Fungicide sensitivity screening of *Corynespora cassiicola* in US soybean and cotton

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DEDICATION

I dedicate my work to my mom and dad. Thank you for giving me the opportunity and the guidance to tackle any problems.

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ABSTRACT

Target spot, caused by the fungus *Corynespora cassiicola*, is a foliar disease of cotton and soybean. Target spot has become a disease of concern in soybean and cotton production systems. Data for fungicide sensitivity and understanding potential impact on yield is varied for *C. cassiicola*. Fungicide resistance is also a concern in *C. cassiicola* with the increase in resistant pathogens in soybean. With the lack of knowledge about baseline fungicide sensitivity and the concern of fungicide resistance the objectives of this study were: (i) to evaluate fungicide products and mixes in small plot soybean and cotton trials, (ii) develop baseline fungicide sensitivity data on *C. cassiicola* isolates from Tennessee soybean and cotton, (iii) investigate the interaction of the insecticide malathion with demethylation inhibitor fungicides on target spot management, and (iv) screen *C. cassiicola* isolates for possible fungicide resistance mutations that are non-sensitive to quinone outside inhibitor (QoI) fungicides.

Small plot field trials were conducted in soybean and cotton in 2018-2020 to evaluate different fungicide products on management of target spot. A wide variety of fungicides were used in soybean and cotton. Miravis Top was the only product to protect yield and decrease target spot severity in the soybean trials. In cotton, fungicides provided no impact to disease severity and yield in 2018 and 2020, whereas Miravis Top protected yield and decreased disease severity in 2019. Pyraclostrobin, thiophanate methyl, and azoxystrobin had the highest

EC50 values when evaluating baseline sensitivity. In vitro and field evaluations suggest that products that contain FRAC groups 7 and 3 have the potential to better protect yield from target spot compared to products containing groups 11 or 1 fungicides. Due to the presence of the G143A mutation in two *C. cassiicola* isolates from soybean, fungicide resistance to the QoI fungicides has been confirmed in Tennessee. The interaction between malathion and two DMI fungicides was evaluated over three separate trials: in small plots, in a detached leaf assay, and *in vitro.* Malathion had no efficacy on management of *C. cassiicola* and also did not impact the efficacy of the two DMI fungicides.

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CHAPTER I. REVIEW OF LITERATURE

Corynespora cassiicola

Corynespora cassiicola is in the kingdom Fungi, phylum Ascomycota, class Dothiomycetes, subclass Pleosporomycetidae, order Pleosporales, and family Corynesporacaceae (Robert et. al 2005). *C. cassiicola* (Berk. and M. A. Curtis) C. T. Wei is the causal pathogen of target spot, and has been documented worldwide, but is primarily found in tropic and subtropical regions (Holliday 1980, Dixon et al. 2009). The host range of *C. cassiicola* is widely diverse, infecting more than 530 plant species across 380 genera including monocots, dicots, and ferns (Smith 2008). Economic crops that are infected by *C. cassiicola* include: cotton, rubber tree, tomato, tobacco, and soybean (Ma and Michailides 2005; Schnabel et al. 2004). The diseases attributed to *C. cassiicola* are mainly foliar, but infections can also occur on the fruits, stems, and roots of different hosts (Jones et al. 1991). *C. cassiicola* is mostly reported as a necrotrophic fungus (Lopez et al., 2018) where the pathogen destroys the host cell to utilize host nutrients and overwinter on infected debris and seeds (Almeida et al. 2001). It has also been described as an endophyte (Déon et al. 2012; Dixon et al. 2009) and as a saprophyte (Cai et al. 2006).

C. cassiicola colonies are whitish to gray in mycelial color in the first few days, and then turn dark gray over time on potato-dextrose agar (PDA) (Lopez et al. 2018). Spore morphology can be highly variable based on the substrate used for spore harvest (MacKenzie et al. 2018). Generally, conidiophores are erect, branched, dark brown in color, can be singly or in swollen basal cell clusters, around 1-20 pseudosepta that are 4-11 by 44-135 µm in size. Conidia are

singular or in chains of 2-6, smooth, brown in color, with a central hilum at the basal end, 3-20 pseudosepta, are slightly curved or straight, and can be 39-520 µm in length (Hartman 2015). A distinct feature of the conidia is the presence of a hilum, which will appear darker and thicker than the rest of the conidia (Ellis and Holliday 1971). *C. cassiicola* reproduces asexually and can produce chlamydospores, allowing the pathogen to overwinter in soil and plant debris in unfavorable conditions (Olive et al 1945). Pathogens that survive on soybean and cotton debris constitute the source of primary inoculum (Almeida et al. 2001). Target spot is a polycyclic disease with a short reproduction cycle where the pathogen can complete many generations in a single growing season (Agrios 2005; MacKenzie et al. 2018).

