborne and Stein 2007). Additionally, greatest infection levels of grains by *F. graminearum* and *F. avenaceum* occur at 28 – 29°C,

while infection levels for *F. culmorum* are highest at 26.5°C (L. Shah et al. 2018a). Composition of the fungal population and environmental variability may explain why certain species are found at different time points during the growing season and why one species is more prevalent during a given season than another. Literature for FHB epidemics in North Carolina sorghum fields has not been extensively monitored or updated for over 30 years (Leslie et al. 1990; Hagler et al. 1986). An experiment conducted in 1986 detected *F. equiseti*, *F. moniliforme*, *F. solani*, and *F. chlamydosporum* from sorghum tissue in North Carolina (Leslie et al. 1990).

Variability in mycotoxin production may also contribute to species complex variation and reduced control options. FGSC produces some trichothecenes, but toxin profiles differ among and within phylogenetic species (Moretti 2017). DON levels are reportedly higher in fields that receive strobilurin-based fungicide applications, thus restricting the number of efficacious chemical control options available for FHB management (Vogelgsang et al. 2019). Additionally, fumonisin production is not unique to the *Fusarium* genus, as some *Aspergillus* species are also capable of producing this toxin (Moretti 2017). Anthesis occurring during warm and moist conditions poses implications for mycotoxin prevention and disease management. Mycotoxin production occurs during caryopsis formation or grain storage when high humidity and warm temperatures occur (Little et al. 2012). Fungi produce mycotoxins as a result of interactions between genes and external stimuli or stressors, such as host responses, temperature and humidity fluctuations, and environmental changes. For example, in *F. graminearum*, an *in vitro* assay was performed identifying the production of hydrogen peroxide prior to deoxynivalenol (DON) production and concluding that DON production results from an oxidative stress response (Audenaert et al. 2010). DON is also a virulence factor that aids FHB pathogens in their infection and colonization (Dweba et al. 2017).

Fusarium spp. Mycotoxins

Emphasis on mycotoxin detection and management for sorghum has been limited since sorghum is not usually grown for human consumption in the United States. In a North Carolina sorghum field study conducted from 1981 through 1985, both zearalenone and deoxynivalenol were detected at harvest (Hagler et al. 1986). *Fusarium* species in the FHB complex can produce mycotoxins including trichothecenes (deoxynivalenol [DON], nivalenol [NIV], diacetoxyscirpenol [DAS], T-2 toxin, and HT-2 toxin), fumonisins (FUM), moniliformins, zearalenone (ZEA or ZON), fusaric acid, fusarin (fusarin C), beauvericin and enniatins, and fusaproliferin (Funnell-Harris and Pedersen 2011; Nguyen et al. 2017; Moretti 2017; Divakara et al. 2014; Glenn 2007; Desjardins and Proctor 2007). Mycotoxins impact United States production and value of grains because of potentially destructive effects, causing \$406 million in losses in barley to DON contamination from 1993 through 1998, \$200 million in losses in wheat to DON contamination from 1993 through 2000, and \$18 million per year in losses in maize for the swine industry (Moretti 2017). The potential for mycotoxin accumulation impacts sorghum globally by contamination with trichothecenes, namely DON, fumonisin, and zearalenone; a thorough list of *Fusarium* spp. that are known to produce these mycotoxins is shown in Table 1.1 (Hagler et al. 1986; Leslie et al. 2005; Bhat R. et al. 2000).

There are more than 200 chemically unique trichothecene mycotoxins that have been assigned to four groups (A, B, C, and D) largely based on substitutions at the C-8 position or on other positions around the core structure (Moretti 2017). Type A trichothecenes contain a hydroxyl, an ester, or an absence of an oxygen substituted at the C-8 position. Type B trichothecenes contain a carbonyl group at the C-8 position. Type C trichothecenes are a lesser group of mycotoxins produced by several fungal genera, and type D trichothecenes are produced

by *Stachybotrys* and other fungal genera considered to be indoor mold hazards (Moretti 2017). *Fusarium* species produce types A and B trichothecenes (Moretti 2017). Monitored examples include diacetoxyscirpenol, nivalenol, T-2 toxin, and deoxynivalenol (DON), but more than 40 naturally occurring trichothecenes have been described (Glenn 2007). The trichothecene biosynthetic gene cluster was discovered in *F. sporotrichoides* and includes genes for terpene cyclase production that play a role in fungal primary and secondary metabolism (Desjardins and Proctor 2007). Trichothecenes induce toxicity in animals and humans by inhibiting ribosomal protein synthases, which interferes with translation and the generation of new proteins essential to cellular function (Desjardins and Proctor 2007). In *F. graminearum*, trichothecene biosynthesis and regulatory genes have been mapped to four unlinked loci (Desjardins and Proctor 2007). This means that trichothecene biosynthesis and regulatory genes can produce mycotoxigenic compounds independently of one another. In the United States, *Fusarium crookwellense*, *F. culmorum*, *F. graminearum*, *F. poae*, *F. pseudograminearum*, and *F. sporotrichoides* produce one or more types of trichothecene mycotoxin (Glenn 2007). However, these species have toxin profiles that are unique to each phylogenetic species. The types of trichothecenes each species produces may be shared with or absent from other *Fusarium* species. DON exhibits both acute and chronic toxicity in livestock (Bertero et al. 2018). United States DON regulatory limits are 1,000 ppb in grain produced for food and 5,000 ppb – 10,000ppb in grain produced for animal feed (Moretti, 2017).

Fumonisin inhibit ceramide synthases, important enzymes in sphingolipid metabolism, resulting in fatal diseases in livestock and induce apoptosis-like programmed cell death in plants (Divakara et al. 2014). There are at least 28 known fumonisin homologues, but the most important group is the B series: B₁ (FB1), B₂ (FB2), and B₃ (FB3) (Bertero et al. 2018). Horses

and pigs are especially sensitive to FB1 toxicity, as the central nervous system (leukoencephalomalacia), liver, and heart are targeted in horses, and pigs may exhibit acute porcine pulmonary edema (PPE) (Rheeder et al. 2002; Nelson et al. 1993). Fumonisin presence slows livestock and agricultural commodity production. United States FUM regulatory limits are 2,000 ppb – 4,000 ppb in grain produced for food and 5,000 ppb – 10,000ppb in grain produced for animal feed (Moretti, 2017). The fumonisin biosynthetic gene cluster was discovered in *F. verticilloides* and includes genes for the production of polyketide synthases, which are enzymes essential to fumonisin production (Desjardins and Proctor 2007). In the United States, *Fusarium* species that produce fumonisins include *F. fujikuroi*, *F. thapsinum*, *F. proliferatum*, and *F. verticillioides*, which can be found on maize and other cereal crops grown for animal and human consumption (Glenn 2007; Moretti 2017).

Zearalenone and its metabolites function as endocrine disruptors exhibiting strong estrogenic activities (Bertero et al. 2018). Zearalenone binds to estrogen receptors and causes hyperoestrogenism syndrome. They are of greater significance to monogastric livestock than to ruminants, although symptoms of zearalenone sensitivity have been observed in young animals whose ruminal systems are not fully developed. It is also reported to be hepatotoxic, hematotoxic, immunotoxic and genotoxic (Bertero et al. 2018). Presently, there are no United States regulatory limits for zearalenone in grain produced for food and livestock feed (Moretti, 2017). *Fusarium graminearum* is a known zearalenone producer on cereal crops which means it could be problematic on sorghum (Wegulo et al. 2013; Bertero et al. 2018). The mycotoxigenic potential of FHB pathogens in sorghum could have negative impacts that resonate beyond the scope of direct crop loss, including international trade and animal welfare impacts.

Fusarium Identification & Characterization

Variation in morphological characteristics of *Fusarium* species requires immense time and mycological expertise making rapid identification difficult (Wagacha and Muthomi 2007; Divakara et al. 2014). Common morphological characteristics of *Fusarium* species include white mycelia, pigment production in certain media, and canoe-shaped macroconidia. Morphological variations namely pigment, spore-type, and mycotoxin production occur within and between species. For example, *F. thapsinum* may produce violet, cream yellow, or cream pigmentation on potato dextrose agar. *Fusarium thapsinum* isolates that do not produce yellow pigmentation are visibly indistinguishable from *F. verticillioides* (Pena et al. 2018). *Fusarium thapsinum* also produces fumonisin in low levels and moniliformin and fusaric acid in higher quantities, whereas *F. verticillioides* produces higher levels of fumonisins and lower levels of moniliformin (Pena et al. 2018). Identification of *Fusarium* to species level is important because the variation within and between species influences the sustainability of control and management options, particularly those focused on cultural and integrated pest management practices. More recently, nucleic acid assays, such as polymerase chain reaction (PCR) have been developed for aiding the identification of *Fusarium* spp. (Divakara et al. 2014). *Fusarium* spp. are identified using the translation elongation factor-1 α (*tef-1 α*) gene marker (Divakara et al. 2014; O'Donnell et al. 1998). The *tef-1 α* gene encodes for a necessary factor of translational protein machinery in *Fusarium* and differs between species; this enables species-specific identification of *Fusarium* (Divakara et al. 2014; O'Donnell et al. 1998). Multiplex assays have been developed to identify mycotoxin-producing strains of *Fusarium* (Divakara et al. 2014; O'Donnell et al. 1998). In wheat, barley, oat, and maize cropping systems, mycotoxigenic *Fusarium* species are genotyped and chemotyped to construct genetic and mycotoxigenic profiles. This has yet to be done for

isolates from sorghum in the southeastern United States and would provide a baseline for future FHB research in sorghum and for management decisions.

Management of FHB in Sorghum

FHB management relies on cultural and chemical practices that target interactions between the pathogen with its environment and host. No singular management technique provides complete FHB control. Primarily, overwintering and primary inoculum is harbored in crop debris and may cause problems for subsequent growing seasons. A non-host crop that does not consistently perpetuate or harbor disease propagules can be used for crop rotations, lowering soil inoculum levels, and thereby potentially reducing disease incidence (Leplat et al. 2013; Pirgozliev et al. 2003; L. Shah et al. 2018a). For example, in wheat following maize, elevated head blight severity and mycotoxin accumulation is observed (Akinsanmi et al. 2004; Champeil et al. 2004; Leplat et al. 2013). Crop rotation is an important management tool, and the use of tillage practices, fertilizers, herbicides, resistant cultivars, and fungicide chemistries provide additional reinforcement for disease management.

Tillage affects pathogen development, dispersal and survival. Inversion tillage decreases the risk of FHB infection compared to non-inversion tillage (Leplat et al. 2013; Blandino et al. 2012). Agricultural systems using non-inversion tillage generally contain larger amounts of infested crop residues and have higher incidences of FHB (Leplat et al. 2013; Blandino et al. 2012). Tillage may also enhance decomposition rates of infested residues. There is faster and more complete decomposition of crop residues using inversion tillage than decomposition at the soil surface in non-inversion till systems, and the amount of *F. graminearum* inoculum is higher for unburied residue compared to buried residue (Leplat et al. 2013). Slower decomposition at

the soil surface provides nutrients for a longer time, supporting *F. graminearum* development (Leplat et al. 2013). There is evidence that no-tillage systems coupled with favorable environmental conditions for FHB dispersal and development promote DON accumulation (Blandino et al. 2012; L. Shah et al. 2018a). Tillage practices also influence weed populations, and their variability can, ultimately, serve as a reservoir for FHB pathogens (Mourellos et al. 2014). However, tillage in agricultural systems is harmful to the environment, so the role of weeds and how they influence the FHB disease cycle should be considered for disease management (Leplat et al. 2013).

Fertilizers and herbicides can contribute to both the vigor of the plant and vigor of the pathogen. Fungi in the FHB complex are able to use all forms of nitrogen, but alternate forms of nitrogen can affect the development, reproduction and survival of FHB pathogens (Leplat et al. 2013; Pirgozliev et al. 2003). Herbicide applications for weed management may also reduce disease pressure by removing inoculum sources; however, there is little information how herbicides might directly affect FHB pathogens or their saprophytic survival (Leplat et al. 2013).

Resistant cultivars joined with other cultural and chemical practices are valid options for controlling FHB in wheat and maize cropping systems. Currently there are no sorghum cultivars with complete FHB resistance, although resistance to *Fusarium* stalk rot pathogens is available (Funnell-Harris et al. 2014, 2016). In wheat and barley, cultivars with a shorter growing period are grown in order to prevent overlap between anthesis, the critical FHB infection stage, and weather conditions conducive for FHB disease development (L. Shah et al. 2018a). Sorghum cultivars having shorter growing periods are a mechanism for avoiding FHB, and earlier planting dates also promote avoidance of FHB during anthesis (L. Shah et al. 2018a). The discovery of cold tolerant sorghum cultivars is being investigated, and some cultivars show promise of cold

tolerance; cold-tolerant cultivars could mitigate crop exposure to disease propagules and avoid infection by allowing plant development during the overwintering stage of *Fusarium* spp. (M. L. Wang et al. 2017).

Fungicides are also integrated into FHB management plans for small grains. Few fungicides are registered for managing FHB in sorghum in the United States, but several chemistries are registered for FHB management on wheat and maize (Cowger et al. 2016). Application timing and fungicide mode of action are important to managing FHB with fungicide applications. In wheat, the efficacy of fungicide applications is dependent on timing since registered fungicide classes are incapable of translocating quickly (L. Shah et al. 2018a). This is especially difficult when panicles do not emerge or flower synchronously (Wegulo et al. 2015). Additionally, panicle emergence coincides with weather conducive for FHB infection, which is typically sub-optimal spraying conditions (L. Shah et al. 2018a; Wegulo et al. 2015). Demethylase inhibitors (DMI's) and strobilurins (QoI's) are primary classes of fungicides evaluated for FHB on wheat and/or maize in the United States. The succinate dehydrogenase inhibitors (SDHI's) are relatively new to the market and are of increasing importance due to the lack of cross resistance to other SDHI fungicides (J. Wang et al. 2017).

DMI's, also known as triazoles, block sterol biosynthesis in fungi. Triazoles used in wheat FHB management include prothioconazole, tebuconazole, and metconazole. DMI fungicides reduce FHB incidence and mycotoxin production (Scarpino et al. 2015; L. Shah et al. 2018a). In wheat, combinations of tebuconazole + prothioconazole (Prosaro) and metconazole (Caramba) are the two most effective fungicides for FHB control and DON reduction, if sprayed up to one week after anthesis (D'Angelo et al. 2014). Benzimidazole fungicides have also been implemented for FHB control in wheat. Since the 1970's, carbendazim, a benzimidazole

fungicide, was determined to lower disease incidence in wheat panicles by 70% and reduce mycotoxin accumulation in grain for *F. graminearum*, *F. culmorum*, and *F. avenaceum* (L. Shah et al. 2018a; Yin et al. 2009). Now, however, resistance to carbendazim has been detected in *Fusarium* spp. populations (Liu et al. 2019; Chen et al. 2019; Chen and Zhou 2009). There is also evidence that *F. graminearum* produces more deoxynivalenol when exposed to prothioconazole at sub-lethal doses (Audenaert et al. 2010). Despite providing control for FHB and mycotoxin accumulation, DMI's fail to provide complete control. Thus, alternative effective fungicide classes are desired for management.

QoI's, also known as strobilurins, have been commercially available since the 1990's (Fernandez-Ortuno et al. 2010). This class of fungicides affects the cytochrome bc1 complex in fungal mitochondria by preventing the transfer of electrons at the quinol oxidation (Qo) site, thereby minimizing adenosine triphosphate (ATP) production and preventing spore germination and mycelial growth (Fromme et al. 2017; Dubos et al. 2011). QoI's are considered an ineffective chemistry for FHB control and can increase DON production (Pirgozliev et al. 2002; Wegulo et al. 2015). Despite past evidence that azoxystrobin exhibits some ability to lower FHB and DON compared to nonsprayed controls although azoxystrobin applications were less effective than metconazole applications on wheat (Pirgozliev et al. 2002), more recent evidence suggests QoI applications, whether applied singularly or in mixes, should not be administered after flag leaf emergence in wheat and barley due to an increase in DON compared to nonsprayed controls (Paul et al. 2018). *Fusarium graminearum* also exhibits natural resistance to trifloxystrobin (Dubos et al. 2011; L. Shah et al. 2018a). Trials using mixtures of strobilurins and triazole fungicides resulted in less FHB disease symptoms but increased DON during growing years with conditions conducive for disease development (Blandino et al. 2006; Paul et al. 2018).

SDHI fungicides are used to manage a broad range of plant pathogens on several cropping systems (J. Wang et al. 2017). These fungicides operate with a single-site mode of action that inhibits energy metabolism in fungi. Because of this mode of action, most fungicides in this group are classified as medium to high risk for selecting SDHI-resistant isolates from fungal populations. However, fluopyram, an SDHI fungicide, exhibits a lower cross-resistance potential to other SDHI's because the molecule binds to a different target site in the succinate dehydrogenase enzyme (J. Wang et al. 2017; Fraaije et al. 2012).

Conclusions

Despite the crop production potential for various market applications, sorghum production in North Carolina is primarily marketed for animal feed and human food. The physiological attributes of sorghum support its ability to withstand drought stress and maintain a high production potential. The reproductive and grain maturing growth stages are an entryway for pathogen colonization and infection, especially by FHB causal agents. The diversity of FHB pathogens and their mycotoxins in North Carolina has not been extensively examined or characterized in sorghum production. Identifying FHB pathogens to species level can determine which mycotoxins might be produced. Although, in cereals, DON, fumonisin, and zearalenone constitute three mycotoxins of concern, there is little research on the prominent mycotoxins associated with FHB in sorghum and how current management strategies influence mycotoxin accumulation in grain. Current strategies for managing FHB are concentrated around cultural and chemical practices. Few small grain FHB chemistries are registered for FHB in sorghum, and optimal application timings on sorghum are understudied but necessary for management. Implications of applying certain chemistries include influencing mycotoxin levels and types

associated with FHB infested grain at harvest. The research objectives of this project are to 1) examine the diversity, pathogenicity, and fungicide sensitivity of *Fusarium* isolates collected from sorghum fields in North Carolina and 2) evaluate the efficacy of fungicides at three application timings for management of FHB disease severity, disease progression, and mycotoxin accumulation of DON, fumonisin, and zearalenone in sorghum.

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Tables and Figures

Table 1.1. *Fusarium* spp. known to produce trichothecene, fumonisin, and zearalenone mycotoxins in cereal crops.

Mycotoxin	Known Producers
Trichothecene	<i>F. graminearum</i> , <i>F. sporotrichioides</i> , <i>F. langsethiae</i> , <i>F. armeniacum</i> , <i>F. acuminatum</i> , <i>F. sambucinum</i> , <i>F. venenatum</i> , <i>F. poae</i> , <i>F. culmorum</i> , <i>F. crookwellense</i> , <i>F. pseudograminearum</i> , <i>F. boothii</i> , <i>F. equiseti</i> , <i>F. kyushuense</i>
Fumonisin	<i>F. verticillioides</i> , <i>F. thapsinum</i> , <i>F. proliferatum</i> , <i>F. fujikuroi</i> , <i>F. subglutinans</i> , <i>F. napiforme</i> , <i>F. nygamai</i> , <i>F. globosum</i> , <i>F. andiyazi</i>
Zearalenone	<i>F. graminearum</i> , <i>F. equiseti</i> , <i>F. crookwellense</i> , <i>F. semitectum</i> , <i>F. culmorum</i> , <i>F. graminearum</i> , <i>F. pseudograminearum</i>

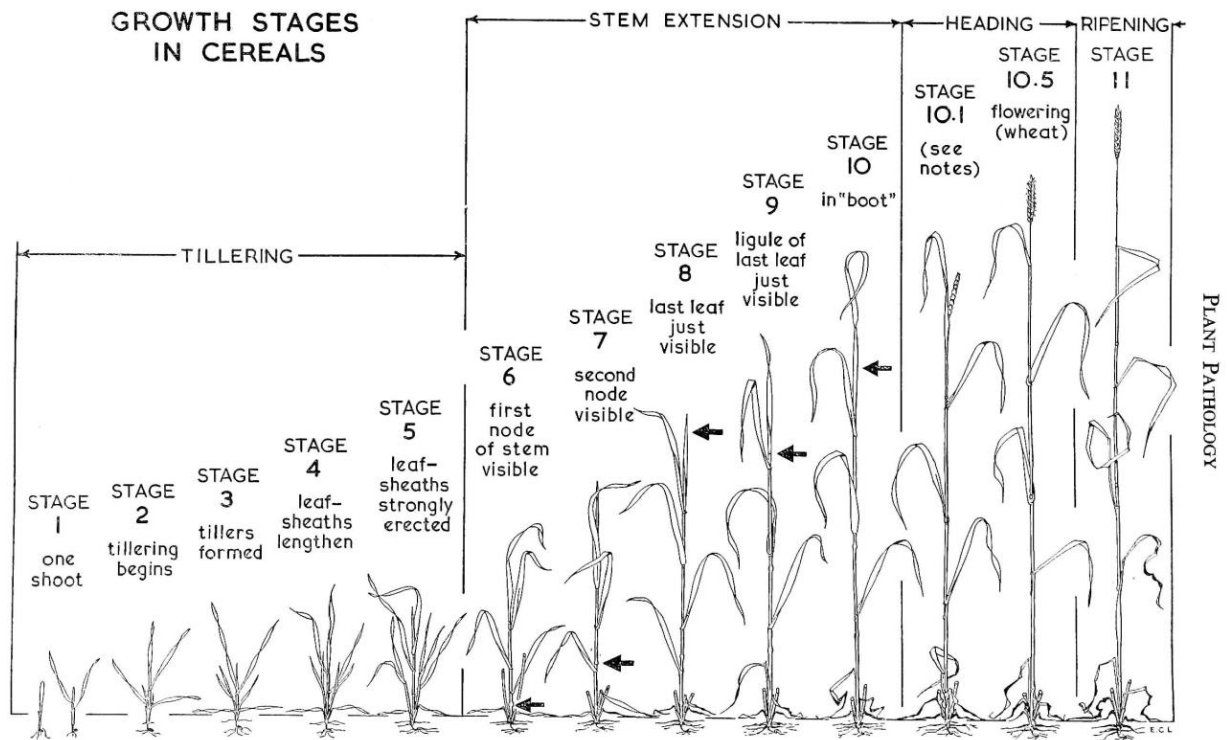


Figure 1.1. Growth stages in cereal crops, comparable to growth stages in sorghum (Large 1954). Stage numbers described in text begin with zero (Stage 1 in figure).



Figure 1.2. Fusarium head blight (FHB) reddening and necrosis associated with *Fusarium* spp. infection sorghum panicle in 2017.

CHAPTER II. Diversity, Fungicide Sensitivity and Pathogenicity of *Fusarium* Isolates in North Carolina Sorghum Fields

ABSTRACT

Fusarium head blight (FHB) has not been fully characterized in sorghum, and identification of *Fusarium* isolates affecting North Carolina sorghum fields has yet to be completed. Infected grain sorghum was collected from 10 field locations across 2017-2018. *Fusarium* isolates (n=166) were morphologically characterized by spore measurements, presence of chlamydospores, hyphal color, and pigment production on potato dextrose agar (PDA). Translation elongation factor (TEF), ribosomal protein subunit II (RPB2), and beta-tubulin (TUB2) sequences were obtained for 139 *Fusarium* isolates. Phylogenetic analysis revealed isolate grouping to *F. graminearum*, *F. verticillioides*, *F. fujikuroi*, *F. proliferatum*, and members of the *F. incarnatum-equiseti* species complex (FIESC). Detached leaf assays were conducted using representative isolates from each field location and an additional isolate (*F. sporotrichioides*) from wheat for a total of 11 isolates, which were evaluated on sorghum varieties SH80G4 and Pioneer 84P80. *Fusarium sporotrichioides* had the highest aggressiveness despite no significant differences of isolate pathogenicity. Fungicide sensitivity assays were conducted with the same representative isolates with the addition of a *F. graminearum* isolate collected from sorghum (n=12) for response to concentrations of tebuconazole, prothioconazole, fluopyram, and trifloxystrobin. All isolates were sensitive to tebuconazole and prothioconazole, insensitive to fluopyram, and isolate sensitivity was variable to trifloxystrobin.