The pathogen is becoming more prevalent in temperate regions, particularly on soybean and cotton (Boosalis and Hamilton 1957; Malvick 2004; Seaman et al. 1965). Recently there has been a surge of first reports of target spot affecting cotton in U.S states, including Georgia, Louisiana, Alabama, Mississippi, and Tennessee (Fulmer et al 2014, Price et al 2012, Conner et al 2013, Butler et al. 2016). In soybean, the increased occurrence of target spot is becoming a problem to growers when not properly controlled. Disease incidence and severity has been increasing due to an increase in conservation tillage, use of susceptible varieties, lack of crop rotation, and changes in weather patterns (Avonzi et al. 2014, Koenning et al. 2006)

Symptomology and Impact on Yield

Initial visible symptoms of target spot include a small reddish spot which can expand into a circular or irregular red-brown lesion, causing a zonate or 'target-like' pattern (Koenning et al. 2006; Faske and Kirkpatrick 2014). Target spot disease symptoms also include necrosis, with or without a yellow halo (Pernezny and Simone 1993), due to production of a host specific toxin, cassicolin (Barthe et al. 2007; Kurt 2004). On a majority of hosts, the pathogen can be characterized by high virulence and rapid expansion of the necrotic lesions with the yellow halo (Chase 1987; McRitchie and Miller 1971; Ross 2007). Disease development of *C. cassiicola* is favored in areas of warm temperatures and high moisture and humidity (Chase 1982; Date et al. 2004) particularly in the southern U.S. In the southern U.S., *C. cassiicola* infects soybean and cotton leaves but can also infect the hypocotyls of soybean seedlings (Seaman et al. 1965; Faske 2015). Premature defoliation is the main yield limiting factor due to target spot, with defoliation being greater with more severe infection (Godoy et al. 2015). In soybean, yield losses attributed to target spot can be as high as 40% (Koenning et al. 2006; Faske and Kirkpatrick 2014). Similarly, target spot causes significant damage in cotton due to premature defoliation of leaves (Lakshmanan et al. 1990). In cultivars that are highly susceptible, defoliation in the lower to mid canopy can reach upwards of 75 percent, reducing yields of seed cotton by 336 kg/ha (Fulmer et al. 2014).

C. cassiicola is becoming relevant and concerning as a plant pathogen precisely because of its increasing occurrence on several high economic value

crops. Several strategies have been indicated to control target spot, such as effective fungicides, crop rotation, and resistant varieties. *C. cassiicola* also presents a high-risk to becoming resistant to fungicides (FRAC 2019). The development of resistant/tolerant germplasm seems to be a feasible strategy for disease management (Fernando et al. 2009) but it is important to take into consideration the variability of *C. cassiicola* isolates in order to develop effective resistance to target spot. As the disease becomes more important on cotton and soybean, there is an increasing need of more research on the phytopathogenic fungus. Studies that deal with different aspects of the pathogen, interaction with host plants, molecular approaches focused on their diversity, efficiency of chemical control and their risk of resistance, and alternative paths to integrated pest management (IPM) are required to overcome this disease.

Fungicide Resistance

Fungicides are essential in maintaining healthy, reliable, and high-quality agricultural products. Up until the 1970s, there were sporadic cases of fungicide resistance long after some products have been on the market. Resistance to these products was infrequent despite overall widespread usage. However, since the introduction of systemic fungicides in the 1960s, the time it takes for fungicide resistance to occur has been relatively short (Brent 1995). Fungicide resistance can be stable and is an inheritable mutation passed down from fungicide-selected fungi, resulting in a reduction of sensitivity to the specific fungicide. Resistance can be the result of single or multiple gene mutations in the fungus.

Resistant isolates typically arise from a very low natural rate of genetic mutation, and these isolates are less affected or not inhibited at all by a labeled application rate of a fungicide. Since the fungicide can effectively control sensitive isolates, resistant isolates may become dominant in pathogen populations under selection pressure of fungicide use over time, therefore disease control failures may eventually occur. The ecological fitness of fungicide-resistant fungal isolates will determine the persistence of resistant genotypes once they are selected. In many instances, since resistant isolates may have lower fitness than sensitive isolates, they cannot survive well in the absence of fungicide selection pressure. In this case, the frequencies of resistant isolates in pathogen populations will decrease once the fungicide applications cease. Alternatively, resistant isolates can be as fit as sensitive isolates and persist for a long time even without any use of the fungicides. This has exemplified by the resistance of several phytopathogenic fungi to benzimidazole or strobilurin fungicides (Koenraadt et al. 1992; Baraldi et al. 2003).

Fungicide resistance can be conferred by various mechanisms (Gisi et al. 2000; Gullino et al. 2000; Fluit et al. 2001; MgGrath 2001), including: (I) an altered target site, which reduces the binding of the fungicide; (II) the synthesis of an alternative enzyme capable of substituting the target enzyme; (III) the overproduction of the fungicide target; (IV) an active efflux or reduced uptake of the fungicide; and (V) a metabolic breakdown of the fungicide. In addition, some unrecognized mechanisms could also be responsible for fungicide resistance.

Quinone Outside Inhibitors

Quinone outside inhibitor (QoI) fungicides are FRAC Class 11 fungicides that contain the strobilurin fungicide group. QoIs inhibit mitochondrial respiration of fungi by binding to the cytochrome *bc1* enzyme (complex III) at the quinone outside site (Bartlett et al. 2002). Soon after introduction to the market in 1996, resistant isolates were detected in field populations to several plant pathogens (Bartlett et al. 2002). In most cases, QoI resistance was conferred by a point mutation in the mitochondrial cytochrome b (cyt b) gene. The mutation leading to an amino acid change from phenylalanine to leucine at codon 129 (F129L), or from glycine to alanine at codon 143 (G143A) of the cyt b gene conferred QoI resistance without any negative effects on enzyme activity (Bartlett et al. 2002). Strobilurin fungicides are subject to being overcome due to their single site specificity; thus, a point mutation at the target site, which alters the expected amino acid (F129L, G137R, G143A, Y279), may confer fungicide resistance (Fisher & Meunier, 2008). To date, the G143A mutation has been correlated with QoI resistance in a wide variety of fungi from many hosts (FRAC 2021). QoI-resistant isolates with mutations at different codons have been shown to have different resistance levels. Kim et al. (2003) reported that resistance levels of isolates of *Pyricularia grisea* containing the F129L mutation were significantly lower than isolates having the G143A mutation. Pathogens may acquire resistance to QoI fungicides due to target site mutations, but may also overcome the action of the fungicide, at least in vitro, by the production of the alternative oxidase enzyme (Avila-Adame & Koller 2003; Bartlett et al. 2002). There are no

visually detectable differences between QoI resistant and QoI sensitive isolates of *C.sojina*; and while greenhouse inoculations suggest that resistant isolates demonstrate greater initial virulence, given 1-2 weeks the resulting disease severity did not differ according to the QoI sensitivity of the isolate (Zhang 2012). The first report of QoI resistance for *Cercospora sojina,* the causal pathogen of frogeye leaf spot in soybean, in the U.S. came out of Tennessee in 2010 (Zhang et al. 2012). *C. cassiicola* has been reported to be resistant to QoIs in multiple crops including: cucumber, tomato, and soybean (Duan et al. 2019; MacKenzie et al. 2020; Rondon and Lawrence 2020).

Methyl Benzimidazole Carbamates

Methyl Benzimidazole Carbamates (MBC) are a fungicide class (FRAC Class 1) containing the benzimidazoles and thiophanates (FRAC 2019). FRAC classifies Group 1 fungicides as having a high risk for fungicide resistance as well cross resistance within the group. Thiophanate methyl (TM) belongs to the MBC class of fungicides, which inhibit growth of fungi by interfering with microtubule assembly during mitosis (Howard and Aist 1977). FRAC also considers TM to be high risk for selecting resistant individuals in fungal plant pathogens (FRAC 2019). This resistance is due to a mutation in the β-tubulin gene leading to high levels of resistance in vitro and in vivo (Monma et al. 2003). Resistance to MBC fungicides was first documented in 1969, soon after their introduction, and resistance to MBC fungicides of approximately 130 fungal pathogens of agronomic, fruit, horticultural, turf grass, and vegetable crops has

been confirmed (FRAC 2019). 11 isolates of *C. cassiicola* recovered from soybean in Brazil were either moderately or highly resistant to carbendazim with the range of EC_{50} values being 1-575 µg/mL (Xavier et al. 2013). Recently, a double mutation of *β-tubulin* conferring resistance to benzimidazoles was reported for *C. cassiicola* isolates. In addition, a strong cross resistance was observed among the MBC fungicides: carbendazim, benomyl, and thiabendazole on *C. cassiicola* isolates as a result of different *β-tubulin* mutations (Duan et al., 2019).

Demethylation Inhibitors

Demethylation inhibitors (DMI) are FRAC class 3 fungicides that contain triazole fungicides (FRAC 2019). FRAC classifies DMIs as medium risk for fungicide resistance so resistance management is encouraged. DMIs inhibit the sterol C-14 α-demethylation of 24-methylenedihydrolanosterol, which is the precursor to the production of ergosterol in fungi (Brent 1995). Ergosterol impacts permeability and fluidity of cell membranes in fungal cells. There are several commercially successful fungicides containing DMIs in the 1970s and in the 1980s, resistance problems have been reported in numerous fungi (Delye et al.1997, 1998; Koller and Wilcox 1999; Ma et al. 2002).

Two methods of resistance have been identified in phytopathogenic fungi in response to DMIs: mutations in the 14α-demethylase gene (*CYP51*) and overexpression of CYP51 (Ma and Michailides 2005). *Uncinula necator*, the causal agent of grape powdery mildew, was observed to have a single mutation

leading to a substitution at the Y136F codon that was found in all isolates with high resistance factors, but was not found in isolates with low resistance factors (Delye et al. 1997). Additionally, several other amino acid alterations in the CYP51 genes were shown to be associated with DMI resistance in laboratory-induced resistant isolates of *Penicillium italicum* (Joseph-Horne and Hollomon,1997), and *Ustilago maydis* (Butters et al. 2000). However, studies on *Venturia nashicola* (Cools et al. 2002) and *T. yallundae* (Wood et al. 2001) showed that DMI resistance in field isolates of these fungi was not related to amino acid changes in the CYP51 proteins. Therefore, alternative mechanisms other than mutations in the target gene could be responsible for DMI resistance.