INTRODUCTION

The U.S. is a major global producer of sorghum, exporting \$1,368,422,000 of sorghum in 2016 (FAOSTAT 2016). Sorghum is not commonly grown in the Southeastern United States and has been previously suggested as a replacement for corn due to lower input requirements (Hagler et al. 1986). Sorghum is also tolerant of drought conditions, with water and nutrient efficiencies higher than corn, supporting high yield potential (Zegada-Lizarazu and Monti 2012b). Sorghum produced in North Carolina is used for animal feed and can be used for human consumption; however, sorghum-based feed for swine has been linked to reproductive issues resulting from mycotoxin-contaminated grain (Hagler et al. 1986). Organisms that are commonly associated with mycotoxin-contaminated grain include members of the genera *Aspergillus*, *Penicillium*, and *Fusarium* (Nguyen et al. 2017). *Fusarium* spp. are pathogenic to a variety of hosts, especially members of Poaceae (Marburger et al. 2015; Moretti 2017). Fusarium head blight (FHB), caused by a disease complex of *Fusarium* spp., is significant to sorghum producers in the Southeastern U.S. because the warm temperatures and high humidity favor disease development.

Fusarium head blight causes similar symptoms in sorghum compared to other small grain systems. Symptoms include reddening of susceptible tissues on the panicle, such as florets, developing grain, and rachis branches, that progresses to necrotic regions across infected panicles (Frederiksen and Odvody 2000). Furthermore, FHB pathogens can colonize heads asymptotically (Funnell-Harris and Pedersen 2011). This can be problematic because the mycotoxigenic potential of *Fusarium* spp. is diverse and abundant regardless of symptom development (Funnell-Harris and Pedersen 2011; Funnell-Harris et al. 2010).

The causal agent of FHB in sorghum was originally thought to be primarily *Fusarium moniliforme* (Klittich and Leslie 1992; Castor and Frederiksen 1980; Leslie et al. 2005);

however, other pathogenic *Fusarium* spp. have been isolated from infected and contaminated sorghum in several production regions. For example, *F. roseum* [*Gibbosum* and *Semitectum*] in North Carolina (Hagler et al. 1986), *F. thapsinum* in Australia, South Africa, and Nebraska (Kelly et al. 2017; Stack and Pedersen 2003; Leslie et al. 2005), members of *F. incarnatum-equiseti* species complex (FIESC) in Australia (Kelly et al. 2017), *F. semitectum* in Nebraska (Stack and Pedersen 2003), and *F. proliferatum* in Nebraska (Stack and Pedersen 2003) were isolated and determined to be pathogenic in sorghum. Additionally, members of *Fusarium fujikuroi* species complex (FFSC) have been collected from sorghum in Argentina, Australia, Brazil, India, South Africa and the U.S. (Pena et al. 2018; da Silva et al. 2006; Klittich and Leslie 1992; Sreenivasa et al. 2008; Kelly et al. 2017; Leslie et al. 2005). *F. graminearum* species complex (FGSC) in Argentina (Pena et al. 2018), the predominant FHB causal species in wheat (Del Ponte et al. 2015), and members of the FIESC - *F. semitectum* in Argentina and *F. equiseti* in Kansas (Pena et al. 2018; Leslie et al. 2005) were isolated from sorghum. Furthermore, members of the *F. oxysporum* species complex (FOSC) and *F. sporotrichioides* in Argentina and India have also been collected from sorghum (Pena et al. 2018; Sreenivasa et al. 2008). However, no research has been published on FHB causal pathogens or isolate pathogenicity and aggressiveness for *Fusarium* spp. in North Carolina sorghum fields since 1986 (Hagler et al. 1986). Moreover, isolate pathogenicity and virulence may be influenced by the mycotoxigenic profile of each species and their interactions with the local environment, so survey data are needed to examine the FHB population species and strains affecting sorghum in North Carolina.

Stalk rot and grain mold diseases in sorghum are more common in major sorghum production regions than FHB (Pena et al. 2018); however, conditions for FHB development are prevalent in the southeast. Pathogenicity, the ability of an organism to infect a host, pathogen

aggressiveness, and quantitative traits in a pathogen lifecycle influence infection severity (Van der Plank 1963, 1968; Pariaud et al. 2009). Pathogenicity and pathogen aggressiveness assays in sorghum seedling roots and shoots have been evaluated for *F. andiyazi*, *F. nygamai*, *F. pseudonygamai*, *F. thapsinum*, and *F. verticillioides* from Namibia and South Africa, all of which were previously identified as *F. moniliforme* (Leslie et al. 2005). All isolates were confirmed to be pathogenic to sorghum, and *F. thapsinum* was the most aggressive to sorghum seedlings of the five species followed by *F. andiyazi*, *F. verticillioides*, and *F. nygamai* and *F. pseudonygamai*, which had similar aggressiveness (Leslie et al. 2005). Stalk rot pathogenicity assays showed that *F. verticillioides*, *F. thapsinum*, *F. nygamai*, *F. proliferatum*, *F. andiyazi*, *F. pseudoanthophilum*, *F. brevicatenuatum*, and *F. pseudonygamai* on three sorghum genotypes were able to infect sorghum stalks and disease scores (lesion length and nodes crossed) were significantly different from control treatments (Tesso et al. 2010). Numerous FHB pathogenicity tests have been performed for *Fusarium* isolates from wheat spikes (Brennan et al. 2003; Akinsanmi et al. 2004); however, few pathogenicity or aggressiveness assays have been conducted for sorghum FHB isolates using sorghum.

Similarly, studies of trichothecene-producing *Fusarium* species, including *F. culmorum* and *F. graminearum*, suggest deoxynivalenol (DON) production contributes to pathogenesis (Wagacha and Muthomi 2007; Wegulo et al. 2015). For instance, *F. culmorum* of the DON chemotype is more aggressive to barley seedlings, and exhibits more aggressiveness towards wheat than the nivalenol (NIV) chemotype (Wagacha and Muthomi 2007). Conversely, *F. graminearum* can be more aggressive than some other members of the FGSC, and the species and trichothecene type combination influence strain aggressiveness to wheat (Wagacha and Muthomi 2007). *F. graminearum* of the 15-ADON type exhibits a higher aggressiveness than *F.*

asiaticum or *F. graminearum* of the NIV type (Del Ponte et al. 2015). Due to the lack of information on FHB isolates from the Southeast U.S., there is a need for further characterization of the *Fusarium* species that infect sorghum and species-chemotype frequencies in this environment.

An additional problem is the lack of host resistance in sorghum to FHB. Without FHB-resistant varieties, producers are reliant on chemical control as a management strategy for FHB in sorghum. Currently, there are no baseline sensitivities for *Fusarium* isolates collected from FHB-infected sorghum to fungicides commonly used for disease management in other cereal crops, which are currently not labeled for FHB control in sorghum. Internationally, *Fusarium* spp. populations in wheat and barley have been examined for sensitivities to demethylase inhibitors (DMI) and quinone outside inhibitors (QoI). Isolates from Serbian wheat are more sensitive to DMI fungicides than QoI fungicides (Rekanovic et al. 2010). *F. graminearum* mycelial growth from Brazilian wheat populations was most sensitive to metconazole, prothioconazole, and tebuconazole, which supports the common practice of using triazole fungicides for managing *F. graminearum* (Avozani, Reis, et al. 2014). *F. graminearum* isolates are also sensitive to moderately-sensitive to trifloxystrobin, azoxystrobin, kresoxim-methyl, and pyraclastrobin (Avozani, Tonin, et al. 2014), although application is not recommended due to increased DON contamination associated with strobilurin exposure. Developing baseline sensitivities of *Fusarium* spp. isolates relevant to sorghum production is important to develop an integrated pest management strategy that includes fungicides for sorghum FHB.

The specific objectives of this study were to 1) determine the diversity of *Fusarium* spp. that cause FHB in North Carolina produced sorghum, 2) evaluate the pathogenicity of *Fusarium* spp. on sorghum, and 3) identify fungicide sensitivity of *Fusarium* spp. strains to fungicides

commonly used in grain production. My hypotheses were that 1) several *Fusarium* spp. will be collected from North Carolina sorghum fields, 2) no differences will be observed in isolate aggressiveness, and 3) isolates will be sensitive to tebuconazole and prothioconazole.

MATERIALS AND METHODS

Isolate Collection

Sorghum panicle samples (n = 155) were collected from FHB-affected fields across North Carolina. Symptomatic panicles were obtained from six fields in Johnston County, one field in Pasquotank County, one field in Edgecombe County, one field in Nash County, and one field in Wake County during the 2017 and 2018 growing seasons. In 2017, sorghum growers and North Carolina county extension agents were solicited to identify FHB-affected fields and provided field locations for sampling. From each field, three to five symptomatic panicles were collected randomly from the affected areas. Sorghum was not extensively grown in North Carolina in 2018, and only two fields were sampled that year by collecting 12-15 random symptomatic panicles.

Fusarium Isolation

In a sterile working environment, three sorghum grains from each panicle were surface sterilized for one minute in 10% bleach solution (HDX Germicidal Bleach, 8.25% sodium hypochlorite). Grains were then rinsed in sterile deionized (DI) water for 60 seconds, air dried on sterile paper towels, and then plated onto water agar. Plates were sealed with parafilm and stored at 25°C for approximately 48 hours. Growing tips of fungal isolates were aseptically transferred from the water agar to plates of antibiotic-amended (100 mg/L ampicillin, and 100 mg/L

streptomycin sulfate) potato dextrose agar (ABPDA) (Difco, Detroit, MI), which were then incubated at 25 °C for four to seven days. Fungal isolates not exhibiting typical *Fusarium* growth characteristics were discarded.

Morphological Characterization of Field-Collected Fusarium isolates

Remaining *Fusarium* isolates were morphologically characterized based on spore type (macroconidia, microconidia, and chlamydospores, if present), average spore length (µm), average spore width (µm), hyphal color, and pigment production on ABPDA (Appendix Table A.1). Presence of chlamydospores was noted. Conidial measurements were obtained from 14-day old isolates grown on ABPDA by imaging conidia with a Nikon camera attached to the Nikon Eclipse compound microscope. The NIS-Elements software (Nikon, Tokyo, Japan) imaging program was used to measure length and width of 50 separate conidia from each *Fusarium* isolate. Eleven known, locally-collected *Fusarium* reference isolates from wheat and corn were also obtained from Dr. Christina Cowger (USDA-ARS, Raleigh, NC) and Dr. Thiago Marino (formerly North Carolina State University, Raleigh, NC) to serve as control isolates, including *F. graminearum*, *F. acuminatum*, *F. avenaceum*, *F. armeniacum*, *F. sporotrichioides*, and *F. verticillioides*; these were characterized alongside the field survey collected isolates.

Molecular Characterization of Field-Collected Fusarium isolates

DNA Collection of Field-Collected Fusarium isolates

Fungal suspensions from each field-collected *Fusarium* isolate (Appendix Table A.1) were used for subsequent molecular characterization. To create fungal suspensions, five ml DI water was added to a petri dish containing each isolate. Sterile rubber policemen were used to

scrape spores and hyphae into the water. From the suspension, one ml of mycelia, spores, and sterile water was used for DNA extractions. DNA was extracted according to the DNeasy Power Soil DNA extraction kit protocol (Qiagen, Germantown, MD).

PCR of Field-Collected Fusarium isolates

Previous molecular studies for *Fusarium* species used internal transcribed spacer (ITS) region to examine phylogenies; however, ITS is insufficient for distinguishing closely related species in the *Fusarium* genus, and translation elongation factor - 1 α (TEF) in combination with a multilocus phylogenetic analysis can provide species-level resolution (Karlsson et al. 2016; Geiser et al. 2004; O'Donnell and Cigelnik 1997). The multilocus approach used in this study incorporated β -tubulin (TUB2) and ribosomal protein subunit II (RPB2) for phylogenetic resolution (Mulè et al. 2004; O'Donnell et al. 2012). Polymerase chain reactions (PCR) for each isolate were performed for TEF EF1/EF2 (Divakara et al. 2014; O'Donnell et al. 1998), β -tubulin T1/T22 (Taylor et al. 2016; O'Donnell and Cigelnik 1997), and RPB2 5f2/11ar (Cerón-Bustamante et al. 2018; O'Donnell et al. 2010) to examine species-level resolution and relatedness among species using protocols as outlined for each primer set. Each 25 μ l PCR reaction included 8.75 μ l Accustart II PCR Toughmix (Quantabio, Beverly, MA), 10 mM final concentration of forward and reverse primers, and 2.5 μ l extracted DNA. PCR cycling conditions for each region varied for optimal amplification of regions (Table 2.1). PCR products were visualized in a 1.0% agarose gel prepared in 1X TBE buffer. To the starting well of each gel row, 0.5 μ l MassRuler 10 Kb ladder (Thermo Scientific, Waltham, MA) was added, and the PCR product with 4.0 μ l of loading dye/GelRed dyemix (Biotium, Fremont, CA) was added to the

remaining wells. Gels were run at 100 V for 60 min and imaged using a BioRad Gel Imager (Bio-rad, Hercules, CA).

PCR Product Cleanup

PCR products were cleaned following a modified Illumina 16S PCR cleanup protocol (Illumina, San Diego, CA). PCR products in a 96-well plate were vortexed for 10 s and centrifuged at 1,000 g for 1 min. An initial stock of 80% molecular grade ethanol was prepared for PCR product cleanup. Ampure XP bead reservoir was aliquoted into each tube of a 0.2 µl 12-well strip. Using a 12-channel multichannel pipette, each PCR sample in the 96-well plate received 20 µl of room temperature beads. The mixture was pipetted up and down 15 times and incubated at room temperature for 5 min. Following initial incubation, the tubes were placed on a magnetic stand for 2 min or until the supernatant cleared. Using a 12-channel multichannel pipette set to 100 µl and new pipette tips for each tube, the supernatant was removed from each sample. Two wash steps were performed where 200 µl of 80% molecular grade ethanol was pipetted to each tube. Tips were discarded between rows. Samples were incubated for 30 s, and supernatant was removed after each wash. After the final wash step, the 1-10 µl 12-channel multichannel pipette was set to 4 µl and excess supernatant was removed from the tubes. The samples air dried for 4 min and were removed from the magnetic stand. Beads were resuspended with addition of 26.25 µl of TRIS-HCl pH 8.5 and vortexed for 20 s. The samples were incubated for 2 min at room temperature and then transferred back to the magnetic stand for a final 2-min incubation. The cleaned PCR product (25 µl) was transferred to a new 96-well plate. After DNA concentrations were determined using a NanoDrop (Thermo Fisher Scientific,

Waltham, MA), samples were diluted as needed and sent for sequencing at North Carolina State University Genome Sciences Laboratory (Raleigh, NC).

Sequence and Phylogenetic Relatedness Analysis

Forward and reverse sequences for isolates that amplified well were trimmed in Geneious Prime version 2019.2.1 (<https://www.geneious.com>) and aligned for BLASTn analysis of TEF, TUB2, and RPB2. Gene sequences were then aligned using MAFFT v7.388 alignment (Kato 2002; Kato and Standley 2013). Forward and reverse RPB2 and TUB2 sequences that did not align well were concatenated. The three genes for each isolate were concatenated and masked with 0% gaps, meaning nucleotides without missing data were kept for analyses. *Fusarium* reference sequences were retrieved from NCBI (Table 2.2). Phylogenetic analysis for 139 isolates with sequences for the three genes was conducted via RAxML-NG version 0.9.0 (Kozlov et al. 2019) using high-performance computing (HPC) for fast and accurate results. Isolates were analyzed with the maximum likelihood method GTR-GAMMA substitution model with a bootstrap random number seed 12345 with 20 bootstrap replications. The highest scoring maximum likelihood tree was selected, imported to Geneious, and rooted according to the outgroup *Neonectria coccinea*.

Fusarium Isolate Pathogenicity Assay

A completely randomized, factorial experiment was carried out with two sorghum varieties and *Fusarium* isolate as the factors with four replications (n = 96), and two repetitions in time to examine the aggressiveness of 11 representative isolates (Table 2.3). FHB susceptible varieties Southern Harvest 80G4 (Meherrin, Severn, NC) and Pioneer 84P80 (Pioneer, Johnston

Co., IA) were selected based on observed symptoms in the 2017 sorghum Official Variety Trial test in Rocky Mount, NC. Plants were grown in clay pots, 15.24 cm in diameter, filled with 1.4 liters of Sungro Professional Horticultural Mix 4P (Sungro Horticulture, Agawam, MA) under a 13-hour photoperiod for four weeks. After 29 days, the third and fourth leaves from the base of the plant were harvested to set up a detached leaf assay. Leaf pieces were cut to five cm in length and sterilized with a 10% bleach solution (HDX Germicidal Bleach, 8.25% sodium hypochlorite) for one minute, followed by two 30-second rinses in sterile deionized water, and then dried on sterilized paper towels. Once sterilized, 9 cm diameter filter paper (Fisherbrand, FisherScientific, Pittsburg, PA) was placed in the bottom of each petri plate with five ml of sterile deionized water. Leaves were placed on the filter paper abaxial side up and inoculated with one 5.0 mm agar plug from the leading edge of a *Fusarium* isolate grown on ABPDA, mycelium side facing the leaf. A representative isolate was chosen from each collection source (Table 2.3). The plates were sealed with parafilm and placed on two trays in a completely randomized design spread across four trays and stored in a PRECISION 815 incubator (Thermo Scientific, Waltham, MA) at 25°C in dark conditions. Lesion lengths were assessed in mm at 48, 72 and 96 hours with a digital caliper and recorded for data analysis.

The area under disease progress curve (AUDPC) was calculated for each treatment using ‘reshape2’ package (Wickham 2007). Data were analyzed using a gamma distribution with the ‘glm’ function (R Core Team, 2019). A random error term was added to each observation drawn from a gamma distribution with shape parameter $k = XX$ and scale parameter $\theta = YY$ to approximate measurement error because for some treatment groups, all observations were zero. Means for each treatment were calculated and compared using the ‘emmeans’ function (R Core

Team, 2019) and p-values were calculated with a Tukey correction to account for multiple comparisons.

Mycelial Growth Inhibition Assay

A completely randomized fungicide sensitivity assay was conducted with twelve representative *Fusarium* isolates (Table 2.3) and three fungicides from DMI and SDHI modes of action. Fungicide stock solutions were prepared from technical grade fluopyram (98.13% Bayer Crop Science, Durham, NC), prothioconazole (98.40% Bayer Crop Science, Durham, NC), and tebuconazole (95.40% Bayer Crop Science, Durham, NC) dissolved in molecular grade acetone. Full-strength PDA (39g/1000ml DI H₂O, Difco, Detroit, MI) was autoclaved, cooled to 50-55 °C, and amended to 10, 1, 0.1, 0.01, and 0.001 µg a.i. ml⁻¹ with one ml of each stock solution. Control plates were prepared with 0.1% vol/vol acetone. Approximately 20 ml of molten media was dispensed per plate. Media was stored in a cold room at 4°C under dark conditions until used in the assay.

Four replicates of each fungicide concentration and isolate combination (n = 216) were inoculated with one 5-mm diameter plug taken from the leading edge of an isolate culture. Plugs were placed at the intersection of the two perpendicular lines traced on the underside of the petri plate. Plates were stored at room temperature (20-25 °C), and two perpendicular mycelium diameter measurements (mm) were recorded at 24, 48, 72, and 96 hr post inoculation (hpi). The 5 mm plug was subtracted from each diameter measurement. Relative mycelial growth was calculated by dividing colony diameter by the mean colony diameter of the acetone control plates and multiplied by 100 for data normalization.

No biological hormesis (increased relative mycelial growth at low concentrations compared to control plates) was observed; however, these data were run with four-parameter log-logistic and four-parameter Brain-Cousens models to justify using only the log-logistic model (Noel et al. 2018). Furthermore, the “mselect ()” function was utilized comparing seven dose response models to check the model of best fit for these data. The five-parameter log-logistic and Brain-Cousens models were excluded from determining best fit because it was beyond the scope of this study.

Relative and absolute EC50 estimates and confidence intervals were obtained by fitting percent relative growth against the log concentration using the symmetric four-parameter log-logistic model for tebuconazole, prothioconazole, and fluopyram (Ritz and Streibig 2005; Ritz et al. 2016) in R statistical software (RStudio, R Core Team, 2019) and specifying type = “relative” and “absolute” within the “ED” function of the “drc” package (Ritz et. al., 2015). Relative and absolute EC50 estimates were reported for tebuconazole, prothioconazole, and fluopyram. Standard error and confidence intervals for each relative and absolute estimate were obtained by specifying interval = “delta” within the “ED” function of the “drc” package. Relative EC50 estimates are the inflection point on dose-response curves, while absolute EC50 estimates are the concentration at which the response is 50% of the maximal response (Noel et al. 2018). The definition of absolute EC50 estimates is more consistent with the Fungicide Resistance Action Committee (FRAC) definition of EC50 where it is defined as the fungicide dose that inhibits growth of an isolate by 50% compared to a non-fungicide-amended control (<https://www.frac.info/fungicide-resistance-management/background>).

Fluopyram Mycelial Growth Inhibition Assay

A second completely randomized fungicide sensitivity assay was conducted with twelve representative *Fusarium* isolates (Table 2.3) and one fungicide, fluopyram (SDHI). Fluopyram stock solutions were prepared from technical grade fluopyram (98.13% Bayer Crop Science, Durham, NC) dissolved in molecular grade acetone. Full-strength PDA (39g/1000ml DI H₂O, Difco, Detroit, MI) was autoclaved, cooled to 50-55 °C, and amended to 100, 10, 1, 0.1, 0.01, 0.001, 0.0001, and 0.00001 μg a.i. ml⁻¹ with one ml of each stock solution. Control plates were prepared with 0.1% vol/vol acetone. Approximately 20 ml of molten media was dispensed per plate. Media was stored in a cold room at 4 °C under dark conditions until used in the assay.

Four replicates of each fungicide concentration and isolate combination (n = 108) were inoculated with one 5-mm diameter plug taken from the leading edge of an isolate culture. Plugs were placed at the intersection of the two perpendicular lines traced on the underside of the petri plate. Plates were stored at room temperature (20-25 °C), and two perpendicular mycelium diameter measurements (mm) were recorded at 24, 48, 72, and 96 hr post inoculation (hpi). The 5mm plug was subtracted from each diameter measurement. Relative mycelial growth was calculated by dividing colony diameter by the mean colony diameter of the acetone control plates and multiplied by 100 for data normalization.

No biological hormesis (increased relative mycelial growth at low concentrations compared to control plates) was observed; however, these data were run with four-parameter log-logistic and four-parameter Brain-Cousens models to justify using only the four-parameter log-logistic model (Noel et al. 2018). Furthermore, the “mselect ()” function was utilized comparing seven dose response models to check the model of best fit for these data. The five-parameter log-logistic and Brain-Cousens models were excluded from determining best fit because it was

beyond the scope of this study. Relative EC50 estimates were not attempted since only one isolate had mean relative growths that extended below 50%. Absolute EC50 estimates and confidence intervals were attempted by fitting percent relative growth against the log concentration using the symmetric four-parameter log-logistic model for fluopyram (Ritz and Streibig 2005; Ritz et al. 2016) in R statistical software (RStudio, R Core Team, 2019) and specifying type = “absolute” within the “ED” function of the “drc” package (Ritz et. al., 2015). Standard error and confidence intervals for each absolute EC50 estimate were obtained by specifying interval = “delta” within the “ED” function of the “drc” package.