Changes in the expression level of CYP51 can contribute to development of DMI resistance. There are several different mechanisms known to increase CYP51 expression in fungi. In the clinical fungus *Candida glabrata*, increased expression of CYP51, which was responsible for DMI resistance, resulted from an increase in copy number of the CYP51 (Marichal et al., 1997). In a study on field DMI-resistant isolates of *Penicillium digitatum*, Hamamoto et al. (2000) found that amino acid sequences of CYP51 from six isolates (three DMI resistant and three DMI sensitive) were identical. However, a unique 126-bp sequence in the promoter region of CYP51 gene was tandem repeated five times in resistant isolates and was present only once in sensitive isolates.

Succinate Dehydrogenase Inhibitors

Succinate dehydrogenase inhibitors (SDHI) are FRAC Class 7 fungicides. FRAC classifies SDHIs as being medium to high risk for resistance and should be managed for fungicide resistance (FRAC 2019). SDHIs act on the ubiquinone binding site of the mitochondrial complex II, which inhibits fungal respiration (Avenot and Michailides 2010). Early SDHIs were used in agriculture in the 1960s and were used against diseases caused by basidiomycetes such as rusts and *Rhizoctonia* (Zhang et al. 2009). Newer active ingredients are characterized by a broad spectrum of fungal activity on various crops (Stammler et al. 2007; Yanase et al. 2007). Due to the specificity of the mode of action, frequent and widespread use has caused selection of resistance among field pathogen populations (Dekker 1995).

SDH consists of four subunits, the hydrophilic flavoprotein (SdhA), the iron-sulphur protein (SdhB), and two lipophilic transmembrane C- and D-subunits (SdhC and SdhD). Studies on the molecular mechanisms responsible for the acquisition of resistance to SDHI fungicides, such as carboxin, flutolanil or fenfuram in some resistant isolates of bacteria and Basidiomycete and Ascomycete fungi have shown that mutations which lead to amino acid substitutions in the SdhB, SdhC or SdhD subunits of SDH confer laboratory resistance (Keon et al. 1991; Broomfield & Hargreaves 1992; Matsson et al. 1998; Skinner et al. 1998; Matsson & Hederstedt 2001; Ito et al. 2004; Li et al. 2006; Shima et al. 2008; Miyamoto et al. 2010). Miyamoto et al. (2010) confirmed

resistance to boscalid in *C. cassiicola* in 214 isolates recovered from cucumber, with EC_{50} values as high as 30 µg/mL.

Development of fungicide resistance is an increasingly important issue, but plant diseases have a negative impact on yield in all crops with the ability to cause even worse losses if no control measure is implemented. While large-scale crop production is heavily dependent on effective fungicide applications, other forms of management must be used to decrease the pressure on fungicides. Disease resistant or tolerant varieties are among the most cost-effective tools available to growers (Stevenson et al. 2007). According to Hagan et al. (2016, 2017), sizable differences in defoliation due to target spot infection were observed among cotton varieties planted in Alabama. Yield losses associated with target spot defoliation were as high as 448 kg/ha using a susceptible variety (PHY499 WRF) in southwest Alabama (Hagan et al. 2015).

With the lack of knowledge about baseline fungicide sensitivity and the concern of fungicide resistance the objectives of this study were: (i) to evaluate fungicide products and mixes in small plot soybean and cotton trials, (ii) develop baseline fungicide sensitivity data on *C. cassiicola* isolates from Tennessee soybean and cotton, (iii) investigate the interaction of the insecticide malathion with DMI fungicides on target spot management, and (iv) screen *C. cassiicola* isolates for possible fungicide resistance mutations that are non-sensitive to QoI fungicides.

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CHAPTER II. FUNGICIDE EVALUATION IN TENNESSEE COTTON AND SOYBEAN FOR MANAGEMENT OF TARGET SPOT (*CORYNESPORA CASSIICOLA*)

Abstract

Target spot, caused by the fungus, *Corynespora cassiicola,* is a foliar disease of cotton and soybean. Target spot has always been a disease of note in soybean, but over recent years has increased in prevalence in cotton. This had led to more fungicide applications being applied in both soybean and cotton. Currently, little data exists to support fungicide recommendations for growers in Tennessee. The objective of this study was to evaluate fungicide products and mixes on the management of target spot in Tennessee cotton and soybean. In soybean, this experiment took place over 3 years in 3 different locations that were chosen based on historical disease pressure. Cultivars with ranging susceptibilities to target spot were evaluated along with 5 fungicide mixes across multiple fungicide classes. In cotton, this experiment took place over 3 years with many different fungicide products and application timings. For soybean, target spot was less severe when soybean was planted after wheat. Cultivars with moderate to high tolerance against target spot consistently yielded better than highly susceptible cultivars. Products containing succinate dehydrogenous inhibitors (SDHIs), such as Miravis Top and Priaxor, had the best efficacy on target spot, but Miravis Top consistently enhanced yield protection. For cotton, target spot was reduced in 2019 by Miravis Top and was reduced in 2020 by all fungicide products. Defoliation and lint yield were not impacted by fungicide choice or application timing. The results of these studies suggest that target spot can be reduced in soybean and cotton by using fungicide mixtures containing SDHIs, but the impact on yield is greater in soybean than in cotton.