Spore Germ-tube Inhibition Assay

A completely randomized germ-tube inhibition assay was conducted with twelve representative *Fusarium* isolates (Table 2.3) and one QoI fungicide. The fungicide stock solutions were prepared from technical grade trifloxystrobin (98.60% Bayer Crop Science, Durham, NC) dissolved in molecular grade acetone. A 100 µg ml⁻¹ SHAM stock solution was prepared in histological grade methanol. Full-strength PDA (39g/1000ml DI H₂O, Difco, Detroit, MI) was autoclaved, cooled to 50-55 °C, amended with one ml salicylhydroxamic acid (SHAM, 99% Sigma Aldrich, St. Louis, MO) and amended to 10, 1, 0.1, 0.01, and 0.001 µg a.i. ml⁻¹ with one ml of each stock solution. Two control plates were prepared with 0.1 % vol/vol acetone and 0.1% vol/vol acetone with 100 µg ml⁻¹ SHAM. Approximately 20 ml of molten media were dispensed per plate. Media was stored in a cold room at 2-8 °C until inoculation. Conidia were collected from one-month-old *Fusarium* isolates grown on PDA petri plates under a 12-hr light cycle and adjusted to a 3.3×10⁵ spore concentration.

For inoculation, suspensions were vortexed briefly and 200 μ l of spore suspension was pipetted onto each of four replicate plates of each concentration by isolate combination (n = 72). Plates were covered with parafilm and stored on the benchtop at room temperature (20-25 °C) for 24 hr. A conidium was considered germinated if the germ tube was at least half the length of the spore (Xu et al. 2019). Relative spore germination was calculated for data normalization by dividing number of spores germinated by the mean number of spores germinated on the control plates multiplied by 100. Relative and absolute EC50 were estimated by fitting relative spore germination against the concentration or log concentration using the best model for each isolate. Additionally, the “mselect ()” function was used to compare seven selected dose-response models to determine the best-fitting model in the “drc” package version 3.0-1 (Ritz and Streibig 2005; Ritz et al. 2016; Cedergreen et al. 2005) in R statistical software (RStudio, R Core Team, 2019). Relative and absolute estimates were determined by specifying type = “relative” or type = “absolute” within the “ED” and “drm” functions of the “drc” package. Standard error and confidence intervals for each relative and absolute estimate were obtained by specifying interval = “delta” within the “ED” function of the “drc” package. Relative and absolute EC50 estimates were reported for tebuconazole, prothioconazole, and fluopyram. Standard error and confidence intervals for each relative and absolute estimate were obtained by specifying interval = “delta” within the “ED” function of the “drc” package.

RESULTS

Characterization of Field-Collected Fusarium isolates

Hyphal color, pigment production on PDA, and spore types varied between isolates of the different *Fusarium* strains (Appendix Table A.1). Each isolate produced spores for

measurements (Table 2.2). Masked alignment with 0% missing data of TEF, RPB2, and TUB2 *Fusarium* sequences resulted in the analysis of 370 bp of an approximate total of 3,344 bp. Phylogenetic analysis of masked alignments of 10% and 1% missing data were examined; however, field collected isolates did not group with the NCBI isolates, making phylogenetic inference more challenging. A *Fusarium* phylogeny using 0% masked alignment is represented in the tree with the highest maximum likelihood shown (Figure 2.1). Bootstrap values ranged from 76 to 100.

Known isolates collected from wheat and corn were grouped with the same reference isolates with which they were identified prior to confirmation in this study (Figure 2.1). Of the isolates included in the phylogenetic analysis, the majority (65%) of *Fusarium* isolates collected from sorghum grouped with *FIESC* reference isolates. Additionally, 25% of isolates grouped with *FFSC*, and 8% of isolates grouped with *FGSC*. Based on BLASTn analysis of TEF, of the 2017-2018 isolates, 67% were identified in *FIESC*, 25% identified in *FFSC*, and 8% in *FGSC* (Figure 2.2) which agreed with phylogenetic analysis.

Fusarium Isolate Pathogenicity Assay

There was no significant effect of variety or isolate on lesion length ($P > 0.05$). Non-inoculated controls for both trials had no lesion development at the media plug site. Lesions were not observed for isolates S1-3C-1 (*FIESC*) and S2-1C-2 (*FIESC*) on either SH80G4 or Pioneer 84P80 varieties in trial one. Effect of trial was significant ($P < 0.05$). All isolates generated a lesion in at least one trial and were different compared to the non-treated control (Figure 2.3). The largest log-mean AUDPC value was 7.3 from FA2 (*F. equiseti*) on Pioneer 84P80 when data were analyzed accounting for the effect of trial (Table 2.4).

Mycelial Growth Inhibition Assay

Isolates were most sensitive to tebuconazole and prothioconazole, with complete or majority of colony inhibition observed at the highest dosage concentration between 1 and 10 ppm. The four-parameter log-logistic model distinguished absolute and relative EC50 estimates for tebuconazole and prothioconazole data (Table 2.5, 2.6). Ranges for tebuconazole relative EC50 estimates were from 0.01 to 0.42 ppm and absolute EC50 estimates ranged from 0.00063 to 0.33 ppm between tested isolates. Ranges for prothioconazole relative EC50 estimates were between 0.057 and 10 ppm, and absolute EC50 estimates ranged from 0.00038 to 6.05 ppm. Isolate insensitivity to fluopyram at mid-range doses was observed, and isolates with relative growths that did not decrease below 50% at high concentrations had either no relative and absolute estimates or estimates outside the experimental dosage range. Tebuconazole had the lowest overall confidence intervals for absolute and relative EC50 estimates, followed by prothioconazole and then fluopyram (Tables 2.5, 2.6, and 2.7), as indicated by dose response curves in Figures 2.4, 2.5, and 2.6.

Fluopyram Mycelial Growth Inhibition Assay

Isolate insensitivity to fluopyram was observed for all isolates, and complete inhibition was not observed for any isolate. One isolate, S2-1C-2 (*FIESC*) had an absolute EC50 estimate of 6.5 ppm. Absolute EC50 estimates for the remaining isolates could not be determined because relative growth values did not extend below 50% (Table 2.8). Additionally, fluopyram precipitation from solution was observed at 100 ppm at the media amendment stage, so isolate responses to 100 ppm were excluded from estimate determination. Isolate dose-response curves

in this study are not similar to the curves observed in the previous mycelial growth inhibition assay and do not exhibit growth uniformity among fungicide sensitivity assays (Figure 2.7).

Spore Germ-tube Inhibition Assay

Two isolates, FA2 (*F. equiseti*) and S2-1C-2 (*FIESC*), were sensitive to trifloxystrobin and absolute EC50 estimates were 0.1 ppm and 0.03 ppm respectively. C-46-19C (*F. sporotrichioides*; from wheat) showed no sensitivity to trifloxystrobin. EC50 estimates (ppm) could not be determined for nine isolates (Table 2.9). C-46-19C showed consistent germination at all trifloxystrobin concentrations including the highest concentration (10 ppm). S4-2C-2 did not have differences in relative spore germination between each fungicide concentration. Dose-response curves for the eight isolates that generated relative EC50 estimates, as shown in Figure 2.8, indicate that EC50 estimates could not be determined for 9 isolates. Relative spore germination values did not extend below 50%, values below 50% relative spore germination showed no differences between the concentrations, and relative spore germination values began increasing at higher trifloxystrobin concentrations.

DISCUSSION

Fusarium isolates collected from FHB-infected sorghum fields in North Carolina over the 2017 and 2018 growing seasons predominantly belonged to the *FIESC*, but isolates grouping to *FFSC* and *FGSC* were also identified. *FIESC* isolate identification to species-level is unachievable due to unavailable reference sequence information in NCBI. These findings are in contrast with isolates identified in maize and small grains in North Carolina where *F. graminearum*, *F. culmorum*, *F. avenaceum*, and *F. verticillioides* are the predominant species

infecting spikes and kernels (Walker et al. 2001; Hagler et al. 1986). Additionally, recovery of sorghum stalk rot pathogen *F. thapsinum* was not confirmed (Funnell-Harris et al. 2016). The results of this study are limited to the environmental conditions prevalent during sampling and the reference isolates included in analysis were limited to characterized species for which published sequences of TEF, RPB2, and TUB2 genes were available, which may be in contrast to previous studies. Collection from additional sorghum fields over several years with variable environmental conditions could provide a clearer understanding of the abundance and diversity of *Fusarium* species causing FHB in sorghum fields in North Carolina.

Although aggressiveness amongst isolates was insignificant for *Fusarium* spp. evaluated in this study, C-46-19C (*F. sporotrichioides*; from wheat) was the most aggressive, followed by F-4D3 (*F. verticillioides*; from maize), 104C-1 (*F. proliferatum*), FA2 (*F. equiseti*), RM5-3 (*FIESC*), S3-4C-1 (*F. fujikuroi*), and S5-1C-1 (*F. verticillioides*; from sorghum). Isolate aggressiveness is important because it can be influenced by type and prevalence of mycotoxin production and may provide insight into the efficacy of management strategies. For example, the most aggressive species in this study, *F. sporotrichioides*, may produce type A trichothecenes, T-2 and HT-2, that are acutely toxic to animals, and moniliformin (Bottalico and Perrone 2002; Proctor et al. 2009; Moretti 2017). The least aggressive isolate was S1-3C-1 (*FIESC*). Many members of *FIESC* many produce trichothecenes, moniliformin, and zearalenone (Moretti 2017). However, since *FIESC* were most abundant and the least aggressive isolate was in *FIESC*, additional experiments could clarify whether *FIESC* is of significance to FHB infection and mycotoxin contamination of grain. Furthermore, these sorghum pathogenicity assays with *Fusarium* species recovered from FHB-infected panicles can serve as a foundation for potential FHB resistance breeding evaluations.

Presently, *Fusarium* spp. resistance in sorghum is restricted to stalk and crown rot (Funnell-Harris et al. 2014; Funnell and Pedersen 2006; Funnell-Harris et al. 2016); however, this study shows that isolate aggressiveness to leaf tissue varies significantly in isolates obtained from infected panicles. A potential limitation of this evaluation of pathogenicity is that the resistance response of a variety may differ within sorghum panicles compared to leaves. Similar pathogenicity studies on wheat resistances to FHB have been examined using *Microdochium nivale*, a contributor to the disease complex, using detached leaves (Browne and Cooke 2004), and another study using detached leaves from several varieties of oats and wheat to evaluate *F. langsethiae* (isolates obtained from oat and wheat), *Microdochium nivale*, *Microdochium majus*, *F. poae*, and *F. culmorum* isolate pathogenicity (Imathiu et al. 2009). In contrast to the findings in these sorghum bioassays, the wheat and oat studies indicated that whole-plant resistance can be examined using detached leaf assays, but the results may not detect potential susceptibility factors or accurately reflect resistance in the panicles (Browne and Cooke 2004; Imathiu et al. 2009). This study was also not exhaustive of isolates and host resistance, as only 11 isolates and two varieties were tested. Other existing varieties may exhibit alternative resistance to FHB infection by other isolates. Expanding the number and type of isolates tested against a handful of other varieties may provide additional insight to the extent of *Fusarium* isolate pathogenicity and aggressiveness to sorghum varieties.

In order to effectively control disease, chemical modes of action should be integrated into management programs alongside cultural practices and resistance. This study identified dose responses of collected isolates to fungicide chemistries that may be effective for control of sorghum FHB isolates. The 12 isolates were sensitive to the DMI chemistries tebuconazole and prothioconazole, which agrees with current literature for these modes of action (Avozani, Reis, et

al. 2014; Spolti et al. 2012; Ivic et al. 2011; Spolti et al. 2014; Cowger et al. 2016). Isolate sensitivity to DMI fungicides is expected, but few resistant isolates have been previously identified in other studies (Yin et al. 2009; Spolti et al. 2014; Chen et al. 2019b). EC50 estimates for isolates to tebuconazole and prothioconazole in this study align with previous findings for *F. graminearum*, *F. proliferatum*, and *F. verticillioides* collected from corn and wheat at tested DMI concentrations (Masiello et al. 2019). Isolates were insensitive to fluopyram at higher doses, and absolute EC50 estimates could not be determined at higher doses because relative growths of isolates increased. Higher isolate relative growth may have been influenced by fluopyram precipitating out of solution at 100 ppm, and so relative growth values at 100 ppm were excluded during EC50 estimate determination. Fluopyram field applications are administered at low concentrations, and due to its low binding energy for the succinate dehydrogenase binding site, cross-resistance to other SDHI fungicides may be avoided (J. Wang et al. 2017). Isolate insensitivity to fluopyram after expanding the range of concentrations may suggest an alternative respiration pathway or natural insensitivity. It should be noted that insensitivity of *Fusarium* spp. to trifloxystrobin is common and may respire via a different mechanism (Masiello et al. 2019; Dubos et al. 2011), and a similar mechanism may be present in the SDHI pathway. The remaining isolates showed moderate sensitivities, but absolute EC50 estimates could not be determined. These fungicides, especially DMI chemistries, not only have the potential to reduce FHB severity, they also have the potential to reduce mycotoxin accumulation in infected grain (Avozani, Reis, et al. 2014). Further investigations of field management efficacy are important in the effort to achieve sustainability in sorghum production.

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Tables and Figures

Table 2.1. Polymerase chain reaction cycling conditions for genes, translation elongation factor (TEF), β -tubulin (TUB), and ribosomal protein subunit II (RPB2), used for molecular identification and comparison of *Fusarium* spp. isolates collected in North Carolina sorghum fields in 2017 and 2018 survey collections.

Cycle Step	Cycles	Cycle Temperature		
		TEF ¹	TUB2 ²	RPB2 ³
Initial Denaturation	1	94°C x 3 min	94°C x 3 min	94°C x 3 min
Denaturation	35	94°C x 1 min	94°C x 1 min	94°C x 30 s
Annealing		55°C x 30 s	58.1°C x 30 s	65°C x 35 s
Extension		72°C x 30 s	72°C x 1 min	72°C x 1.5 min
Final Extension	1	72°C x 10 min	72°C x 10 min	72°C x 10 min
Infinite Hold	1	10°C	10°C	10°C

¹Translation elongation factor (Divakara et al. 2014; O'Donnell et al. 1998)

²Beta-tubulin (Taylor et al. 2016; O'Donnell and Cigelnik 1997)

³Ribosomal protein subunit II (Cerón-Bustamante et al. 2018; O'Donnell et al. 2010)

Table 2.2. List of *Fusarium* reference sequences for translation elongation factor (TEF), ribosomal protein subunit II (RPB2), and β -tubulin (TUB2) gathered from NCBI for phylogeny analysis.

Name	Identity	NCBI Accession		
		TEF ¹	RPB2 ²	TUB2 ³
<i>Fusarium incarnatum</i> strain NRRL 31160	<i>Fusarium semitectum</i> ; <i>Fusarium incarnatum</i>	GQ915510.1	GQ915494.1	GQ915444.1
<i>Gibberella pulicaris</i> strain FRC R-07843	<i>Fusarium sambucinum</i>	GQ915512.1	GQ915496.1	GQ915446.1
<i>Fusarium verticillioides</i> strain MRC 826	<i>Fusarium verticillioides</i>	MH582330.1	MH582218.1	FN545365.1
<i>Fusarium oxysporum</i> strain FRC O-1890	<i>Fusarium oxysporum</i>	KF466420.1	KF466409.1	KF466442.1
<i>Fusarium cerealis</i> strain FRC R-09624	<i>Fusarium crookwellense</i>	GQ915505.1	GQ915489.1	GQ915439.1
<i>Fusarium avenaceum</i> isolate SICAUCC 18-0001	<i>Fusarium avenaceum</i>	MK226333.1	MK396098.1	MK253102.1
<i>Fusarium armeniacum</i> strain NRRL 6227	<i>Fusarium armeniacum</i>	HM744692.1	JX171560.1	HQ141667.1
<i>Fusarium venenatum</i> strain FRC R-09186	<i>Fusarium venenatum</i>	GQ915515.1	GQ915499.1	GQ915449.1
<i>Fusarium graminearum</i> strain NRRL 31084	<i>Fusarium graminearum</i>	HM744693.1	JX171644.1	HQ141668.1
<i>Fusarium poae</i> strain FRC T-0962	<i>Fusarium poae</i>	GQ915511.1	GQ915495.1	GQ915445.1
<i>Fusarium sporotrichioides</i> strain NRRL 53434	<i>Fusarium sporotrichioides</i>	HM744687.1	HQ154475.1	HQ141662.1

Table 2.2. (continued).

<i>Fusarium culmorum</i> strain FRC R-09618	<i>Fusarium culmorum</i>	GQ915506.1	GQ915490.1	GQ915440.1
<i>Fusarium equiseti</i> strain NRRL 13405	<i>Fusarium equiseti</i>	GQ915507.1	GQ915491.1	GQ915441.1
<i>Fusarium proliferatum</i> strain ITEM 2287	<i>Fusarium proliferatum</i>	KF466422.1	KF466411.1	KF466444.1
<i>Fusarium fujikuroi</i> strain HKM 41	<i>Fusarium fujikuroi</i>	KF466416.1	KF466405.1	KF466438.1
<i>Fusarium boothii</i> strain NRRL 26916	<i>Fusarium boothii</i>	GQ915503.1	GQ915487.1	GQ915437.1
<i>Fusarium longipes</i> strain NRRL 20695	<i>Fusarium longipes</i>	GQ915509.1	GQ915493.1	GQ915443.1
<i>Fusarium</i> sp. FRC R-06979	<i>Fusarium scirpi</i>	GQ915513.1	GQ915497.1	GQ915447.1
<i>Fusarium langsethiae</i> strain NRRL 53409	<i>Fusarium langsethiae</i>	HM744667.1	HQ154455.1	HQ141642.1
<i>Fusarium kyushuense</i> strain NRRL 25349	<i>Fusarium kyushuense</i>	GQ915508.1	GQ915492.1	GQ915442.1
<i>Fusarium mangiferae</i> strain UMAF 0924	<i>Fusarium mangiferae</i>	KP753402.1	KP753442.1	KP753388.1
<i>Fusarium nygamai</i> strain FRC M-7492	<i>Fusarium nygamai</i>	KF466419.1	KF466408.1	KF466441.1
<i>Fusarium hostae</i> strain NRRL 29642	<i>Fusarium hostae</i>	KF466418.1	KF466407.1	KF466440.1
<i>Fusarium globosum</i> strain NRRL 26131	<i>Fusarium globosum</i>	KF466417.1	KF466406.1	KF466439.1
<i>Neonectria coccinea</i> var. faginata CBS 119160	<i>Neonectria coccinea</i> *	DQ789740.1	DQ789811.1	DQ789883.1

¹Translation elongation factor, ² Ribosomal protein subunit II, ³ Beta-tubulin, *Outgroup

Table 2.3. Representative *Fusarium* spp. isolates collected during 2017 and 2018 sorghum growing seasons in North Carolina and used in pathogenicity and fungicide sensitivity assays. Known isolate *F. sporotrichioides* from wheat was also included in bioassays (C-46-19C).

Isolate Name¹	Year	Location (County)	Host	Identity²
C-46-19C	2017	Stokes	Wheat	<i>F. sporotrichioides</i>
RM 5-3	2017	Nash	Sorghum	<i>FIESC</i>
FA2	2017	Nash	Sorghum	<i>F. equiseti</i>
104C-1	2017	Pasquotank	Sorghum	<i>F. proliferatum</i>
S1-3C-1	2017	Johnston	Sorghum	<i>FIESC</i> *
S2-1C-2	2017	Johnston	Sorghum	<i>FIESC</i>
S3-4C-1	2017	Johnston	Sorghum	<i>F. fujikuroi</i>
S4-2C-2	2017	Johnston	Sorghum	<i>FIESC</i>
S5-1C-1	2017	Johnston	Sorghum	<i>F. verticillioides</i>
F-4D3	2018	-	Maize	<i>F. verticillioides</i>
FU18A-10-1	2018	Johnston	Sorghum	<i>F. fujikuroi</i>
104C-3†	2017	Pasquotank	Sorghum	<i>F. graminearum</i> *

¹ Isolates excluded from pathogenicity assays and used only in fungicide sensitivity assays are indicated by †.

² Isolate identity was confirmed with multilocus sequencing (TEF, TUB2, and RPB2 regions) for all isolates, except 104C-3 and S1-3C-1 where NCBI BLAST of the TEF gene was used (indicated by *).

Table 2.4. Log-mean AUDPC values for each variety-by-isolate combination of two sorghum varieties and 11 *Fusarium* isolates (Table 2.3). Two detached leaf assays performed as two replicates in time were combined for statistical analysis accounting for the effect of trial. Identities were determined from phylogenetic analysis of translation elongation factor (TEF), beta-tubulin (TUB2), and ribosomal protein subunit II (RPB2).

Isolate	Identity	Variety	Mean AUDPC (log) ¹	
			Trial 1	Trial 2
Control	-	SH80G4	1.400 ab ²	1.400 ab
Control	-	Pioneer 84P80	2.760 b	2.760 ab
S1-3C-1	<i>FIESC</i>	SH80G4	1.660 ab	6.193 cdefg
S1-3C-1	<i>FIESC</i>	Pioneer 84P80	1.689 ab	6.540 defg
S2-1C-2	<i>FIESC</i>	SH80G4	1.984 ab	6.933 efg
S2-1C-2	<i>FIESC</i>	Pioneer 84P80	1.635 ab	7.088 fg
S3-4C-1	<i>F. fujikuroi</i>	SH80G4	6.076 cdefg	6.257 cdefg
S3-4C-1	<i>F. fujikuroi</i>	Pioneer 84P80	6.011 cdefg	6.435 cdefg
S4-2C-2	<i>FIESC</i>	SH80G4	4.872 cd	6.407 cdefg
S4-2C-2	<i>FIESC</i>	Pioneer 84P80	0.935 a	6.218 cdefg
S5-1C-1	<i>F. verticillioides</i>	SH80G4	6.022 cdefg	6.318 cdefg
S5-1C-1	<i>F. verticillioides</i>	Pioneer 84P80	5.642 cdefg	6.641 defg
RM5-3	<i>FIESC</i>	SH80G4	5.278 cdef	7.237 g
RM5-3	<i>FIESC</i>	Pioneer 84P80	5.267 cde	7.138 g
FA2	<i>F. equiseti</i>	SH80G4	5.710 cdefg	6.912 efg
FA2	<i>F. equiseti</i>	Pioneer 84P80	4.702 c	7.254 g
104C-1	<i>F. proliferatum</i>	SH80G4	5.595 cdefg	6.309 cdefg
104C-1	<i>F. proliferatum</i>	Pioneer 84P80	6.255 cdefg	6.363 cdefg
C-46-19C	<i>F. sporotrichioides</i>	SH80G4	6.196 cdefg	7.220 g
C-46-19C	<i>F. sporotrichioides</i>	Pioneer 84P80	6.112 cdefg	7.103 g
F-4D3	<i>F. verticillioides</i>	SH80G4	6.042 cdefg	6.277 cdefg
F-4D3	<i>F. verticillioides</i>	Pioneer 84P80	6.216 cdefg	6.571 defg
FU18A-10-1	<i>F. fujikuroi</i>	SH80G4	5.936 cdefg	6.024 cdefg
FU18A-10-1	<i>F. fujikuroi</i>	Pioneer 84P80	5.846 cdefg	6.266 cdefg

¹Data were analyzed in R statistical software with a Gamma distribution using the ‘glm’ function

with a random error term, shape parameter k = XX and scale parameter theta = YY to

approximate measurement error because for some treatment groups, all observations were zero.

(R Core Team, 2019).

²Means of the same letter are not significantly different at $\alpha = 0.05$.

Table 2.5. Relative and absolute EC50 estimates (ppm) and confidence intervals for tebuconazole using 6 concentrations (10, 1, 0.1, 0.01, 0.001, and 0 ppm) and 12 *Fusarium* isolates (Table 2.3) collected 2017-2018 from sorghum, wheat, and corn.

Isolate	Tebuconazole ¹			Absolute	Lower ²	Upper ³	Lack-of-Fit (p-value)
	Relative	Lower ²	Upper ³				
104C-1	0.25	0.09	0.41	0.13	0.063	0.20	0.000
C-46-19C	0.062	0.0057	0.12	0.029	0.0043	0.054	0.0
FA2	0.037	-0.022	0.096	0.012	-0.0042	0.028	0.000
RM5-3	0.057	-0.031	0.14	0.019	-0.0078	0.045	0.00
S1-3C-1	0.14	0.092	0.18	0.11	0.078	0.15	0.000
S2-1C-2	0.42	0.33	0.51	0.33	0.26	0.39	0.00
S3-4C-1	0.077	0.046	0.11	0.049	0.031	0.067	0.000
S4-2C-2	0.087	0.056	0.12	0.081	0.053	0.11	0.00
S5-1C-1	0.014	-0.023	0.052	0.002	-0.002	0.0063	0.00
F-4D3	0.011	-0.038	0.061	0.00063	-0.0014	0.0026	0.00
FU18A-10-1	0.10	-0.11	0.32	0.0081	-0.0053	0.021	0.000
104C-3	0.027	-0.046	0.10	0.0025	-0.0025	0.0076	0.0087

¹ Relative EC50 estimates are based on the inflection point on a dose response curve, while absolute estimates refer to the concentration at which 50% relative growth is observed; both estimates were determined with a four-parameter log-logistic model (LL.4). Confidence intervals were determined using Delta method in R statistical software, and function “modelFit” in the “drc” package (Ritz et al. 2016) was used to perform a Lack-of-Fit test for LL.4 models with the data for each isolate.

^{2,3} Lower and upper ends of confidence intervals for EC50 estimates.

* indicates an EC50 estimate outside of the tested dose range. Cells containing dashes, estimates and parameters outside the tested dose range, and identical estimates and parameters indicate indeterminate EC50 estimates or parameters due to isolate insensitivity or model failure.

Table 2.6. Absolute and relative EC50 estimates (ppm) and confidence intervals were determined for prothioconazole using 6 concentrations (10, 1, 0.1, 0.01, 0.001, and 0 ppm) and 12 *Fusarium* isolates (Table 2.3) collected in 2017 and 2018 from sorghum, wheat, and corn.

Isolate	Prothioconazole ¹			Absolute	Lower ²	Upper ³	Lack-of-Fit (p-value)
	Relative	Lower ²	Upper ³				
104C-1	10.38	-26.76	47.51	6.05	-13.31	25.41	0.00
C-46-19C	8.72	-15.32	32.76	1.82	-1.73	5.37	0.0
FA2	0.71	-2.08	3.50	0.20	-0.64	1.04	0.000
RM5-3	2.32	-4.94	9.58	0.93	-0.55	2.41	0.00
S1-3C-1	1.06	0.66	1.46	1.22	-0.22	2.67	0.0005
S2-1C-2*	45.55	-157.97	249.07	5.43	-14.65	25.51	0.0278
S3-4C-1*	11.06	-25.19	47.32	0.37	-0.58	1.33	0.0045
S4-2C-2	0.19	-0.18	0.57	0.17	-0.15	0.49	0.000
S5-1C-1	0.057	-0.15	0.27	0.0074	-0.016	0.031	0.00
F-4D3	0.24	-0.96	1.46	0.00075	-0.0023	0.0038	0.000
FU18A-10-1	0.35	-1.99	2.69	0.05580	-0.31	0.42	0.00
104C-3	0.085	-0.57	0.74	0.00038	-0.0025	0.0033	0.00

¹ Relative EC50 estimates are based on the inflection point on a dose response curve, while absolute estimates refer to the concentration at which 50% relative growth is observed; both estimates were determined with a four-parameter log-logistic model (LL.4). Confidence intervals were determined using Delta method in R statistical software, and function “modelFit” in the “drc” package (Ritz et al. 2016) was used to perform a Lack-of-Fit test for LL.4 models with the data for each isolate.

^{2,3} Lower and upper ends of confidence intervals for EC50 estimates.

* indicates an EC50 estimate outside of the tested dose range. Cells containing dashes, estimates and parameters outside the tested dose range, and identical estimates and parameters indicate indeterminate EC50 estimates or parameters due to isolate insensitivity or model failure.

Table 2.7. Relative and absolute EC50 estimates (ppm) and confidence intervals were determined for fluopyram using 6 concentrations (10, 1, 0.1, 0.01, 0.001, and 0 ppm) and 12 *Fusarium* isolates (Table 2.3) collected in 2017 and 2018 from sorghum, wheat, and corn.

Isolate	Fluopyram ¹			Absolute	Lower ²	Upper ³	Lack-of-Fit (p-value)
	Relative	Lower ²	Upper ³				
104C-1*	1.91	-182.68	186.51	-	-	-	0.00
C-46-19C*	247.21	-	-	-	-	-	0.00
FA2*	56.49	-	-	6.2e-168	6.2e-168	6.2e-168	0.000
RM5-3*	68.89	-	-	1.093e-72	-	-	0.00
S1-3C-1*	12.64	-393.92	419.21	9.79	-290.54	310.13	0.00
S2-1C-2*	0.029	-0.30	0.36	-	-	-	0.00
S3-4C-1*	530.57	-48386.05	49447.20	-	-	-	0.0
S4-2C-2*	0.14	-16.78	17.06	-	-	-	0.0
S5-1C-1	0.058	-0.55	0.66	0.046	-0.56	0.66	0.0
F-4D3	0.096	-0.082	0.27	0.041	-0.52	0.60	0.0
FU18A-10-1*	33.16	-	-	0	0	0	0.000
104C-3*	5.82	-3330.04	3341.69	0	0	0	0.00

¹ Relative EC50 estimates are based on the inflection point on a dose response curve, while absolute estimates refer to the concentration at which 50% relative growth is observed; both estimates were determined with a four-parameter log-logistic model (LL.4). Confidence intervals were determined using Delta method in R statistical software, and function “modelFit” in the “drc” package (Ritz et al. 2016) was used to perform a Lack-of-Fit test for LL.4 models with the data for each isolate. ^{2,3} Lower and upper ends of confidence intervals for EC50 estimates.

* indicates an EC50 estimate outside of the tested dose range. Cells containing dashes, estimates and parameters outside the tested dose range, and identical estimates and parameters indicate indeterminate EC50 estimates or parameters due to isolate insensitivity or model failure.

Table 2.8. Absolute EC50 estimates (ppm) and confidence intervals were determined for fluopyram using 8 concentrations (10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001 and 0 ppm) and 12 *Fusarium* isolates (Table 2.3) collected in 2017 and 2018 from sorghum, wheat, and corn. Isolate responses at 100 ppm were excluded from analysis due to fluopyram precipitation from solution at the media amendment step.

Isolate	Fluopyram ¹			Lack-of-Fit (p-value)
	Absolute	Lower ²	Upper ³	
104C-1*	-	-	-	-
C-46-19C*	-	-	-	-
FA2*	-	-	-	-
RM5-3*	-	-	-	-
S1-3C-1*	-	-	-	-
S2-1C-2	6.50	-19.61	32.62	0.2707
S3-4C-1*	-	-	-	-
S4-2C-2*	-	-	-	-
S5-1C-1*	-	-	-	-
F-4D3*	-	-	-	-
FU18A-10-1*	-	-	-	-
104C-3*	-	-	-	-

¹ Relative EC50 estimates are based on the inflection point on a dose response curve, while absolute estimates refer to the concentration at which 50% relative growth is observed; both estimates were determined with a four-parameter log-logistic model (LL.4). Confidence intervals were determined using Delta method in R statistical software, and function “modelFit” in the “drc” package (Ritz et al. 2016) was used to perform a Lack-of-Fit test for LL.4 models with the data for each isolate.

^{2,3} Lower and upper ends of confidence intervals for EC50 estimates.

* indicates an EC50 estimate outside of the tested dose range. Cells containing dashes, estimates and parameters outside the tested dose range, and identical estimates and parameters indicate indeterminate EC50 estimates or parameters due to isolate insensitivity or model failure.

Table 2.9. Relative and absolute EC50 estimates (ppm) and confidence intervals were determined for trifloxystrobin using 6 concentrations (10, 1, 0.1, 0.01, 0.001, and 0 ppm) and 12 *Fusarium* isolates (Table 2.3) collected in 2017 and 2018 from sorghum, wheat, and corn.

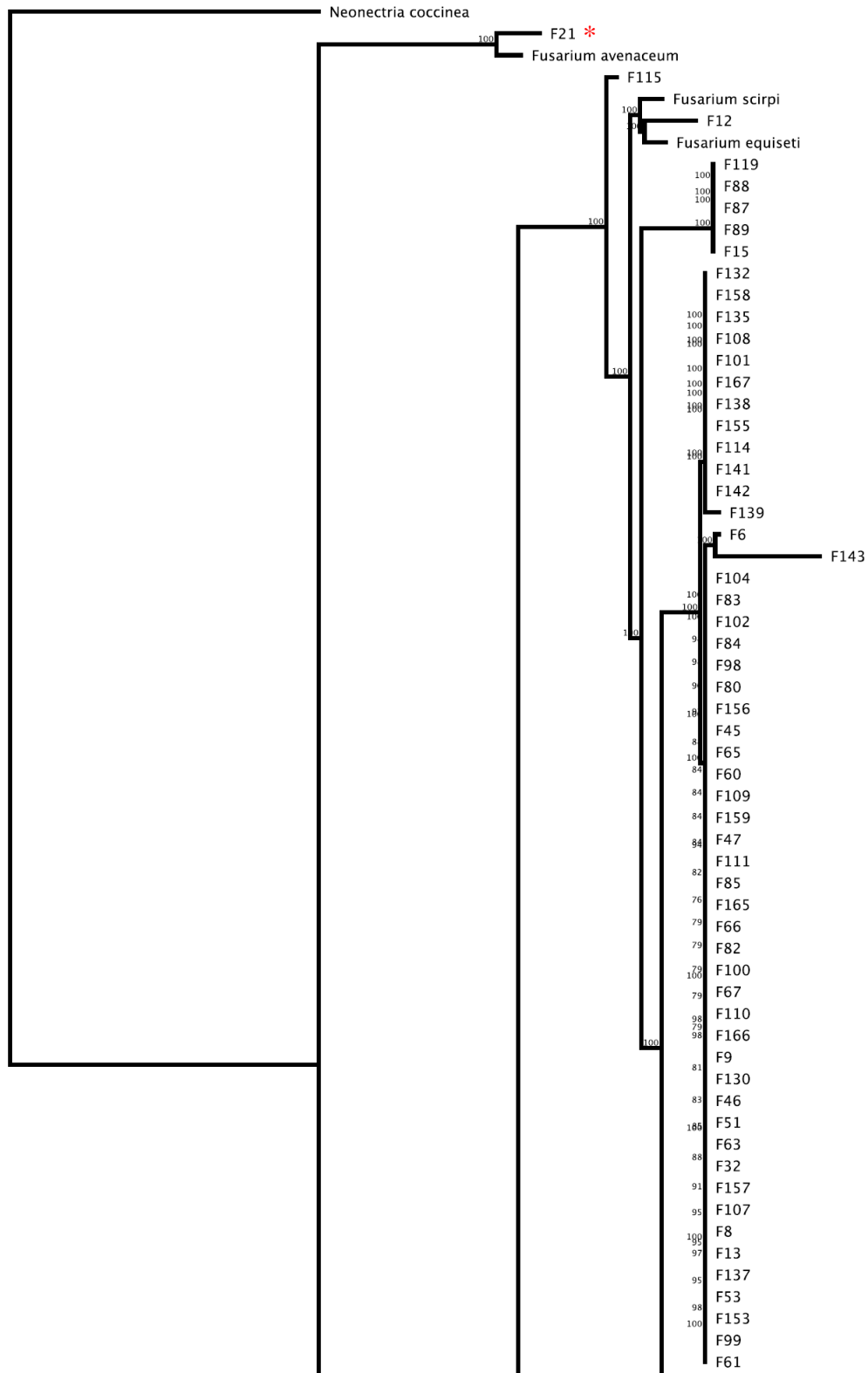
Isolate	Trifloxystrobin ¹			Absolute	Lower ²	Upper ³	Lack-of-Fit (p-value)
	Relative	Lower ²	Upper ³				
104C-1*	0.0021	0.0004	0.0038	-	-	-	0.3996
C-46-19C*	-	-	-	-	-	-	-
FA2	0.099	-0.90	1.10	0.11	-1.017	1.24	0.0624
RM5-3*	4.02e+31	4.02e+31	4.02e+31	-	-	-	0.00
S1-3C-1*	-	-	-	-	-	-	-
S2-1C-2	0.034	-0.16	0.23	0.028	-0.13	0.19	0.7396
S3-4C-1*	0.15	-0.65	0.96	-	-	-	0.3744
S4-2C-2*	-	-	-	-	-	-	-
S5-1C-1*	0.16	-0.15	0.47	-	-	-	0.9976
F-4D3*	0.24	-0.54	1.033	-	-	-	0.1343
FU18A-10-1*	26.13	-	-	7.05e-250	7.05e-250	7.05e-250	0.001
104C-3*	-	-	-	-	-	-	-

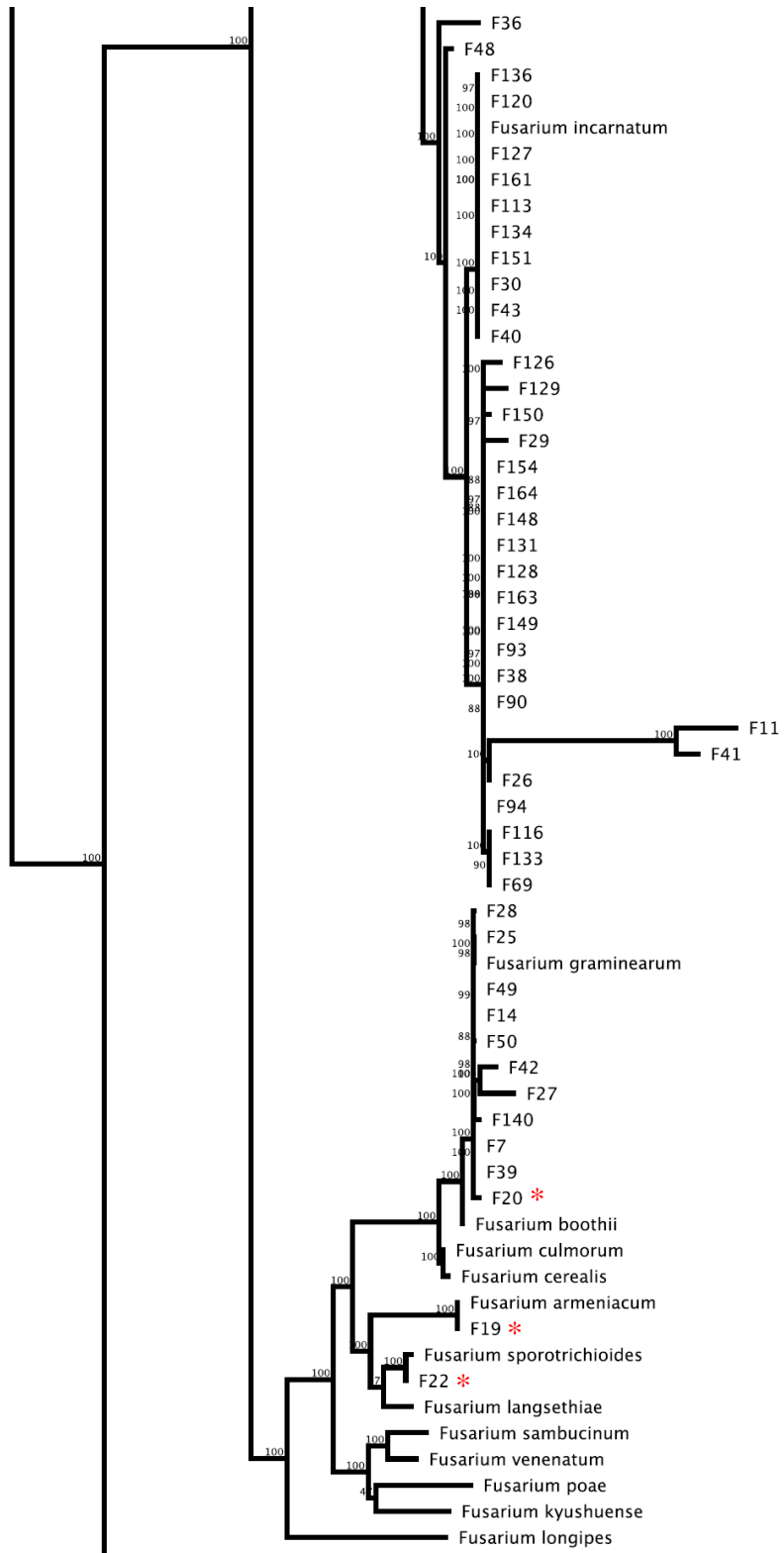
¹ Relative EC50 estimates are based on the inflection point on a dose response curve, while absolute estimates refer to the concentration at which 50% relative growth is observed; both estimates were determined with a four-parameter log-logistic model (LL.4). Confidence intervals were determined using Delta method in R statistical software, and function “modelFit” in the “drc” package (Ritz et al. 2016) was used to perform a Lack-of-Fit test for LL.4 models with the data for each isolate.

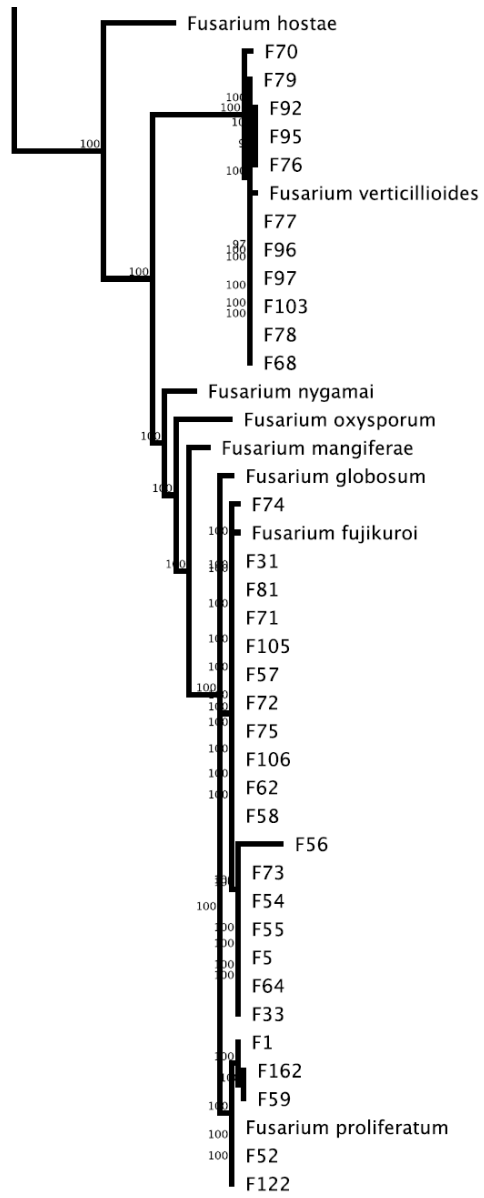
^{2,3} Lower and upper ends of confidence intervals for EC50 estimates.

* indicates an EC50 estimate outside of the tested dose range. Cells containing dashes, estimates and parameters outside the tested dose range, and identical estimates and parameters indicate indeterminant EC50 estimates or parameters due to isolate insensitivity or model failure.

Figure 2.1. RAxML phylogenetic tree was constructed using *Fusarium* spp. translation elongation factor, beta-tubulin, and ribosomal protein subunit II sequences mask-aligned with 0% gaps run with 12345 seed and a bootstrap value of 20. Isolates were collected from North Carolina sorghum fields during 2017-2018; wheat and corn isolates were obtained from Dr. Christina Cowger (USDA-ARS) and Dr. Thiago Marino (formerly NCSU) (Appendix Table A.1). Identity *Fusarium* sequences were obtained from the NCBI Database (Table 2.2). Reference sequences are marked with *. The tree is in three segments.







0.06

2017-2018 *Fusarium* Species Complex Prevalence

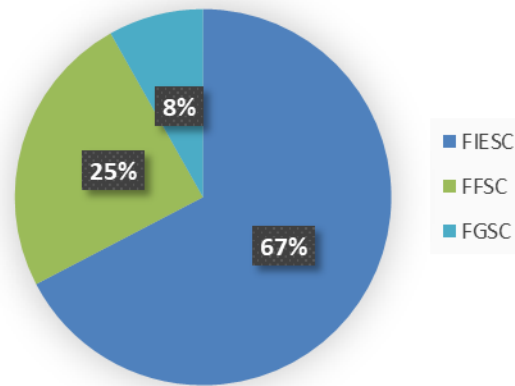


Figure 2.2. Species complex prevalence for 144 collected *Fusarium* isolates from North Carolina sorghum fields in 2017-2018. Identifications based on BLASTn analysis using translation elongation factor (TEF) sequences. 22 isolates were excluded from the calculation due to insufficient TEF sequences (11) or obtained as known reference isolates (11). FIESC = *Fusarium incarnatum-equiseti* species complex, FFSC = *Fusarium fujikuroi* species complex, and FGSC = *Fusarium graminearum* species complex.