Introduction

Target spot, caused by the ascomycete fungus *Corynespora cassiicola* (Berk. & M. A. Curtis) C.T. Wei*,* is a foliar disease in soybean and cotton. The host range of *C. cassiicola* is diverse, infecting more than 500 plant species worldwide (Smith 2008). Economic crops that are affected by *C. cassiicola* include: cotton, soybean, tomato, and rubber (Ma and Michailides 2005). Diseases are mostly foliar, but fruit, stems, and roots can be infected depending on the host (Jones et al. 1991). *C. cassiicola* is reported as a necrotrophic fungus that destroys the host cell to utilize host nutrients and commonly overwinters on debris and litter from the previous growing season (Almeida et al. 2001). *C. cassiicola* that survive specifically on soybean and cotton debris constitute the source of primary inoculum (Almeida et al. 2001).

Historically, target spot of cotton and soybean has been of minor concern until recently. Surges in first reports of target spot infecting cotton in the southeastern US have led to more research into studying the disease (Butler et al. 2016, Campbell et al. 2012, Fulmer et al. 2012). In certain areas massive yield losses have occurred in soybean and cotton. In Alabama, lint yield was reduced by 224 kg/ha and was attributed to a massive defoliation event caused by target spot (Hagan et al. 2015). Edwards Molina et al. (2019) reported yield losses upwards of 40% when target spot severity was at least 50% in susceptible soybean in Brazil. According to Sumabat et al. (2018), it is unclear why target spot is increasing in incidence and severity.

Although there are IPM practices that can reduce the incidence and severity of target spot, fungicide applications are the most effective way at managing this disease. Widespread fungicide efficacy trials are important to understand which classes of fungicides can be used to manage target spot (Edwards Molina et al. 2019). Edwards Molina et al. (2019) reported that soybean yields were increased and target spot severity was reduced in plots that were treated with foliar fungicides. Hagan et al. (2017) observed decreased defoliation due to target spot in cotton in Alabama when plots were treated with the fungicide Headline (FRAC, Group 11). While efficacy trials are important, the rise in fungicide resistance must also be taken into account. FRAC (2021) reports that *C. cassiicola* is a medium to high risk pathogen for development of fungicide resistance, with reports of resistance to quinone outside inhibitor (QoI, FRAC, Group 11) already appearing in tomato and soybean (MacKenzie et al. 2020; Rondon and Lawrence 2019).

In Tennessee, fungicide applications are not readily used to treat target spot in cotton and soybean due to the lack of efficacy data. With target spot becoming more prevalent in TN cotton and soybean and the possibility of the development of fungicide resistance, field fungicide trials are important for monitoring the development of resistance over time. The objective of this study is to evaluate a number of different fungicide products for the management of target spot across soybean and cotton field trials.

Materials and Methods

Soybean Field Trial Setup

In soybean, this experiment was conducted in three locations from 2018-2020. Each location was selected based on different crop rotations and disease pressure. The high pressure area was the Milan Research and Education Center (MREC) located in Milan, TN and is continuous soybean with irrigation and a high pressure of both target spot and frogeye leaf spot. The moderate pressure area was a grower field 13.4 km east of Jackson, TN. This field was in a multi-year rotation between corn and soybean, non-irrigated and had moderate to high pressure of target spot and frogeye leaf spot. The low pressure area was planted at the West Tennessee Research and Education Center in Jackson, TN in 2018 and 2019 and was planted in a grower field 13.4 km east of Jackson in 2020. Both locations are double-cropped with wheat and soybean each year and had little to no pressure from target spot and frogeye leaf spot. All locations were planted in three cultivars based on disease resistance to target spot. The susceptible cultivar used in 2018 was AG39X7 (Asgrow, Bayer Crop Science, Research Triangle Park, NC, USA), AG49X6 in 2019, and AG53X0 in 2020. The moderately susceptible cultivar used all three years was AG43X7. The tolerant cultivar used in 2018 was AG44X6 and AG46X6 in 2019 and 2020. Cultivars were changed due to availability from year to year.

Experiments were arranged in a randomized complete block design with a 2x2 factorial arrangement of treatments consisting of 3 cultivars and 5 fungicide treatments and untreated check. Soybeans were planted on 76 cm rows with a

plant density of 24 seeds per meter and plots were 9 m long by 3 m wide and was replicated 4 times at each location. Weed and insect control was based on recommendations from the University of Tennessee Extension Service. Planting dates varied from year to year: June 4th-June 14th in 2018, May 21st-June 14th in 2019, and June 2nd-June 24th in 2020. The center two rows of each plot were harvested with an Almaco two-row combine and yield was adjusted based on 13 percent moisture in ARM 2020 (Gylling Data Management, Brookings, SD, USA).

Fungicide applications were made with a Lee Spider Sprayer (LeeAgra Inc, Lubbock, TX, USA) fitted with TeeJet 8002 flat fan spray tips during the R3 growth stage as described by Fehr et al. (1971). Applications were made at 140 L/ha, at a pressure of 241 KPA, and at a speed of 5.6 km/h. Fungicides included single product and tank mixes including multiple modes of action as shown in Table 1. Visual disease ratings were taken twenty-one days after fungicide application and again at twenty-eight days after application on a 0-100% scale with 0 = no disease present and 100 = total infection of the canopy. Visual ratings were taken for frogeye leaf spot and target spot. Percent target spot and yield were measured and responses were analyzed with a mixed model approach using JMP 9.14. Cultivar, fungicide, and cultivar x fungicide were treated as fixed effects; whereas random effects were environment, product, and replication. Means were separated using Tukey's least significant difference test ($P \leq 0.05$).

Cotton Field Trial Setup

In cotton, this experiment was conducted at the West Tennessee Research and Education Center (WTREC) in Jackson, TN from 2018-2020. This location is a continuous cotton crop each year with no recent rotation. Cotton cultivars were planted on 3 May 2018, 1 May 2019, and 20 May 2020. Weed and insect control along with cotton growth management recommendations of the University of Tennessee Extension service were followed. In 2018 and 2019 the cultivar planted was PHY 490 W3FE (Bayer Crop Science, Research Triangle Park, NC, USA) and Nextgen 4936 (Americot, Lubbock, TX, USA) in 2020. All cultivars that were used were susceptible to target spot. The trial was setup in a randomized complete block design with effects being fungicide treatment and application timing as shown in Table 2.

Fungicide applications were made with a Lee Spider Sprayer (LeeAgra Inc, Lubbock, TX, USA) fitted with TeeJet 8002 flat fan spray tips at three different application timings: 3rd week of bloom, 5th week of bloom, and onset of disease. Applications were made at 15 GPA, at a pressure of 35 PSI, and at a speed of 5.6 km/h. Visual disease ratings were taken twenty-one days after fungicide application and again at twenty-eight days after application on a 0-100% scale with 0 = no disease present and 100 = total infection of the canopy. Defoliation was assessed on a 0-100% visual scale based on amount of leaves lost throughout cotton canopy. Yield was calculated on a lint yield (lbs/acre) basis. Percent target spot, defoliation, and yield were measured and responses were analyzed with a mixed model approach using JMP 9.4. Year, timing,

cultivar, and fungicide were treated as fixed effects; whereas random effects were block and block x year. Means were separated using Tukey's least significant difference test (P≤0.05).

Results

Soybean Field Trial

This experiment evaluated the efficacy of five different fungicide products and tank mixes on the management of target spot in three different soybean field locations over three years. Yield and target spot were statistically impacted by the interaction of susceptibility x fungicide (P<0.0001). In both instances, the highly susceptible cultivars that were left untreated yielded lower and had a higher target spot percentage than treated plots (Data not shown). Target spot percentage was significantly affected by location (P<0.0001). The on-farm location ($\bar{x}$=6.29%) had the highest target spot percentage followed by MREC ($\bar{x}$=3.16%), with the field planted after wheat with the lowest amount of target spot pressure ($\bar{x}$=1.11%). This led us to evaluate each location separately.

MREC

Varietal susceptibility to target spot was highly significant in the MREC location (P<0.0001). Varieties that were highly susceptible to target spot ranged from 3.9 – 13.9% target spot severity ($\bar{x}$=7.1%). There was only one moderately susceptible variety used across all three years ($\bar{x}$=1.8%) (Figure 1). The two low susceptibility varieties ranged from 0.5 – 1.1% target spot severity ($\bar{x}$=0.6%). Fungicide treatment was also highly significant at the MREC location (p<0.0001).

The treatments Topsin XTR ($\bar{x}$=1.3%), Miravis Top ($\bar{x}$=1.4%), Quadris Top SBX ($\bar{x}$=2.6%), and Priaxor + Tilt ($\bar{x}$=5.30%) statistically reduced the severity of target spot compared to the untreated check ($\bar{x}$=5.3%) (Figure 2).The treatment Domark ($\bar{x}$=5.4%) was not different from the untreated check.

When evaluating yield, there was no statistical differences among varieties with differing susceptibilities to target spot (P=0.33). Yields ranged from 3181 – 3254 kg/ha among all varieties (Figure 3). Fungicide treatment was highly significant when evaluating yield protection (P<0.0001). The treatments Miravis Top ($\bar{x}$=3410 kg/ha), Quadris Top SBX ($\bar{x}$=3287 kg/ha), Priaxor + Tilt ($\bar{x}$=3213 kg/ha), and Topsin XTR ($\bar{x}$=3207 kg/ha) protected yield the best compared to the untreated check ($\bar{x}$=2996 kg/ha) (Figure 4); whereas Domark ($\bar{x}$=3153 kg/ha) did not protect yield.