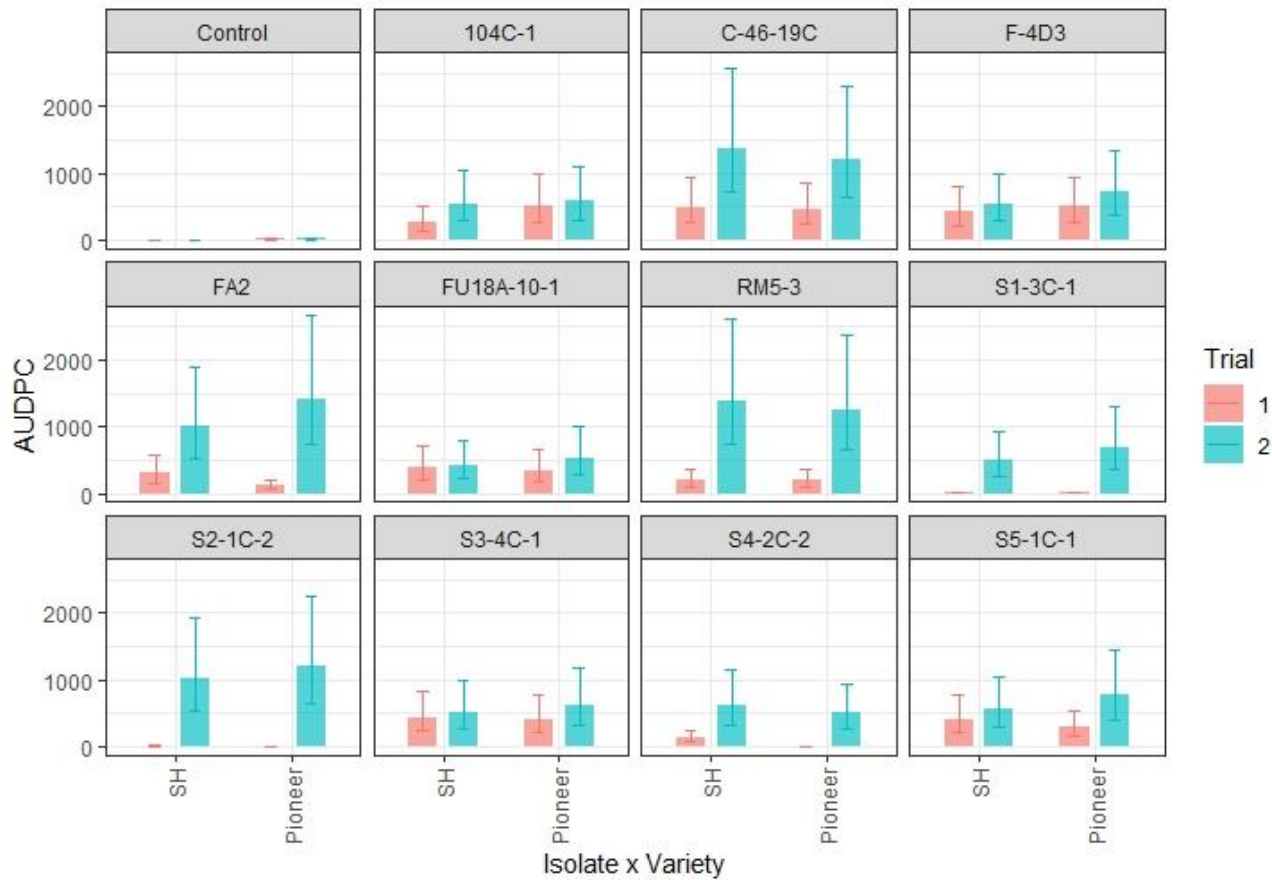


Figure 2.3. Calculated mean AUDPC values and associated 95% confidence intervals for each of 11 *Fusarium* isolates with SH80GH4 and Pioneer 84P80 sorghum varieties. Log-mean AUDPC values for each treatment are listed in Table 2.4. Species identities for each isolate are: 104C-1 = *F. proliferatum*; C-46-19C = *F. sporotrichioides*; F-4D3, S5-1C-1 = *F. verticillioides*; FA2 = *F. equiseti*; S3-4C-1, FU18A-10-1 = *F. fujikuroi*; and S1-3C-1, S2-1C-2, S4-2C-2, RM5-3 = *FIESC*.

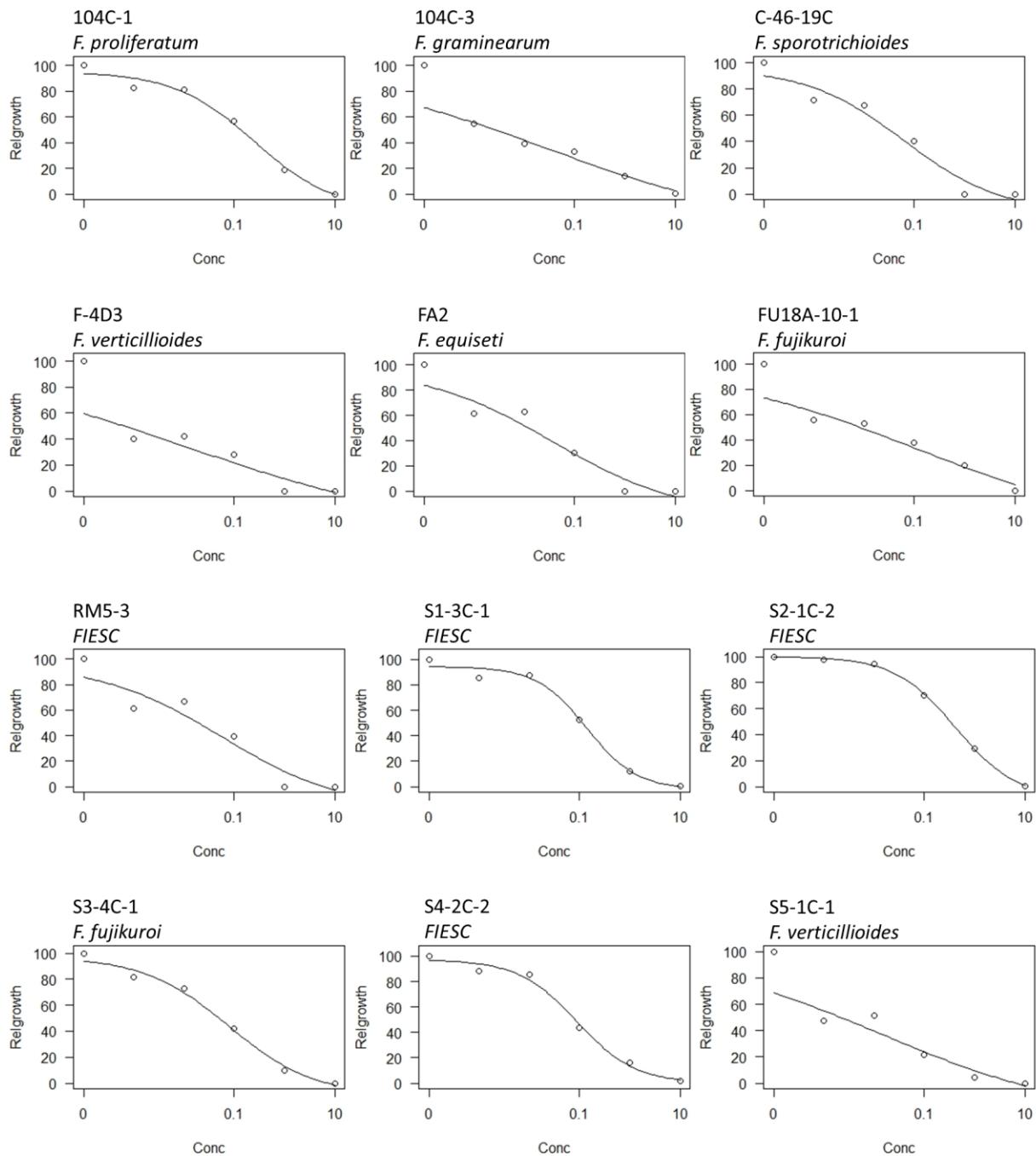


Figure 2.4. Dose-response curves generated with a four-parameter log-logistic model in R statistical software for tebuconazole with six tested concentrations (10, 1, 0.1, 0.01, 0.001, and 0 ppm) and each of the 12 *Fusarium* isolates examined for fungicide sensitivity (Table 2.5).

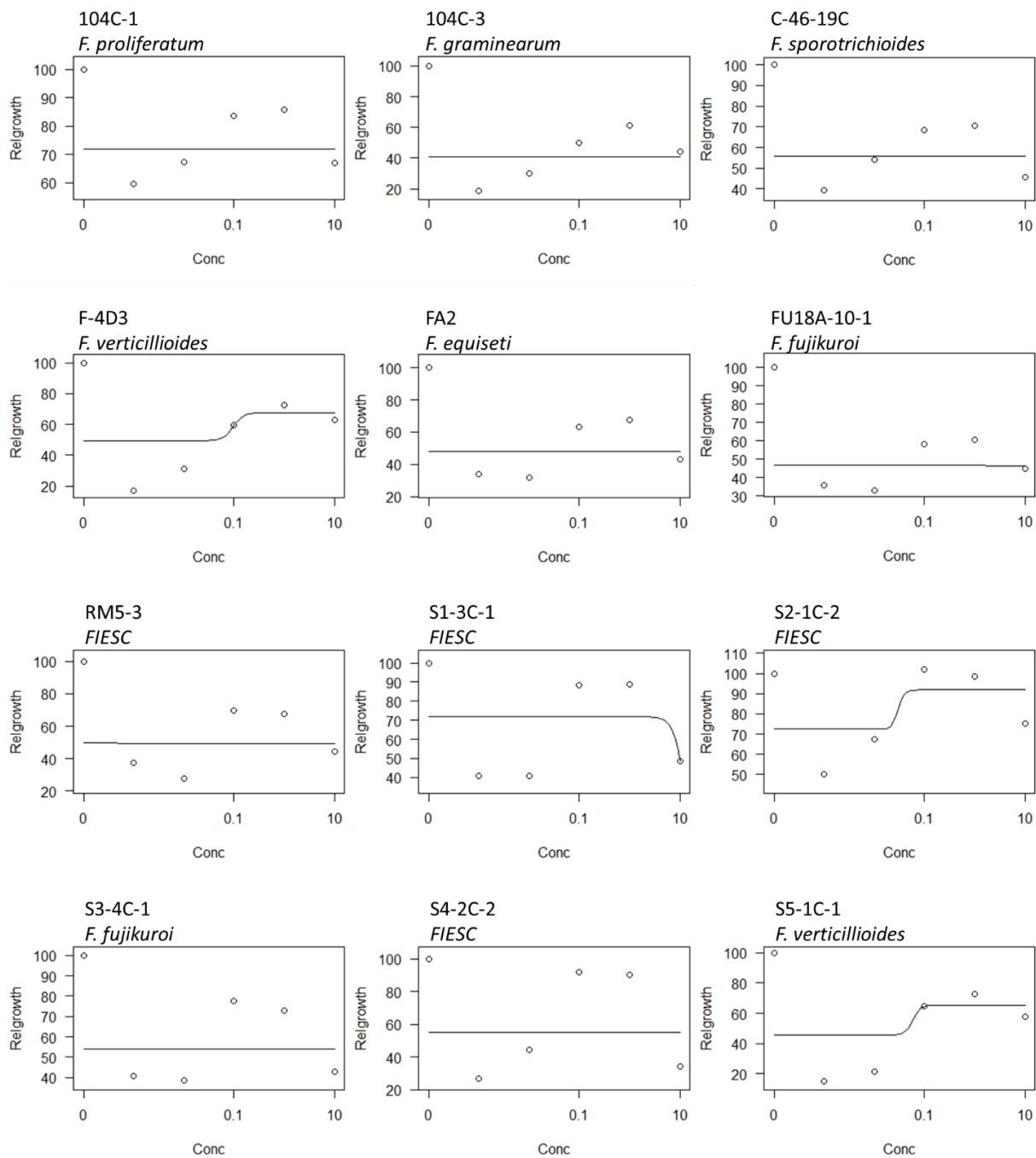


Figure 2.5. Dose-response curves generated with a four-parameter log-logistic model in R statistical software for fluopyram with six tested concentrations (10, 1, 0.1, 0.01, 0.001, and 0 ppm) and 12 *Fusarium* isolates examined for fungicide sensitivity.

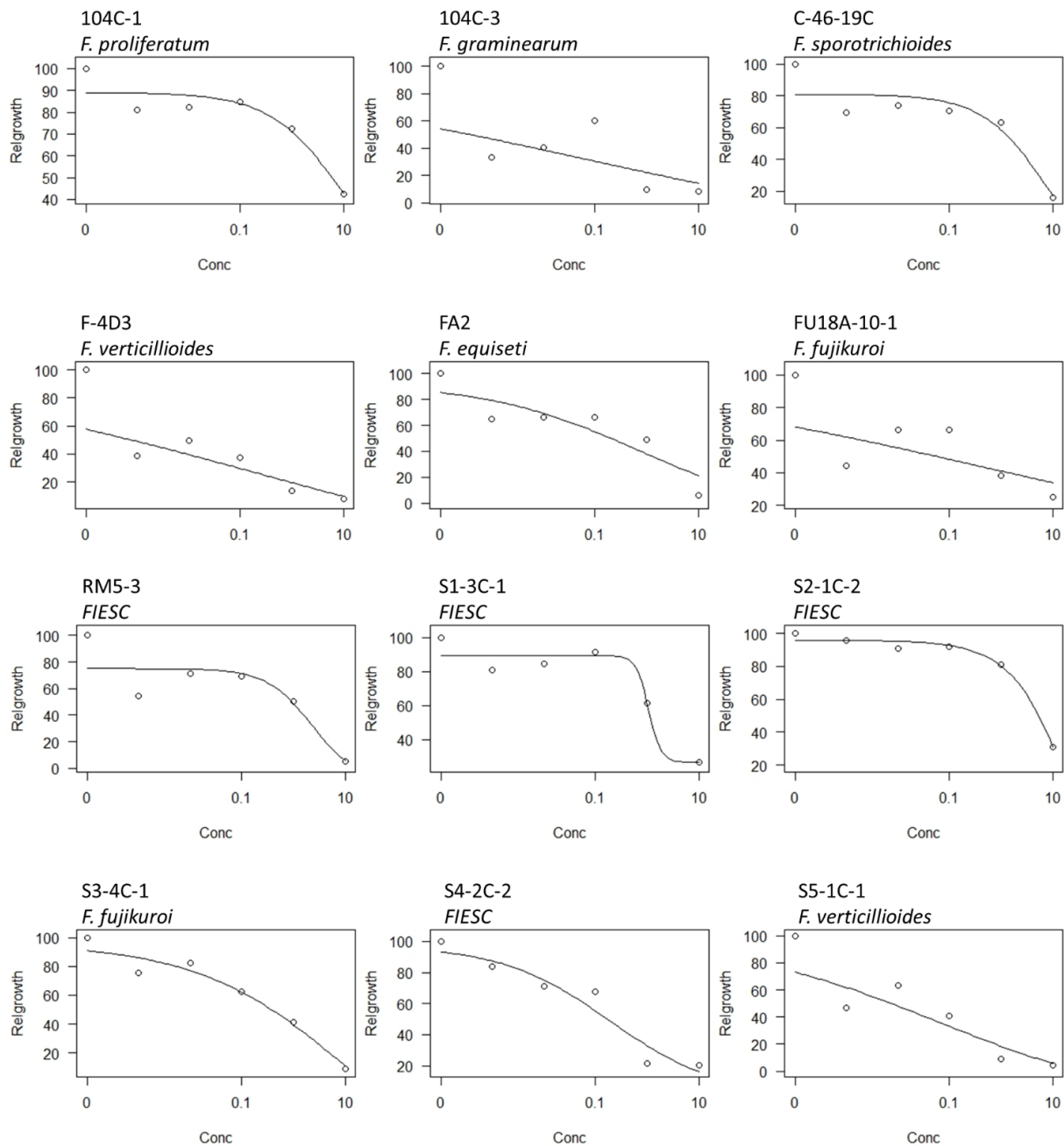


Figure 2.6. Dose-response curves generated with a four-parameter log-logistic model in R statistical software for prothioconazole with six tested concentrations (10, 1, 0.1, 0.01, 0.001, and 0 ppm) and 12 *Fusarium* isolates examined for fungicide sensitivity.

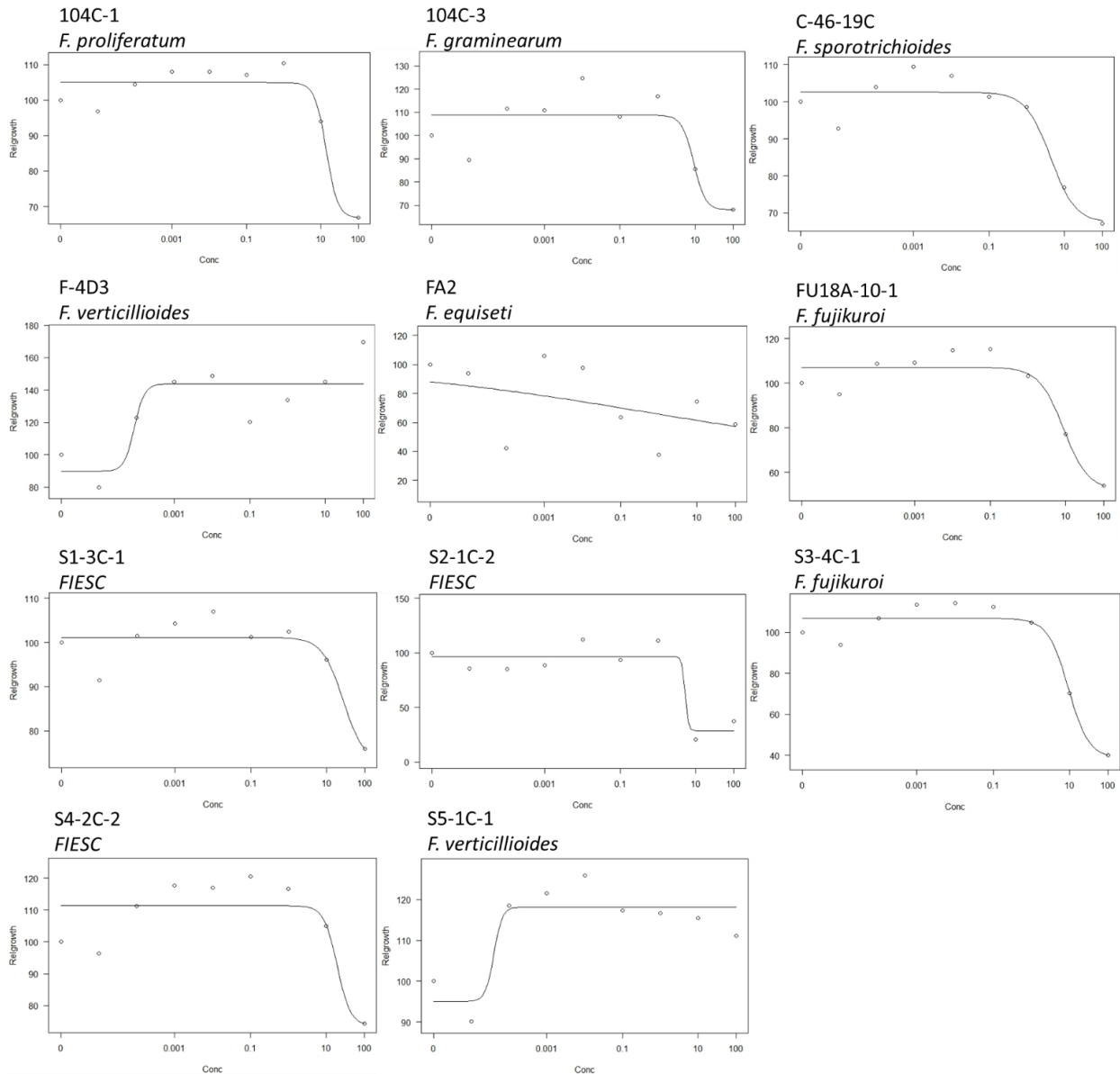


Figure 2.7. Dose-response curves generated with a four-parameter log-logistic model in R statistical software for fluopyram assay with nine tested concentrations (100, 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001, and 0 ppm) and 11 of the 12 *Fusarium* isolates examined for fungicide sensitivity.

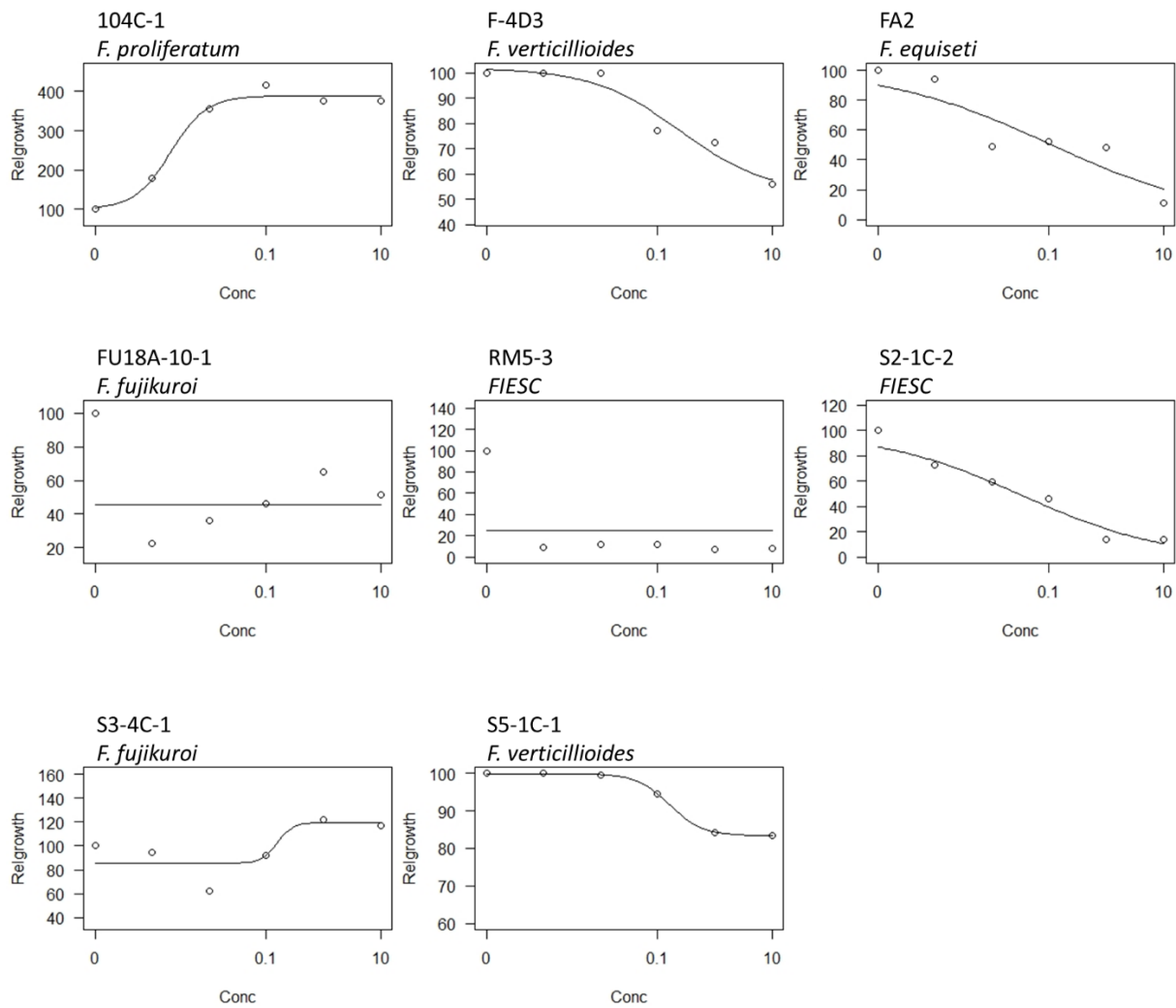


Figure 2.8. Dose-response curves generated with a four-parameter log-logistic model in R statistical software for trifloxystrobin with six tested concentrations (10, 1, 0.1, 0.01, 0.001, and 0 ppm) and 8 of the 12 *Fusarium* isolates examined for fungicide sensitivity.

CHAPTER III. Fungicide Timing Evaluations for Fusarium Head Blight Management

ABSTRACT

Sorghum grain disease management is limited by lack of available fungicides, specifically for fungi associated with the Fusarium head blight (FHB) disease complex. Understanding timing of fungicide applications is integral to implementing effective management strategies. In this project, fungicide timing experiments were conducted to examine the effects of fungicide selection and application timing on disease severity, AUDPC, yield and production of deoxynivalenol (DON), fumonisin (FUM), and zearalenone (ZEA) at harvest. The efficacy of four fungicides at three application timings was evaluated for FHB management in 2018 and 2019 at Lake Wheeler Road Facility Research Station (2018 and 2019) and the Upper Coastal Plain Research Station (2019). The fungicides prothioconazole (Proline), prothioconazole + trifloxystrobin (Delaro), fluopyram + trifloxystrobin (Luna Sensation), and prothioconazole + tebuconazole (Prosaro) were applied at 187 L/ha at heading, anthesis or soft-dough stages of sorghum growth. Mycotoxins were semi-quantitatively analyzed with ELISA kits. Severity and AUDPC of treatments in 2018 were not significantly different than non-treated controls. There were no significant differences in 2018 among treatments for DON, FUM, and ZEA levels ($P > 0.05$). In 2019, significant differences were observed for AUDPC and final disease severity ($P < 0.05$), but mean severities of treated plots were not lower than mean severities for non-treated plots. Significant differences between treatments were observed for DON levels between locations, and effect of location was significant ($P < 0.05$). FUM levels for samples at Lake Wheeler were below the limit of detection (LOD) of the ELISA kits, and samples were excluded from analysis. FUM levels at Upper Coastal showed no significance differences among the treatments. ZEA levels from both locations were combined for analysis; a

significant difference between Proline at anthesis and Prosaro at soft-dough was detected, but these means were not significantly different from control treatment means. Additional treatment replications and improved inoculum pressure in future experiments will target variable disease severity and low mycotoxin levels observed in these experiments. Further field experiments examining these chemistries across additional site years and with other varieties are required before concluding these fungicides may not be important for sorghum production.

INTRODUCTION

Fusarium head blight (FHB) is a disease in sorghum caused by several *Fusarium* spp., including mycotoxigenic *Fusarium graminearum* and *Fusarium verticillioides* species complexes. Current strategies for FHB management in North Carolina sorghum production are limited. Sorghum producers typically apply fertilizer and herbicides, but fungicides are not a standard application in the Southeast United States (Post, personal communication), and few are labeled for use on FHB in sorghum. Furthermore, conservation tillage systems are being used in sorghum rotations to prevent erosion; however, pathogen populations, including *Fusarium* spp., can build to high levels in remaining plant debris from this management strategy and in environments when conditions are conducive for disease development (Mourelos et al. 2014; Scarpino et al. 2015; Steinkellner and Langer 2004; Rojas et al. 2018; Rudd et al. 2001; Dill-Macky and Jones 2000). Current strategies for managing FHB in wheat and maize management may be applicable to managing the disease in sorghum. Chemical control options are available to combat FHB disease severity and mycotoxin contamination in barley and wheat systems (Rudd et al. 2001; Scarpino et al. 2015; L. Shah et al. 2018b; Caldwell et al. 2017; Limay-Rios and Schaafsma 2018).

Minimization of FHB severity and mycotoxin contamination in sorghum production is limited by the number of available chemistries that are registered for FHB control. Demethylase inhibitor (DMI) fungicides are the primary form of chemical management of FHB in wheat and ear rot (ER) in maize (Masiello et al. 2019; Becher et al. 2010; D. A. Shah et al. 2018). Industry standards are Caramba (metconazole; BASF, Durham, NC), Prosaro (prothioconazole + tebuconazole; Bayer Crop Science LP, St. Louis MO) and Proline (prothioconazole; Bayer Crop Science LP, St. Louis MO), which consist of DMI fungicide active ingredients (Cowger et al.

2016; Paul et al. 2010). Recommended application timings are at 50% anthesis (Feekes stage 10.5.1) in wheat because anthesis is the stage of greatest susceptibility to *Fusarium* infection (Caldwell et al. 2017; Yoshida et al. 2012; D'Angelo et al. 2014). The use of QoIs for FHB management systems is not recommended because it can increase deoxynivalenol (DON) concentrations in wheat and barley (Pirgozliev et al. 2002; Paul et al. 2018). While these products have been tested in wheat production systems, efficacy and optimal fungicide application timing in sorghum is presently unknown.

The objectives of this study were to determine the effects of fungicide type and application time on disease severity, yield, and mycotoxin production. My hypotheses were that (1) there were no differences among treatment timings for disease severity, yield, or DON, FUM, and ZEA production at harvest, and (2) fungicides containing DMI active ingredients would reduce mycotoxin production.

MATERIALS AND METHODS

Field Inoculum Preparation

A virulent *Fusarium graminearum* isolate (C-38-32A), collected from wheat in North Carolina, was increased on ten plates of ¼ strength PDA. Five to six sterilizable airflow spawn bags (Fungi Perfecti LLC, Olympia, WA) were filled with 1.2 kg cracked corn and 800 ml deionized water. Cracked corn was sealed inside bags to rehydrate it for 24 hours at room temperature (23°C average). Two times in succession, the bags were autoclaved for 1.5 hrs at 115.6°C (240°F) on slow setting and cooled to room temperature for 24 hrs without being opened. Ten 7-mm plugs from the *F. graminearum* isolate were added to each bag and incubated

for 14 days at 25°C. Bags were moved periodically during incubation to ensure even infection of kernels. Infected corn was air dried for 2-3 weeks or until completely dry.

Field Experimental Design

Fungicide selection and timing experiments were conducted in a field with sandy loam soil at the Lake Wheeler Road Facility Research Station (35°43'37.3" N, 78°40'47.9" W; Lake Wheeler) and at a field with Norfolk loamy sand at the Upper Coastal Plain Research Station (35°53'22.9"N 77°40'43.0"W; Upper Coastal). Sorghum was planted on 2 Jul 2018, 22 May 2019, and 1 Jul 2019 with 38.1 cm row spacing (Lake Wheeler) and 76.2 cm row spacing (Upper Coastal) and 6.096 m (2018) and 7.62 m (2019) long rows with a planting density of 395,200 seed/ha (160,000 seed/A). Row spacing was adjusted in the second year to improve weed management.

Fusarium-colonized corn was spread across plots at 10 g/m² by hand at booting. The test was arranged in a randomized complete block design factorial with four replicates, with a total of 12 fungicide timing treatments and one unsprayed control treatment. Fungicides were applied with a CO₂-pressurized backpack sprayer with an application volume of 187 L/ha at 103.42 kPa (15 psi) at crop heading (Feekes stage 10.5; entirety of the head emerged from sheath), anthesis (Feekes stage 10.5.1; 50% of head flowering), or soft-dough (Feekes stage 11.2; majority of kernels white and milky inside, others mealy but dry) (Table 3.1). Application rates were selected based on labeled spray volumes for FHB in corn or wheat or the most closely related labeled pathogen and/or crop. For example, the label rate for *Botrytis cinerea* on lemongrass was selected for applying Luna Sensation.

Field Experiment Data Collection and Sampling

In the 2018 growing season, disease severity was assessed on ten randomly selected heads per plot weekly beginning at Feekes stage 10.5 and ending at stage 11.3, excluding 14 Sept 2018 due to disruption of the trial by Hurricane Florence. In the 2019 growing season, individual plants were repeatedly assessed for disease severity as percent of total head symptomatic in order to follow disease progress on each head throughout the FHB epidemic. Harvest occurred on 15 Oct 2018, 17 Oct 2019 (Lake Wheeler), and 3 Sep 2019 (Rocky Mount). Yields were assessed from each whole plot. From each 2018 plot, a 250 mL subsample of grain was collected for mycotoxin analysis. A subsample size of at least 1 kg was collected per plot for mycotoxin analysis from both field sites in 2019. Subsamples were stored in a 2-8°C walk-in storage room from harvest until further processing.

Mycotoxin Extraction

Each grain subsample from field plots was mixed thoroughly and analyzed for presence of deoxynivalenol (DON), fumonisin (FUM), and zearalenone (ZEA). Excess plant debris was removed by filtering sample contents through a number four sieve. Grain from the 2018 site year was transferred to paper bags and placed in the Isotemp Oven 100 Series Model 116G (Fisher Scientific, Waltham, MA) at 40.6 °C for 72 hours. Grain from the 2019 Upper Coastal site was dried for seven days in an air-circulating trailer, while the Lake Wheeler field site was dried in the field for one additional week prior to harvest, achieving 14% grain moisture for Upper Coastal samples and around 16% grain moisture for Lake Wheeler samples. Dried samples were ground such that 95% of the sample passed through a 20-mesh sieve using a Laboratory Mill (Pertin Instruments, Stockholm, Sweden). Extraction and semi-quantitative detection for DON,

FUM, and ZEA content were performed following instructions provided in ELISA kits (Romer Labs, Singapore). FUM and ZEA were extracted in a fume hood by combining 10 g of ground sample to 50 mL of 70% methanol (1:5 ratio of sample to extraction solution). Mixtures were shaken for three minutes on a 2100 Platform Shaker at 230 rpm (Eppendorf, Hamburg, Germany). After settling, the top layer of extract was filtered through a Whatman #1 filter paper, and pH was adjusted between 6 and 8 according to manufacturer protocols. Subsequently, FUM samples were diluted in deionized water, and ZEA samples were diluted in 70% methanol, according to manufacturer recommendations. Samples were stored at 2-8°C in 50 mL Falcon tubes until ELISA quantification.

DON was extracted by shaking five grams ground sample in 25 mL deionized (DI) water for three minutes at 230 rpm using a Thermo SCIENTIFIC MAXQ 2000 shaker (Thermo Scientific, Waltham, MA). After allowing the sample to settle, the top layer of extract was filtered through a Whatman #1 filter paper, and the pH was adjusted according to manufacturer recommendations, diluted with DI water, and stored at 2-8°C in 50mL Falcon tubes until ELISA quantification.

Mycotoxin Detection

ELISA was performed according to the manufacturer guidelines for AgraQuant Deoxynivalenol, AgraQuant Total Fumonisin, and AgraQuant Zearalenone Plus ELISA mycotoxin detection kits (Romer Labs, Singapore). Diluted DON extracts for each of the Lake Wheeler and Upper Coastal samples (2019) were evaluated with the undiluted samples to determine limits for DON detection with the ELISA plate reader. Undiluted extracts were used for further analysis because concentrations were less than the 0.2 ppm limit of detection (LOD).

Standards and samples were duplicated per plate as technical replicates, and sample replicates within each treatment were placed on the same plate. Endpoint absorbances were read using a Cyt5 Bio Tek plate reader (Bio Tek Instruments, Winooski, VT) at 450 nm and 630 nm wavelengths. The difference in wavelengths was interpreted as the absorbance for each sample. Median absorbance for technical replicates was determined, and approximate mycotoxin quantity was calculated according to guidelines from Romer Labs. Quantitation of undiluted, 2019 DON samples was determined using standard curves and dilution factor of 1:5, instead of the kit 1:20 standard curves.

Data Analysis

The area under disease progress curve (AUDPC) was calculated for each treatment using ‘reshape2’ package (Wickham 2007) in RStudio version 3.3.1 (R Core Team, 2019). Yield, AUDPC, severity, and mycotoxin data were analyzed with linear mixed effects models using the ‘nlme’ package (Pinheiro et al. 2018) in RStudio version 3.3.1 (R Core Team, 2019). DON values in 2018 that were less than the limit of detection were assigned a value of 0.1 for statistical analysis. Treatment means were calculated and compared using the ‘emmeans’ package (Lenth 2019) in RStudio version 3.3.1 (R Core Team, 2019) and p-values were calculated with a Tukey correction to account for multiple comparisons. AUDPC and final severity data from 2019 field sites were analyzed separately from 2019 yield data, 2019 mycotoxin data, and from the 2018 site year data. AUDPC and final disease severity were log-transformed to best fit the mixed effects model. Estimated marginal means and confidence intervals were back transformed for graphical representation. Response data from both 2019

locations were combined to examine the effect of each treatment. Mycotoxin samples that were below the detectable limit of each kit were excluded from analysis.

RESULTS

2018 Field Trial Results

Fusarium head blight symptoms were first observed on 26 Aug 2018, prior to field inoculation, on some of the early emerging heads at soft-dough grain development. Isolated *Fusarium* species include *Fusarium verticillioides*, *Fusarium fujikuroi*, *Fusarium proliferatum*, and members of the *Fusarium incarnatum-equiseti* species complex (FIESC). There were no significant differences in disease severity, AUDPC, or yield (kg/ha) among treatments (Table 3.2). No significant differences in DON or ZEA were found amongst treatments. Although no significant differences were detected amongst treatments for ZEA, a trend for heading and soft-dough treatment means lower than anthesis can be observed. For FUM, each treatment grouped separately (Table 3.2), but treatments were not significantly different (Figure 3.1).

2019 Field Trial Results

FHB symptoms were first observed 25 Jul 2019 and 24 Aug 2019 for Rocky Mount and Lake Wheeler field sites, respectively. Data were combined for statistical analysis accounting for the effect of location because there was a significant effect of location ($P < 0.001$) to calculate treatment means for log AUDPC, log % Final Disease Severity, Yield, and DON (ppm) (Table 3.3 and 3.4). Statistical analysis of treatments for FUM (ppm) consisted of data from only Rocky Mount; all values for Lake Wheeler were below the limit of detection (LOD < 0.2 ppm; Table 3.4). ZEA (ppb) means for each fungicide-timing treatment across locations were combined in

each model to examine the effect of treatment (Table 3.4). Some values were below the limit of detection for zearalenone, and thereby excluded from analysis resulting in an unbalanced data set. The mean of the AUDPC for the Delaro (prothioconazole + trifloxystrobin) application at heading was higher than and significantly different from the non-treated control at heading (Figure 3.2), while the remaining treatments were not significantly different ($P > 0.05$). The mean of the log final disease severity for the Delaro application at anthesis was higher than and significantly different from control at heading ($P < 0.05$), and the remaining treatments were not significantly different from Delaro at anthesis and control at heading (Figure 3.2). Significant differences in yield were observed between sites (Figure 3.3). Significant differences in DON means among treatments across the two sites were detected ($P < 0.05$) (Figure 3.4). The effect of application timing and location on DON concentration was significant ($P < 0.0001$). FUM was below the detectable limit in every 2019 Lake Wheeler sample, and therefore excluded from further analysis. There were no differences in mean FUM estimates or confidence intervals for the Rocky Mount samples (Figure 3.5). Application timing and fungicide type did not have a significant effect on FUM or ZEA concentrations. Additionally, the only significant difference in ZEA estimates from the combined location analysis was between Proline at anthesis and Prosaro at soft-dough (Figure 3.5).

DISCUSSION

In the field experiments in this study, single fungicide applications do not reduce sorghum FHB AUDPC or final disease severity compared to the non-treated control. This may be the result of the fungicide application window for efficacious and economical fungicide applications being narrower than the timings examined in this study (Cowger et al. 2016).

Differences among treatment means for DON may suggest fungicide active ingredients influence DON accumulation; however, the non-treated control plots, one for each level of timing, were all identical and were assigned a timing for inclusion in data analysis. The variability among the control treatments for final severity, AUDPC, and mycotoxin accumulation is similar to disease severity and mycotoxin accumulation inconsistencies of FHB observed in wheat (Caldwell et al. 2017). Despite potential limitations due to chemical pre-harvest intervals (PHI), subsequent fungicide applications may be necessary to have clearer separation between treatments (Caldwell et al. 2017; D'Angelo et al. 2014; Yoshida et al. 2012). However, DON, FUM, and ZEA detection for both site years was below FDA allowable limits for cereals used for feed and human consumption, which suggests disease pressure was not sufficient or fungicides may not be an important component for sorghum production but may still be considered for efficacy during grain storage. Under storage, the potential for mycotoxin accumulation is still present especially if grain storage conditions are poor (high moisture content, little airflow) or grain is stored for an extended period of time (Vismer et al. 2019; Leslie 2014). Epidemic intensity may not have been sufficient in this study to address mycotoxin differences among treatments. While inoculations at boot stage may not have provided sufficient time for perithecia to develop and ascospores to mature and infect during anthesis (Champeil et al. 2004), an existing *Fusarium* population was observed on early emerging panicles. Additionally, fields surrounding Lake Wheeler were planted with field corn followed by sorghum both years before the field experiment was planted in July. Existing inoculum could have outcompeted the *F. graminearum* isolate used to inoculate the field, which may have reduced mycotoxin production potential compared to the inoculated isolate. Sorghum might also be more susceptible to *FIESC* strains than to *F. graminearum*, and future experiments might examine host susceptibility with several sorghum varieties.

Since fungicide-timing efficacies for FHB control in sorghum have not been previously examined, soft-dough was included as a potential timing in these studies. However, preharvest spray intervals (PHI) determine how late to spray a fungicide for FHB control, and for Caramba and Prosaro in wheat and barley the PHI is 30 days (D'Angelo et al. 2014; McMullen et al. 2012). Therefore, spraying fungicides at soft-dough for FHB in certain sorghum varieties, regardless of efficacy, would likely result in excessive residue levels (D'Angelo et al. 2014; McMullen et al. 2012). Contrary to the literature for wheat FHB control, DON levels in treatments containing trifloxystrobin were too low in this study to draw conclusions. Low DON levels may be caused by timing of fungal infections in the field compared to application timing. Early head emergence, flowering, established FHB pathogen populations, and late-season infection could affect the level and type of mycotoxin detected at harvest (D'Angelo et al. 2014; Yoshida et al. 2008). FUM and ZEA means were not different among treatments, which is consistent with detected levels in wheat (Scarpino et al. 2015); however, very little FUM or ZEA is even detected in wheat produced in the U.S. Subsample volume at harvest and co-extracted compounds, including lipids, carbohydrates, pigments, tannins, extract pH, and solvent composition, may have influenced DON, FUM, and ZEA detection and quantification on the treatment level (Leslie 2014).

Data between the two growing seasons could not be combined for analysis due to environmental factors that influenced disease development and yield. Shortly after planting in 2018, the field experienced saturated conditions followed by Hurricane Florence (14 Sept 2018) and Hurricane Michael (11 Oct 2018), which may have influenced the 2018 site year results. *Fusarium graminearum* infection in wheat and maize requires cool weather followed by warm, humid conditions, and late planting date and excessive moisture received in 2018 may have

impeded *F. graminearum* infection (Ali et al. 2005). An existing population of another *Fusarium* spp. could have out-competed *F. graminearum* inoculum at Lake Wheeler, especially since non-uniform field and panicle development was observed. Future experiments should incorporate inoculated and uninoculated controls to determine indigenous inoculum levels and inoculation success. Additionally, there were no pre- or post- emergent herbicides or insecticides applied to the field in 2018 to manage weed and insect pests, and birds caused damage during grain filling and maturation. Despite inconsistent results in the 2018 experiment, trends were consistent among each site year suggesting these fungicides may not be essential for sorghum production. Further investigations are necessary with additional treatment replications across multiple site years to determine the role of field fungicide efficacy with other sorghum varieties and during grain storage.

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Tables and Figures

Table 3.1. Fungicide name, active ingredient, Fungicide Resistance Action Committee (FRAC) Group, and application rate for the fungicide-timing field experiments carried out in 2018-2019. All fungicide applications were applied at 3.78 L carrier volume of water.

Fungicide	Active ingredient(s)	FRAC Group	Rate (ml/ha)
Delaro 325SC	Prothioconazole + trifloxystrobin	3, 11	584.62
Luna Sensation	Fluopyram + trifloxystrobin	7, 11	555.39
Proline 480SC	Prothioconazole	3	416.54
Prosaro 421SC	Prothioconazole + tebuconazole	3	599.24

Table 3.2. Mean values for final disease severity, AUDPC, Yield, DON (ppm), FUM (ppm), and ZEA (ppb) for the 2018 Lake Wheeler location for control of sorghum Fusarium head blight (FHB) with fungicide selection and timing. Control treatments were excluded from analysis due to the resulting unbalanced data set in 2018.

Fungicide	Timing	Final Disease Severity (%)	AUDPC	Yield (bu/A) ¹	DON (ppm) ²	FUM (ppm)	ZEA (ppb)
Delaro 325SC	Heading	33.74 a ³	707.34 a	6.06 a	0.137 a	3.1 j	726 a
Delaro 325SC	Anthesis	24.64 a	518.47 a	7.75 a	0.159 a	2.8 i	1092 a
Delaro 325SC	Soft-Dough	27.16 a	502.61 a	9.54 a	0.106 a	2.6 f	828 a
Luna Sensation	Heading	32.16 a	456.39 a	7.72 a	0.135 a	3.3 k	768 a
Luna Sensation	Anthesis	27.64 a	582.66 a	6.62 a	0.106 a	2.8 h	987 a
Luna Sensation	Soft-Dough	19.86 a	534.49 a	7.25 a	0.127 a	1.9 d	675 a
Proline 480SC	Heading	24.88 a	528.97 a	6.59 a	0.145 a	2.6 g	826 a
Proline 480SC	Anthesis	23.91 a	494.93 a	5.94 a	0.106 a	1.9 b	892 a
Proline 480SC	Soft-Dough	28.98 a	422.67 a	6.39 a	0.139 a	3.9 l	796 a
Prosaro 421SC	Heading	23.39 a	560.94 a	6.61 a	0.166 a	1.9 c	814 a
Prosaro 421SC	Anthesis	24.63 a	592.44 a	7.92 a	0.126 a	1.8 a	911 a

Table 3.2. (continued).

Prosaro 421SC	Soft-Dough	23.40 a	479.23 a	7.38 a	0.123 a	2.0 e	750 a
Non-treated Control*	-	32.8	624.36	7.58	0.1	3.2	718

¹ Yields based on bushels/acre (using grain sorghum standard weight and moisture 56 lbs/bushel [25.4 kg/bu and 2.47 acre/hectare]).

² DON limit of detection (LOD) is 0.2 ppm. Most of the samples were below the LOD and were assigned the value 0.1 ppm for statistical analyses.

³ Means with the same letter(s) are not significantly different ($P > 0.05$).

* Non-treated control means calculated separate from treatment statistical analyses.

Table 3.3. Mean values for log final disease severity (%) and log(AUDPC) for the 2019 Lake Wheeler and Rocky Mount field sites for control of sorghum Fusarium head blight with fungicide selection and timing.

Fungicide	Timing	Rocky Mount		Lake Wheeler	
		logFinal Disease Severity (%)	logAUDPC	logFinal Disease Severity (%)	logAUDPC
Delaro 325SC	Heading	2.66 ab ¹	5.00 abc	2.81 ab	5.08 bc
Delaro 325SC	Anthesis	2.72 ab	4.92 abc	2.76 ab	5.00 abc
Delaro 325SC	Soft-Dough	2.60 ab	4.73 ab	2.74 ab	4.96 abc
Luna Sensation	Heading	2.62 ab	4.91 abc	2.82 ab	5.11 c
Luna Sensation	Anthesis	2.62 ab	4.83 abc	2.65 ab	4.92 abc
Luna Sensation	Soft-Dough	2.52 a	4.77 abc	2.69 ab	5.01 abc
Proline 480SC	Heading	2.57 ab	5.00 abc	2.60 ab	4.85 abc
Proline 480SC	Anthesis	2.62 ab	4.93 abc	2.66 ab	4.90 abc
Proline 480SC	Soft-Dough	2.69 ab	4.83 abc	2.71 ab	5.06 bc
Prosaro 421SC	Heading	2.59 ab	4.71 a	2.69 ab	4.98 abc
Prosaro 421SC	Anthesis	2.70 ab	4.94 abc	2.56 ab	4.85 abc
Prosaro 421SC	Soft-Dough	2.56 ab	4.82 abc	2.78 ab	5.00 abc
Control	Heading	2.61 ab	4.89 abc	2.49 a	4.75 abc
Control	Anthesis	2.61 ab	4.85 abc	2.75 ab	5.03 abc
Control	Soft-Dough	2.57 ab	4.90 abc	2.89 b	5.10 bc

¹ Means with the same letter(s) are not significantly different ($P > 0.05$).

Table 3.4. Mean values for yield (bushels/Acre), DON (ppm), FUM (ppm), and ZEA (ppb) for the 2019 Lake Wheeler and Rocky Mount field sites for control of sorghum Fusarium head blight with fungicide selection and timing determined using mixed effects models.