Grower Field

There was no statistical difference in the amount of target spot present by year ranging from 5.61% target spot in 2020 to 7.18% in 2018 (P=0.11). Varietal susceptibility to target spot was highly significant in the on-farm location (P<0.0001). Varieties that were highly susceptible to target spot ranged from 7.4 – 15.8% target spot severity ($\bar{x}$=11.9%) (Figure 5). There was only one moderately susceptible variety used across all three years ($\bar{x}$=5.4%). The two low susceptibility varieties ranged from 1.5 – 4.3% target spot severity ($\bar{x}$=1.9%). Fungicide treatment was also highly significant at the on-farm location (p<0.0001) (Figure 6). The treatments Topsin XTR ($\bar{x}$=3.4%), Miravis Top ($\bar{x}$=4.5%), and

Priaxor + Tilt (x̄=5.3%) reduced the severity of target spot compared to the untreated check (x̄=9.2%) (Figure 6). The treatments Domark (x̄=9.2%) and Quadris Top SBX (x̄=7.1%) were not statistically different from the untreated check.

When evaluating yield, moderately susceptible (x̄=3645 kg/ha) varieties outperformed highly susceptible varieties (x̄=3500 kg/ha) (Figure 7). Yields ranged from 3103 – 3917 kg/ha among all varieties. Fungicide treatment was highly significant when evaluating yield protection ($P<0.0001$) (Figure 8). The treatments Miravis Top (x̄=3820 kg/ha), Quadris Top SBX (x̄=3652 kg/ha), and Priaxor + Tilt (x̄=3650 kg/ha) protected yield the best compared to the untreated check (x̄=3332 kg/ha); whereas Domark (x̄=3464 kg/ha) did not protect yield (Figure 8).

Wheat Beans

Susceptibility to target spot was significant in the varieties tested with the varieties having high susceptibility (x̄=2.1%) (Figure 9) to target spot having the highest target spot severity compared to the tolerant varieties (x̄=0.4%) ($P<0.0001$). Wheat bean plots had significantly less target spot across all 3 years compared to the other locations, ranging from 0.7 – 1.8% target spot severity. ($P<0.0001$). Fungicide treatment was also highly significant in the reduction of target spot severity ($P<0.0001$). All treatments reduced target spot severity compared to the untreated check (x̄=2.1%) (Figure 10).

For yield, there was no differences amongst varieties with differing susceptibilities to target spot, with yields ranging from 2413 – 2617 kg/ha (P=0.16) (Figure 11). Fungicide treatment also did not significantly affect yield with yields ranging from 2378 – 2583 kg/ha (P=0.82) (Figure 12).

Cotton Field Trial

There was a statistical difference in percent target spot, lint yield, and defoliation from year to year (P <0.001). Target spot pressure was highest in 2018 ($\bar{x}$=16.67%) and 2019 ($\bar{x}$=14.21%) but was low in 2020 ($\bar{x}$=3.43%). This was also the case with defoliation, with it being the highest in 2019 ($\bar{x}$=13.68%) and 2018 ($\bar{x}$=13.36%) and only $\bar{x}$=6.06% in 2020. For 2020 ($\bar{x}$=2801 kg/ha) lint yield was higher than 2018 ($\bar{x}$=1563 kg/ha) and 2019 ($\bar{x}$=1550 kg/ha). Also, due to fungicide treatments changing from year to year, this data set was run by year. Also since every fungicide treatment was not applied at each timing interval, the interaction between timing and fungicide choice was left out.

2018

There was no difference in target spot severity when comparing the different fungicides used (P=0.8953). Target spot severity ranged from 13.75% in the untreated plots to 20% severity for plots treated with the 6 oz. rate of Aproach (Figure 13). All other fungicides treatments fell somewhere between ($\bar{x}$=13.75 - 19.38%). For percent defoliation, there were also no differences amongst treatments (P=0.1364). Plots treated with Miravis Top had the lowest defoliation ($\bar{x}$=10.5%), while plots treated with Aproach at the 6 oz rate had the greatest

defoliation ($\bar{x}$=23.0%) (Figure 13). Lint yield (kg/ha) was also not statistically impacted by fungicide selection (P=0.0877). Lint yields ranged from 1441 – 1653 kg/ha (Figure 14).

Timing of application had no effect on target spot severity (P=0.5231), with severity ranging from 13.75 – 20.00% (Figure 15). Defoliation (%) was not statistically impacted by fungicide application timing (P=0.8440). Defoliation ranged from 10.5 – 15%. Lint yield was also not impacted by fungicide application timing (P=0.4913). Lint yield ranged from 1495 kg/ha for untreated plots to 1654 kg/ha for plots treated at fifth week of bloom (Figure 16).