Fungicide	Timing	Rocky Mount		Lake Wheeler			
		Yield (bu/A) ¹	DON (ppm) ²	Yield (bu/A) ¹	DON (ppm) ²	FUM (ppm) ³	ZEA (ppb)
Delaro 325SC	Heading	87.4 cd ⁴	0.0918 ab	19.7 a	0.1254 abcde	0.581 a	40.4 ab
Delaro 325SC	Anthesis	111.8 d	0.0893 ab	20.2 a	0.1143 abcde	0.790 a	42.7 ab
Delaro 325SC	Soft-Dough	100.9 cd	0.0817 a	24.3 ab	0.1138 abcde	0.535 a	87.4 ab
Luna Sensation	Heading	97.7 cd	0.1047 abc	22.4 ab	0.1325 abcde	0.756 a	71.1 ab
Luna Sensation	Anthesis	89.2 cd	0.0884 ab	20.1 a	0.1155 abcde	0.658 a	37.9 ab
Luna Sensation	Soft-Dough	86.4 cd	0.0894 ab	25.2 ab	0.1103 abcde	0.603 a	76.8 ab
Proline 480SC	Heading	61.8 bc	0.1199 abcde	21.1 a	0.1604 e	0.753 a	109.6 ab
Proline 480SC	Anthesis	87.0 cd	0.0937 ab	20.6 a	0.1344 bcde	0.603 a	47.4 a
Proline 480SC	Soft-Dough	89.9 cd	0.0880 ab	25.0 ab	0.1066 abcd	0.506 a	44.5 ab
Prosaro 421SC	Heading	105.6 d	0.0909 ab	20.3 a	0.1570 de	0.422 a	48.2 ab

Table 3.4. (continued).

Prosaro 421SC	Anthesis	97.0 cd	0.0913 ab	19.7 a	0.1362 bcde	0.561 a	61.9 ab
Prosaro 421SC	Soft-Dough	97.6 cd	0.1022 abc	17.7 a	0.1217 abcde	0.622 a	61.5 b
Control	Heading	88.2 cd	0.1150 abcde	22.0 ab	0.1461 cde	0.571 a	35.0 ab
Control	Anthesis	82.5 cd	0.0955 abc	17.7 a	0.1260 abcde	0.632 a	64.5 ab
Control	Soft-Dough	96.6 cd	0.1005 abc	30.5 ab	0.1216 abcde	0.550 a	49.1 ab

¹ Yields based on bushels/acre (using grain sorghum standard weight and moisture 56 lbs/bushel [25.4 kg/bu and 2.47 acre/hectare]).

^{2,3} DON and FUM limits of detection (LOD) is 0.2 ppm. Values below the LOD were excluded from analysis. FUM means are based on Rocky Mount mycotoxin quantification because all Lake Wheeler values were below the LOD.

⁴ Means with the same letter(s) are not significantly different ($P > 0.05$).

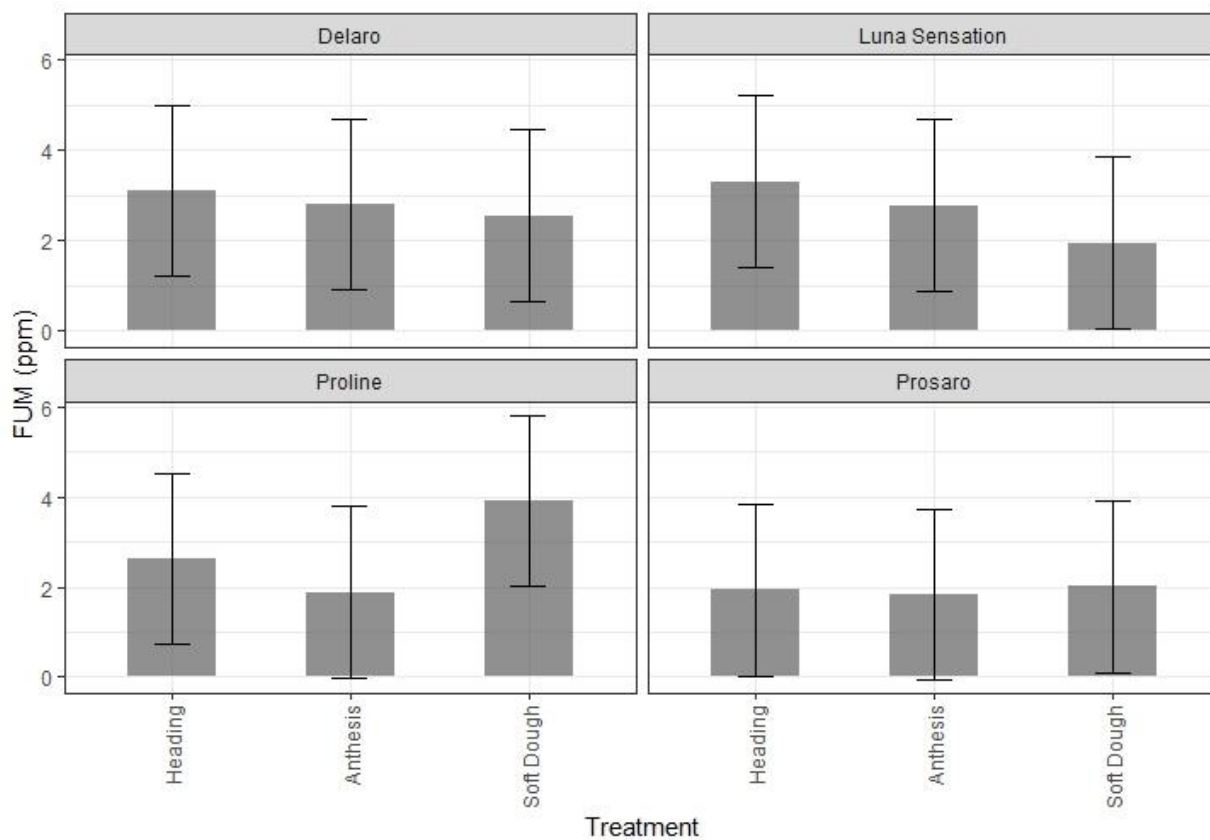


Figure 3.1. FUM means and confidence intervals (ppm) for each fungicide - timing treatment for Lake Wheeler field experiment 2018. Means and confidence intervals were generated from a mixed effects model. Control treatments were excluded from analysis due to the resulting unbalance data set in 2018, but the average FUM concentration for the non-treated control was 3.2 ppm.

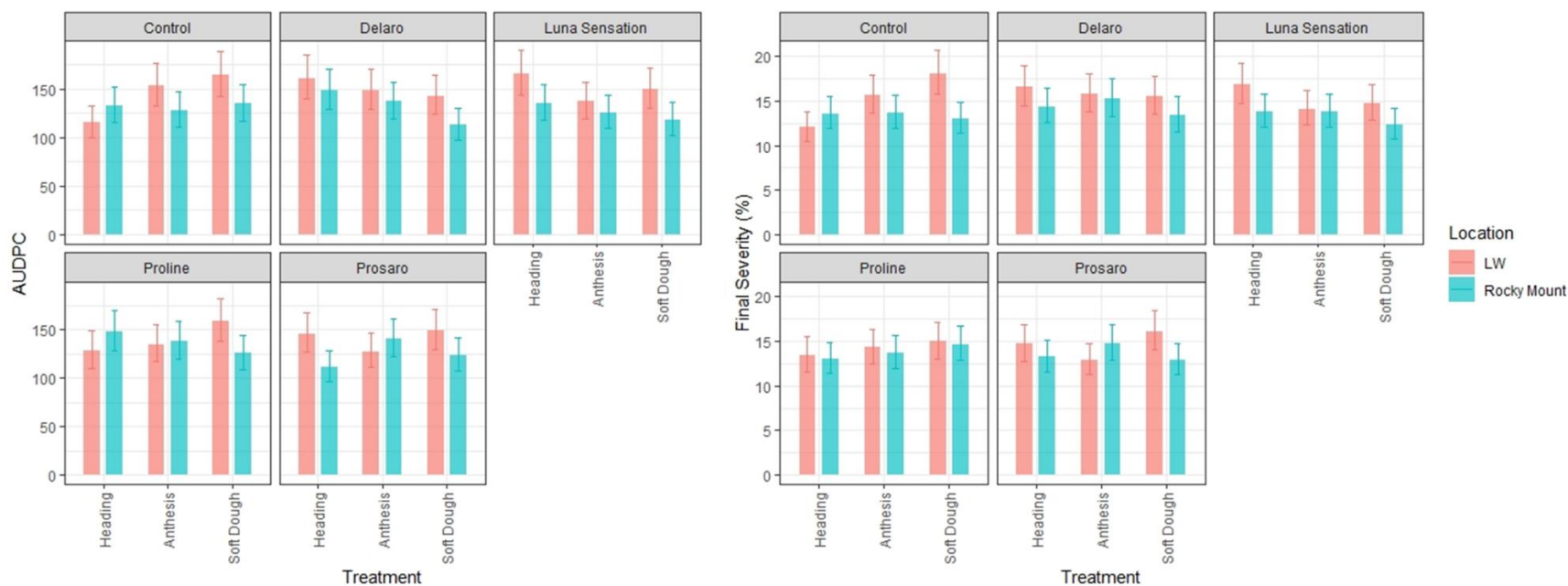


Figure 3.2. AUDPC and % final severity means and confidence intervals for each fungicide - timing treatment for Lake Wheeler (red) and Rocky Mount (blue) 2019 field sites. Means and confidence intervals were generated from a mixed effects model that accounted for differences between the two field sites.

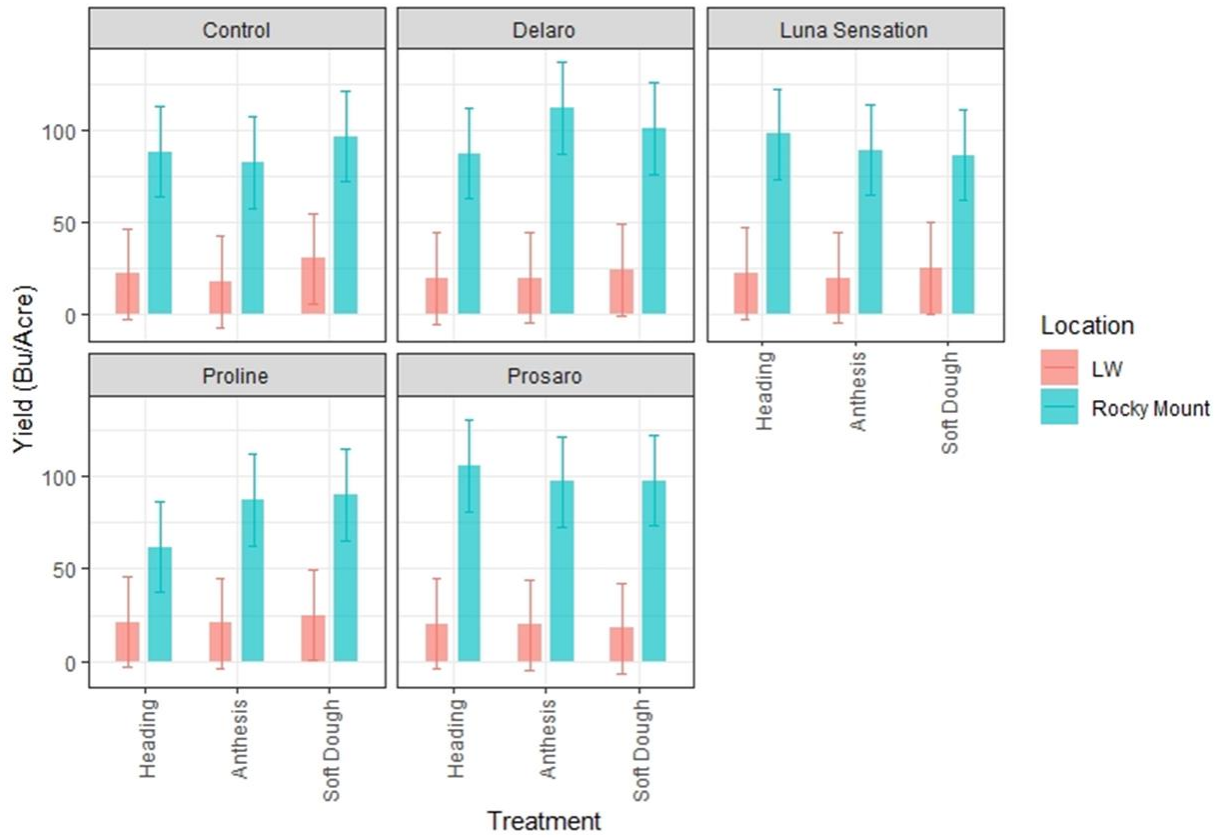


Figure 3.3. Yield (bushels/acre) means and confidence intervals for each fungicide -timing treatment for Lake Wheeler (red) and Rocky Mount (blue) 2019 field sites. Yields based on bushels/acre (using grain sorghum standard weight and moisture 56 lbs/bushel). Means and confidence intervals were generated from a mixed effects model that accounted for differences between the two field sites.

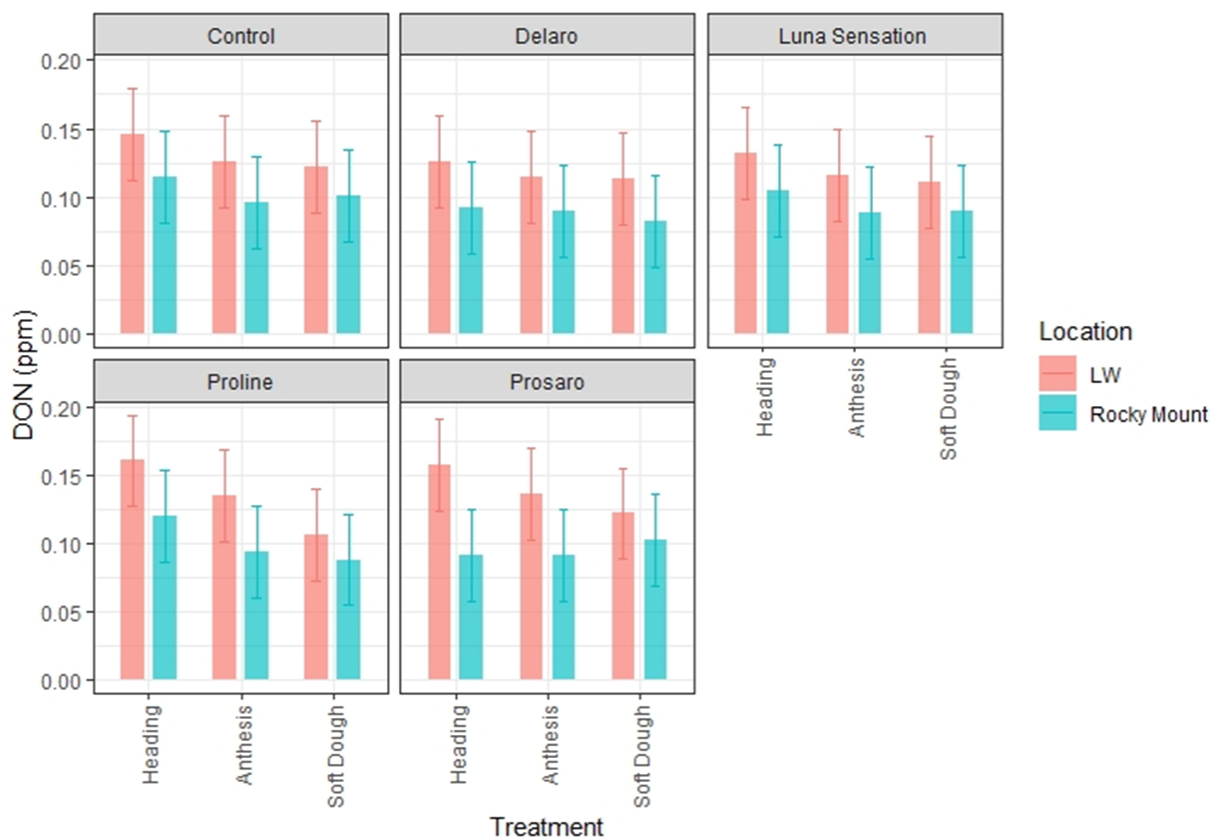


Figure 3.4. DON means and confidence intervals (ppm) for each fungicide - timing treatment for Lake Wheeler (red) and Rocky Mount (blue) 2019 field sites. Means and confidence intervals were generated from a mixed effects model that accounted for differences between the two field sites.

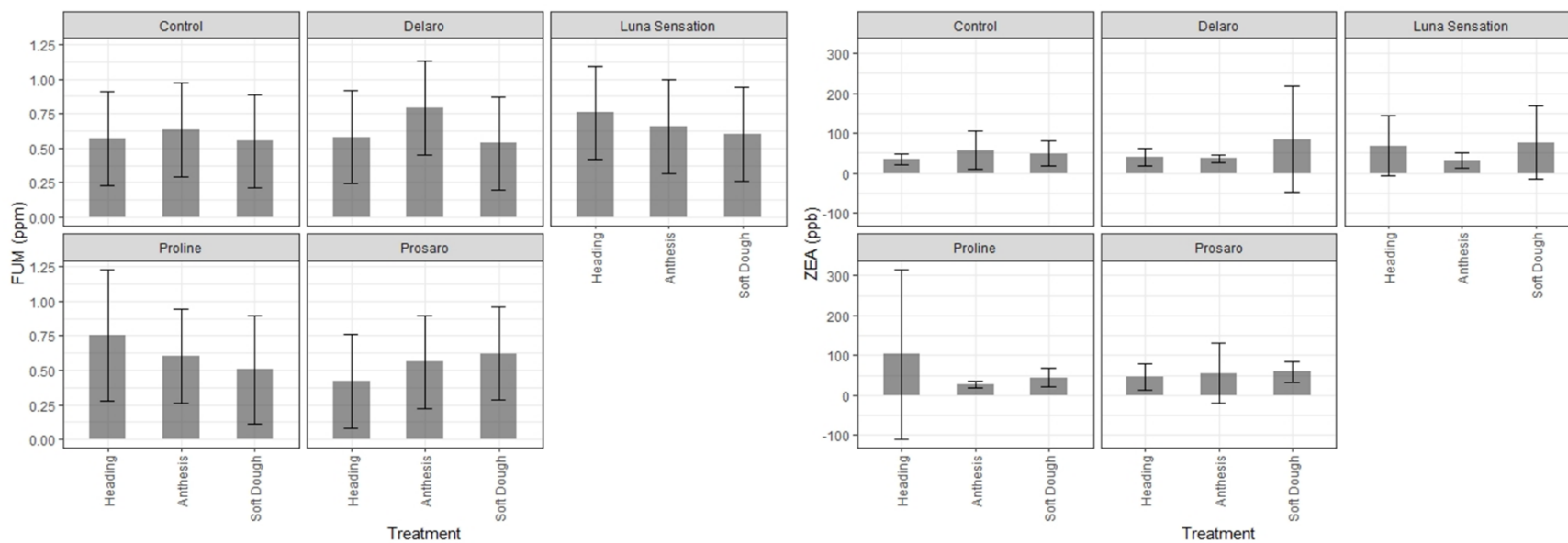


Figure 3.5. FUM means and confidence intervals (ppm) and ZEA means and confidence intervals (ppb) for each fungicide-timing treatment for Rocky Mount 2019 field site. Lake Wheeler 2019 samples were below the limit of detection (LOD <0.2ppm) and excluded from analysis. Statistical analysis was conducted in R using a mixed effects model. ZEA sample concentrations from both field sites were analyzed together.

Appendix

Table A.1. Field names, phylogeny names, locations, average macroconidia lengths(μm), average macroconidia widths (μm), average microconidia lengths(μm), average microconidia widths (μm), presence of chlamydo-spores, host, hyphae color, and pigment production on PDA for all 166 North Carolina *Fusarium* isolates collected from 2017-2018.

Field Name	Phyl. ¹ Name	Location	Macro ² Length	Macro ² Width	Micro ³ Length	Micro ³ Width	Chlamydo-spores	Host	Hyphae Color	Pigment on PDA ⁴
RM 4-2	F25	Nash County	30.62+/-5.24	4.83+/-0.91	-	-	N	sorghum	white/pink	pink
RM 5-2	F26	Nash County	28.41+/-3.85	4.41+/-0.73	-	-	N	sorghum	white/ yellow	peach
RM 5-3	F11	Nash County	29.94+/-3.72	4.38+/-0.86	-	-	Y	sorghum	white	peach/pink
C-1 (C-2,C-3,C-4)	F24	-	17.61+/-2.57	3.72+/-0.46	-	-	N	wheat	brown	brown
C-22-10C	F18	Wayne County	-	-	14.36+/-4.62	4.18+/-0.82	N	wheat	white	purple/blue/ yellow

Table A.1. (continued).

C-43-	F21	Jones	34.40+/ 14C	8.05+/-	-	-	N	wheat	magenta	magenta
C-48-	F23	Forsyth	33.29+/ 20B	4.29+/-	-	-	N	wheat	white/pink	pink
C-30-	F19	Rowan	23.82+/ 7B	3.65+/-	8.46+/-	2.99+/-	N	wheat	white/pink	pink
C-46-	F22	Stokes	21.61+/ 19C	4.73+/-	7.12+/-	6.08+/-	N	wheat	white/pink	brown/magenta/ pink
C-38-	F20	Craven	32.78+/ 32A	5.63+/-	-	-	N	wheat	white/pink	pink/magenta
S1-1C-	F29	Johnston	33.73+/ 1	6.16+/-	14.72+/ -2.56	5.12+/-	N	sorghum	white/bro wn	brown/pink

Table A.1. (continued).

S1-1C-	F30	Johnston	22.96+/ 2	5.08+/- County	-	-	Y	sorghum	white	peach/pink
S1-1C-	F31	Johnston	-	-	8.67+/- 3	3.76+/- County	N	sorghum	white	purple
S1-2C-	F32	Johnston	29.59+/ 1	5.06+/- County	-	-	N	sorghum	white/bro wn	brown
S1-2C-	F33	Johnston	20.29+/ 2	4.6+/- County	9.49+/- -5.18	4.11+/- 0.62	N	sorghum	white/ purple	purple/brown
S1-2C-	F34	Johnston	25.20+/ 3	4.99+/- County	9.74+/- -5.46	4.79+/- 0.65	N	sorghum	white	peach

Table A.1. (continued).

S1-3C-	F35	Johnston	36.05+/ 1	5.48+/- 1.01	-	-	N	sorghum	white/bro wn	brown
S1-3C-	F36	Johnston	30.03+/ 2	6.66+/- 1.07	-	-	N	sorghum	white/ brown	brown/yellow
S2-1C-	F37	Johnston	39.27+/ 1	5.76+/- 0.68	3.47+/- 1.1	3.53+/- 1.07	N	sorghum	white/pink	pink
S2-1C-	F38	Johnston	- 2	-	9.90+/- 1.68	4.07+/- 0.98	N	sorghum	white	peach/brown/ yellow
S2-1C-	F39	Johnston	33.05+/ 3	5.18+/- 0.7	4.44+/- 1.57	3.43+/- 0.79	N	sorghum	white/pink	pink

Table A.1. (continued).

S2-2C-	F40	Johnston	29.96+/ 1	5.16+/- 0.82	-	-	N	sorghum	white/ brown	brown
S2-2C-	F41	Johnston	22.78+/ 3	4.42+/- 0.57	14.6+/- 4.08	3.74+/- 0.7	Y	sorghum	white/bro wn	brown
S2-3C-	F42	Johnston	30.16+/ 1	4.52+/- 0.85	-	-	N	sorghum	white/pink	pink
S2-3C-	F43	Johnston	23.81+/ 2	4.29+/- 0.87	12.41+/ -2.79	2.9+/- 0.62	N	sorghum	white/ brown	peach/brown
S2-3C-	F44	Johnston	34.7+/- 3	3.92+/- 0.82	14.18+/ -3.66	3.47+/- 0.66	N	sorghum	white	peach

Table A.1. (continued).

S3-1C-	F45	Johnston	31.56+/ 1	5.95+/- 0.69	-	-	N	sorghum	white/ brown	brown
S3-1C-	F46	Johnston	35.84+/ 2	5.27+/- 0.57	11.76+/ -1.69	4.69+/- 0.56	N	sorghum	white/ brown	brown
S3-1C-	F47	Johnston	33+/- 3	5.26+/- 0.72	-	-	N	sorghum	white/ brown	brown
S3-2C-	F48	Johnston	27.79+/ 1	4.97+/- 1.04	-	-	N	sorghum	white/ brown	brown
S3-2C-	F49	Johnston	- 2	-	8.73+/- 1.01	3.97+/- 0.55	N	sorghum	white/ pink	pink/magenta

Table A.1. (continued).

S3-2C-	F50	Johnston	37.72+/ 3	5.77+/- 1.05	-	-	N	sorghum	white/ pink	pink
S3-3C-	F51	Johnston	34.87+/ 1	6.28+/- 1.43	-	-	N	sorghum	white/ brown	brown
S3-3C-	F52	Johnston	- 2	-	8.83+/- 1.47	4.28+/- 0.45	N	sorghum	white	blue
S3-3C-	F53	Johnston	40.99+/ 3	6.22+/- 1.03	10.31+/ -1.82	4.28+/- 0.75	N	sorghum	white/ brown	brown
S3-4C-	F54	Johnston	- 1	-	8.42+/- 1.39	3.21+/- 0.54	N	sorghum	white	blue/purple

Table A.1. (continued).

S3-4C-	F55	Johnston	-	-	8.93+/-	3.69+/-	N	sorghum	white	blue/purple
2		County			1.14	0.6				
S3-4C-	F56	Johnston	-	-	9.16+/-	3.75+/-	N	sorghum	white	purple/yellow
3		County			1.54	0.56				
S4-1C-	F57	Johnston	-	-	10.89+/-	4.58+/-	N	sorghum	white	blue/purple
1		County			-2.18	0.69				
S4-1C-	F58	Johnston	28.47+/-	4.74+/-	9.04+/-	3.42+/-	N	sorghum	white	blue/purple
2		County	-9.24	0.7	1.43	0.55				
S4-1C-	F59	Johnston	-	-	9.64+/-	5.4+/-	N	sorghum	white	blue/purple
3		County			1.46	0.93				

Table A.1. (continued).

S4-2C-	F60	Johnston	31.31+/ 2	5.49+/-	11.39+/ -2.18	4.3+/- 0.62	N	sorghum	white/ brown	brown
S4-2C-	F61	Johnston	34.99+/ 3	6.08+/-	-	-	N	sorghum	white/ brown	brown
S4-3C-	F62	Johnston	40.34+/ 1	4.55+/-	9.17+/-	3.92+/-	N	sorghum	white/purp le	blue/purple
S4-3C-	F63	Johnston	39.44+/ 2	5.07+/-	-	-	N	sorghum	white/bro wn	brown
S4-3C-	F64	Johnston	- 3	-	9.03+/-	4.44+/-	N	sorghum	white	blue/purple

Table A.1. (continued).

S4-4C-	F65	Johnston	33.54+/ 1	5.19+/-	8.21+/-	3.95+/-	N	sorghum	white/bro	brown
		County	-7.24	0.71	1.11	0.69			wn	
S4-4C-	F66	Johnston	29.23+/ 2	4.95+/-	-	-	N	sorghum	white/bro	brown
		County	-8.22	0.69					wn	
S4-4C-	F67	Johnston	49.35+/ 3	4.15+/-	11.66+/ -3.04	3.34+/-	N	sorghum	white/bro	brown
		County	-19.78	0.8	0.71				wn	
S5-1C-	F68	Johnston	14.73+/ 1	4.03+/-	7.53+/-	3.34+/-	N	sorghum	white/light	peach
		County	-4.07	0.7	1.58	0.66			pink	
S5-1C-	F69	Johnston	22.82+/ 2	4.87+/-	-	-	N	sorghum	white	pink/peach
		County	-6.62	0.95						

Table A.1. (continued).

S5-1C-	F70	Johnston	-	-	12.18+/-	5.29+/-	N	sorghum	white/purp	purple/yellow
3		County			-2.83	1.36			le	
S5-2C-	F71	Johnston	-	-	8.62+/-	4.33+/-	N	sorghum	white	purple
1		County			1.09	0.47				
S5-2C-	F72	Johnston	-	-	9.62+/-	3.7+/-	N	sorghum	white/purp	yellow
2		County			1.65	0.56			le	
S5-2C-	F73	Johnston	-	-	9.34+/-	4.7+/-	N	sorghum	white/purp	purple/yellow
3		County			1.43	0.92			le	
S5-3C-	F74	Johnston	-	-	8.52+/-	3.84+/-	N	sorghum	white/purp	purple/yellow
1		County			1.78	0.62			le	

Table A.1. (continued).

S5-3C- 2	F75	Johnston County	-	-	7.47+/- 1.06	3.58+/- 0.45	N	sorghum	white	-
106C-1	F8	Pasquata nk County	22.65+/ -7.29	4.85+/- 0.87	11.53+/ -2.85	4.03+/- 0.72	N	sorghum	white/bro wn	pink/brown
106C-2	F9	Pasquata nk County	33.07+/ -4.39	5.56+/- 0.64	-	-	N	sorghum	white	brown
106C-3	F10	Pasquata nk County	8.27+/- 1.58	3.75+/- 0.64	-	-	N	sorghum	white/purp le	purple/yellow
105A-1	F4	Pasquata nk County	19.28+/ -3.91	3.53+/- 0.68	10.3+/- 1.79	3.07+/- 0.49	N	sorghum	white	purple/yellow

Table A.1. (continued).

105A-2	F5	Pasquata	-	-	8.16+/-	3.24+/-	N	sorghum	white	purple/blue
		nk			1.92	0.65				
		County								
105A-3	F6	Pasquata	23.06+/-	5.55+/-	12.91+/-	4.17+/-	N	sorghum	brown/pin	brown/pink
		nk	-4.78	1.14	-2.91	0.67			k/white	
		County								
105C-1	F7	Pasquata	33.04+/-	5.28+/-	-	-	N	sorghum	white	pink
		nk	-5.2	1.18						
		County								
104C-1	F1	Pasquata	-	-	7.32+/-	3.06+/-	N	sorghum	white	purple
		nk			1.67	0.53				
		County								
104C-2	F2	Pasquata	-	-	8.62+/-	3.15+/-	N	sorghum	white	purple
		nk			2.06	0.51				
		County								

Table A.1. (continued).

104C-3	F3	Pasquata	33.43+/ nk	5.06+/- 1.09	-	-	N	sorghum	white/pink	pink/magenta
		County								
FA2	F12	Edgecom	21.52+/ be	4.56+/- 0.71	14.5+/- 3.13	3.83+/- 0.63	N	sorghum	white	peach
		County								
FA3	F13	Edgecom	43.83+/ be	6.76+/- 1.38	-	-	N	sorghum	white/bro wn	peach/brown
		County								
FB1	F14	Edgecom	30.66+/ be	7.21+/- 1.25	-	-	N	sorghum	pink	pink/magenta
		County								
FC1	F15	Edgecom	22.58+/ be	6.53+/- 0.89	-	-	N	sorghum	white	brown
		County								

Table A.1. (continued).

FD1	F16	Edgecom	33.46+/ -3.01	7.49+/- 1.23	-	-	N	sorghum	white	peach/pink/bro wn
		County								
FD3	F17	Edgecom	71.1+/- 15.45	9.79+/- 2.79	-	-	N	sorghum	white	brown/pink
		County								
RM2-1- 1	F27	Nash County	31.15+/ -4.87	5.39+/- 0.59	-	-	N	sorghum	white/pink	pink
RM4-1- 1	F28	Nash County	31.92+/ -5.91	4.93+/- 0.73	-	-	N	sorghum	white/pink	pink
F-1E3	F76	-	17.14+/ -5.39	3.74+/- 0.57	20.89+/ -5.7	6.35+/- 1.32	N	corn	white/purp le	yellow/pink/pur ple
F-4B1	F77	-	14.4+/- 4.19	3.7+/- 0.69	4.71+/- 0.74	2.54+/- 0.54	N	corn	white/purp le	pink/purple/pea ch

Table A.1. (continued).

F-4D3	F78	-	16.15+/ -2.47	4.08+/- 0.87	6.89+/- 1.45	3.07+/- 0.6	N	corn	white	pink/purple
F-1E2	F79	-	19.27+/ -5.79	3.82+/- 0.8	7.9+/- 2.05	3.06+/- 0.64	N	corn	white/purp le	yellow/purple rings
FU18A- 1-1	F80	Johnston County	37.96+/ -7.94	5.84+/- 0.79	4.55+/- 0.53	4.44+/- 0.51	N	sorghum	white/pink	peach/pink
FU18A- 1-2	F81	Johnston County	-	-	8.72+/- 1.13	4.29+/- 0.63	N	sorghum	white	peach
FU18A- 1-3	F82	Johnston County	34.17+/ -8.2	5.33+/- 0.67	-	-	N	sorghum	white/pink	peach/pink

Table A.1. (continued).

FU18A-	F83	Johnston	43.6+/-	6.04+/-	-	-	N	sorghum	white/bro	peach/brown
2-1		County	10.13	0.61					wn	
FU18A-	F84	Johnston	33.44+/-	5.44+/-	-	-	N	sorghum	brown	brown
2-2		County	-5.34	0.57						
FU18A-	F85	Johnston	29.83+/-	4.21+/-	-	-	N	sorghum	brown	brown
2-3		County	-4.5	0.53						
FU18A-	F86	Johnston	-	-	8.37+/-	4.31+/-	N	sorghum	white	purple
3-1		County			1.26	0.77				
FU18A-	F87	Johnston	26.58+/-	6.06+/-	-	-	N	sorghum	white/bro	peach/purple/br
3-2		County	-4.37	0.88					wn	own

Table A.1. (continued).

FU18A-	F88	Johnston	22.33+/ 3-3	6.88+/- 0.74	-	-	N	sorghum	white/pink	peach
FU18A-	F89	Johnston	23.84+/ 3-4	5.61+/- 0.53	8.28+/- 1.16	4.11+/- 0.53	N	sorghum	white/bro wn	brown
FU18A-	F90	Johnston	19.66+/ 4-1	4.91+/- 0.7	-	-	N	sorghum	white	peach/brown
FU18A-	F91	Johnston	33.99+/ 4-2	5.57+/- 0.69	-	-	N	sorghum	white	peach
FU18A-	F92	Johnston	- 5-1	-	7.8+/- 1.33	3.97+/- 0.58	N	sorghum	white/purp le	purple rings

Table A.1. (continued).

FU18A- 5-2	F93	Johnston County	26.07+/ -5.1	4.73+/ 0.7	-	-	N	sorghum	white/pink	peach/pink
FU18A- 5-3	F94	Johnston County	26.5+/ 6.06	5.45+/ 0.76	-	-	N	sorghum	white	peach/brown
FU18A- 5-4	F95	Johnston County	-	-	6.94+/ 1.15	3.05+/ 0.43	N	sorghum	white/purp le	purple
FU18A- 6-1	F96	Johnston County	-	-	6.81+/ 0.92	3.87+/ 0.5	N	sorghum	white/light purple	pink/purple
FU18A- 6-2	F97	Johnston County	-	-	7.17+/ 1.14	3.72+/ 0.49	N	sorghum	white/light purple	pink/purple

Table A.1. (continued).

FU18A-	F98	Johnston	24.8+/-	5.42+/-	-	-	N	sorghum	white/yell	peach/pink
7-1		County	5.36	0.75					ow	rings
FU18A-	F99	Johnston	31.02+/-	5.15+/-	-	-	Y	sorghum	white/bro	brown
7-2		County	-6.91	0.56					wn	
FU18A-	F100	Johnston	44.56+/-	6.17+/-	-	-	N	sorghum	white/bro	brown
8-1		County	-6.72	1.08					wn	
FU18A-	F101	Johnston	16.23+/-	4.84+/-	8.97+/-	4.51+/-	N	sorghum	white/bro	yellow/brown
8-2		County	-2.65	0.63	1.24	0.55			wn	
FU18A-	F102	Johnston	29.74+/-	6.27+/-	7.8+/-	3.66+/-	Y	sorghum	white/bro	brown
8-3		County	-9.85	1.9	1.34	0.42			wn	

Table A.1. (continued).

FU18A-	F103	Johnston	24.75+/ 9-1	5.38+/-	6.16+/-	3.17+/-	N	sorghum	white/bro	brown
		County	-4.95	0.94	0.71	0.65			wn	
FU18A-	F104	Johnston	31.09+/ 9-2	5.94+/-	-	-	N	sorghum	white/bro	peach/brown
		County	-6.06	0.64					wn	
FU18A-	F105	Johnston	- 10-1	-	7.87+/-	4.03+/-	N	sorghum	white	purple
		County			0.94	0.49				
FU18A-	F106	Johnston	- 10-2	-	7.32+/-	2.9+/-	N	sorghum	white	purple
		County			1.22	0.54				
FU18A-	F107	Johnston	23.67+/ 11-1	3.61+/-	-	-	Y	sorghum	white/light	brown
		County	-4.15	0.64					brown	

Table A.1. (continued).

FU18A-	F108	Johnston	14.97+/ 11-2	3.78+/-	8.34+/-	3.29+/-	N	sorghum	white	peach/brown
		County	-2.76	0.57	1.49	0.75				
FU18A-	F109	Johnston	33.81+/ 12-1	4.36+/-	-	-	Y	sorghum	white/bro	brown
		County	-8.62	0.8					wn	
FU18A-	F110	Johnston	25.93+/ 12-2	4.03+/-	-	-	Y	sorghum	white/bro	brown
		County	-7.03	0.65					wn	
FGW-1	F111	Wake	35.47+/ County	4.55+/-	-	-	N	weed	white/bro	peach/brown
			-8.97	0.89					wn	
FGW-2	F112	Wake	-	-	5.79+/-	3.65+/-	N	weed	white	na
		County			0.56	0.52				
FGW-3	F113	Wake	31.69+/ County	4.17+/-	-	-	N	weed	white	peach/pink
			-4.4	0.68						

Table A.1. (continued).

FGW-4	F114	Wake	-	-	9.08+/-	3.3+/-	N	weed	white	peach/light
		County			1.95	0.64				brown rings
FGRP-1	F115	Wake	23.47+/-	3.47+/-	-	-	Y	sorghum	white/bro	peach/brown
		County	-7.74	0.66					wn	
FGRP-2	F116	Wake	25.21+/-	3.96+/-	-	-	N	sorghum	white/bro	peach/brown
		County	-4.67	0.56					wn	
FGRP-3	F117	Wake	37.63+/-	4.85+/-	-	-	N	sorghum	white/bro	peach/brown
		County	-7.28	0.84					wn	
FSP-1	F118	Wake	26.53+/-	4.3+/-	-	-	N	sorghum	white/light	peach/brown
		County	-4.31	0.58				stem	brown	
FSL-1	F119	Wake	37.17+/-	4.74+/-	-	-	N	sorghum	white/bro	peach/pink/bro
		County	-7.91	0.72				stem	wn	wn
FSL-2	F120	Wake	18.67+/-	3.66+/-	7.9+/-	4.53+/-	Y	sorghum	white	peach
		County	-4.32	0.65	1.39	0.82		stem		

Table A.1. (continued).

FSL-3	F121	Wake	25.31+/ -4.54	5.83+/- 0.79	7.92+/- 0.99	4.16+/- 0.58	N	sorghum	white/bro	peach/brown
		County						stem	wn	
FSL-4	F122	Wake	-	-	6.97+/-	3.05+/-	N	sorghum	white/purp	purple
		County			1.03	0.6		stem	le	
FSL-5	F123	Wake	28.01+/ -7.2	4.33+/- 0.71	6.6+/- 1.36	3.12+/- 0.49	N	sorghum	white/bro	peach/brown
		County						stem	wn	
FSLF-1	F124	Wake	-	-	-	-	N	sorghum	white/bro	peach/light
		County						floret	wn	brown
FSLF-2	F125	Wake	-	-	9.37+/-	3.38+/-	N	sorghum	peach	light brown
		County			1.06	0.38		floret		
FSLF-3	F126	Wake	28.1+/- 3.39	3.98+/- 0.63	-	-	N	sorghum	white/bro	peach/brown
		County						floret	wn	
FSLF-4	F127	Wake	23.51+/ -3.32	4.01+/- 0.58	-	-	Y	sorghum	white/bro	peach
		County						floret	wn	

Table A.1. (continued).

FSLF-5	F128	Wake	27.74+/ -8.47	4.81+/ 1.19	-	-	Y	sorghum	white/bro	peach/brown
		County						floret	wn	
FSLF-6	F129	Wake	26.52+/ -4.1	4.49+/ 0.44	14.88+/ -3	3.75+/ 0.71	N	sorghum	white/bro	peach/brown
		County						floret	wn	
LW18-	F130	Wake	22.33+/ -3.96	3.42+/ 0.51	-	-	N	sorghum	white/bro	brown
1-1		County							wn	
LW18-	F131	Wake	-	-	19.41+/ -4.9	3.44+/ 0.54	N	sorghum	white	light pink
1-2		County								
LW18-	F132	Wake	-	-	13.69+/ -3.13	3.2+/ 0.58	N	sorghum	white	peach/yellow
2-1		County								
LW18-	F133	Wake	23.58+/ -3.86	4.46+/ 0.54	-	-	N	sorghum	white	pink/peach/bro
3-2		County							wn	
LW18-	F134	Wake	20.09+/ -5.15	4.51+/ 0.85	-	-	N	sorghum	white/bro	peach/yellow/br
5-1		County							wn	own

Table A.1. (continued).

LW18-	F135	Wake	-	-	13.19+/ -3.93	3.06+/ 0.46	N	sorghum	white	pink
5-2		County								
LW18-	F136	Wake	24.87+/ -4.38	4.32+/ 1.15	-	-	N	sorghum	white	pink/peach
5-3		County								
LW18-	F137	Wake	24.8+/ 2.87	3.47+/ 0.48	15.53+/ -3.41	3.25+/ 0.62	N	sorghum	white	peach/brown
7-3		County								
LW18-	F138	Wake	-	-	12.7+/ 3.55	3.15+/ 0.4	N	sorghum	white/pink	pink/peach
9-1		County								
LW18-	F139	Wake	-	-	13.38+/ -2.38	3.36+/ 0.67	N	sorghum	white/light brown	brown/peach
12-1		County								
LW18-	F140	Wake	37.29+/ -4.33	5.01+/ 0.55	-	-	N	sorghum	white/pink /magenta	magenta
13-3		County								
LW18-	F141	Wake	25.36+/ -6.95	4.04+/ 0.54	15.14+/ -2.9	3.92+/ 0.6	N	sorghum	white	peach/light brown
13-1		County								

Table A.1. (continued).

LW18- 14-3	F142	Wake County	19.49+/ -4.02	4.02+/- 0.71	-	-	N	sorghum	white	peach/light brown
LW18- 4-2	F143	Wake County	-	-	12.76+/ -4.5	2.52+/- 0.8	Y	sorghum	white	peach/dark black spots
LW18- 2-2	F144	Wake County	38.31+/ -6.55	4.08+/- 0.63	-	-	N	sorghum	pink/red/w hite	peach/dark black spots
LW18- 4-1	F145	Wake County	22.54+/ -28.41	3.23+/- 0.81	-	-	Y	sorghum	white/ black	slight peach with dark black spots
LW18- 3-3	F146	Wake County	33.57+/ -15.36	3.9+/- 0.69	5.29+/- 1.04	2.9+/- 0.59	N	sorghum	white/tan	none
LW18- 6-2	F147	Wake County	21.59+/ -1.73	5.11+/- 0.88	-	-	N	sorghum	white/peac h	peach
LW18- 6-3-2	F148	Wake County	30.33+/ -6.48	4.51+/- 0.8	-	-	N	sorghum	white	peach

Table A.1. (continued).

LW18- 4-3-1	F149	Wake County	28.95+/ -4.68	4.23+/- 0.56	-	-	N	sorghum	white/yell ow	peach/pink
LW18- 4-3-2	F150	Wake County	29.29+/ -5.03	4.31+/- 0.63	-	-	N	sorghum	white/yell ow	peach/pink/bro wn
LW18- 8-1	F151	Wake County	21.63+/ -3.18	4.38+/- 0.55	11.74+/ -1.78	3.48+/- 0.56	N	sorghum	white	peach/brown
LW18- 8-3	F153	Wake County	28.25+/ -6.75	4.55+/- 0.79	-	-	N	sorghum	white	peach/pink
LW18- 7-1-1	F154	Wake County	19.3+/- 3.98	3.76+/- 0.63	10.88+/ -1.96	3.01+/- 0.62	Y	sorghum	white/yell ow	peach/brown
LW18- 7-1-2	F155	Wake County	21.27+/ -4.02	3.81+/- 0.45	14.2+/- 2.09	3.4+/- 0.35	N	sorghum	white	tan/brown
LW18- 9-3-1	F156	Wake County	22.08+/ -4.33	3.54+/- 0.56	-	-	N	sorghum	white	peach

Table A.1. (continued).

LW18- 9-3-2	F157	Wake County	29.35+/ -6.68	4.24+/- 0.72	-	-	N	sorghum	white	peach/brown
LW18- 9-2-1	F158	Wake County	22.12+/ -2.99	4.44+/- 0.58	13.41+/ -2.65	3.61+/- 0.51	N	sorghum	white	peach
LW18- 9-2-2	F159	Wake County	23.76+/ -5.12	3.59+/- 0.55	-	-	N	sorghum	white	peach/brown
LW18- 10-3	F160	Wake County	20.22+/ -4.42	3.97+/- 0.69	-	-	N	sorghum	white	dark tan center
LW18- 11-1-1	F161	Wake County	28.76+/ -3.24	5.44+/- 0.88	-	-	Y	sorghum	white/yell ow	peach
LW18- 11-1-2	F162	Wake County	-	-	7.4+/- 0.94	3.64+/- 0.58	N	sorghum	white/yell ow	yellow/purple
LW18- 11-2-1	F163	Wake County	27.07+/ -4.5	3.72+/- 0.68	-	-	N	sorghum	white/yell ow	peach/pink

Table A.1. (continued).

LW18- 11-2-2	F164	Wake County	23.46+/ -4.28	4.11+/- 0.61	-	-	Y	sorghum	white	yellow
LW18- 13-2	F165	Wake County	32.04+/ -6.74	4.45+/- 0.58	-	-	N	sorghum	white/bro wn	brown
LW18- 14-1-1	F166	Wake County	31.81+/ -7.14	4.1+/- 0.83	-	-	N	sorghum	white	peach/pink
LW18- 14-1-2	F167	Wake County	24.74+/ -4.59	4.27+/- 0.73	11.36+/ -2.49	3.67+/- 0.69	N	sorghum	white	yellow/brown

¹Phylogeny name abbreviated “Phyl. Name.”

²Macroconidia abbreviated “Macro.”

³Microconidia abbreviated “Micro.”

⁴Potato Dextrose Agar.