2019

Fungicide product statistically affected target spot percent (P=0.0137). Miravis Top ($\bar{x}$=10.91%) was the only fungicide that statistically reduced the amount of target spot that was present compared to the untreated control ($\bar{x}$=19.86%) (Figure 17). Defoliation was not statistically impacted by fungicide application (P=0.5069) with the range of defoliation percentage was $\bar{x}$=11.66 – 17.62% (Figure 17). Lint yield was also not impacted by fungicide (P=0.1051) with yields ranging from 1396 – 1639 kg/ha (Figure 18).

Fungicides applications at 5th week of bloom ($\bar{x}$=16.34%) had statistically higher amounts of target spot than applications at 3rd week of bloom ($\bar{x}$=8.5%) or at both timings ($\bar{x}$=10%) (P<0.0001). For defoliation, fungicide timing did not impact percent defoliation (P=0.300). Defoliation percentage ranged from 10.62 –

14.46% (Figure 19). Yield was also not impacted by fungicide timing (P=0.8868) with lint yields ranging from 1524 – 1581 kg/ha (Figure 20).

2020

Fungicide selection statistically impacted severity of target spot in 2020 (P<0.001). All treatments reduced the amount of target spot compared to the untreated check ($\bar{x}$=6.88%) (Figure 21). Fungicide selection did not impact amount of defoliation (P=0.6640), with defoliation percentages ranging from 3.06 – 8.13% (Figure 21). Fungicide selection did not impact lint yields (P=0.7394). Lint yields ranged from 2678 – 2882 kg/ha (Figure 22).

Fungicide timing did not statistically impact target spot percentage at the α=0.05 level, but was statistically significant at α=0.1 (P=0.0611). Both the 3rd week of bloom application and the 3rd + 5th week of bloom application reduced target spot percentage. Defoliation (P=0.4175) and lint yield (P=0.6756) were not statistically impacted in 2020. Target spot percentage ranged from 1.72 – 2.6%, defoliation from 4.5 – 6.0%, and lint yield ranged from 2798 – 2836 kg/ha (Figure 23-24).

Discussion

In this study, fungicide efficacy trials on target spot were conducted in both soybean and cotton field trials in Tennessee from 2018-2020. Soybean trials were conducted over three locations to compare the efficacy of fungicides in different disease pressure areas. Cotton trials were conducted over three years to evaluate numerous different fungicides on the management of target spot.

Results from these trials are compared with other trials from different regions to determine the best recommendations for Tennessee cotton and soybean growers.

Target spot has recently re-emerged as a disease of concern in many soybean and cotton growing regions. Since then, fungicide selection and use has been up for debate to help manage severity and defoliation related to target spot infections (Edwards Molina et al. 2019). There appears to be a lot of variability in fungicide efficacy in the results shown here and in other similar trials in other regions. Edwards Molina et al. (2019) reported target spot control in soybean was greater than 75% in plots treated with fluxapyroxad and pyraclostrobin, the active ingredients in Priaxor, but saw low efficacy in plots treated with carbendazim. Depending on which fungicide was used, Reznikov et al. (2019) observed target spot reduction in soybean from 10 – 70%. Our results suggest that there is wide variability in efficacy based on fungicide selection with target spot reduction in soybean ranging from 0% to 76%. Soybean plots treated with Miravis Top, Topsin XTR, or Priaxor +Tilt reduced target spot the most consistently across all three locations, while Domark had less than 17% control. Priaxor + Tilt contains the fungicides fluxapyroxad, pyraclostrobin, and propiconazole and reduced target spot severity by 63%. This result is consistent with Edwards Molina et al. (2019) who observed a reduction in target spot with the fungicide mixtures fluxapyroxad + pyraclostotrobin and fluxapyroxad + pyraclsostrobin + epoxiconazole, at 76 and 75.7% respectively. Miravis Top

contains pydiflumetofen and difenoconazole and reduced target spot severity by 72%. Miravis Top is a relatively new commercial product with limited accessible field data. However, Neves and Bradley (2020) observed a baseline sensitivity of 0.082 µg/mL when *C. cassiicola* isolates were treated with pydiflumetofen, suggesting that Miravis Top has a high efficacy against target spot.

The best fungicide treatment, Miravis Top, protected yield on average of 4-12% compared to the untreated control. The fungicide product Domark had the lowest impact on yield, with only 3.5% greater than untreated control on average. Priaxor + Tilt provided a yield increase on average of 5% at all three locations. Edwards Molina et al. (2019a) reported a yield increase of 20% in plots treated with fluxapyroxad and pyraclostrobin, the active ingredients of Priaxor, in the presence of severe target spot infections. Based on results from Reznikov et al. (2019), target spot severity of 25% or higher can adversely affect soybean yields. Within the parameters of our study a lower threshold (10%) can adversely affect soybean yields, but the magnitude of yield response to fungicide treatments might be lower.

Outside of fungicide applications, one of the most effective methods for disease management is the planting of disease-tolerant cultivars. Zadoks and Schein (1979) define “tolerance” as the internal factors of a plant that allows a cultivar to suffer less damage than others at the same injury levels. Edwards Molina (2019) noticed that the cultivar BMX Potência RR at 50% target spot severity exhibited only 11% yield loss, while the cultivar M9144 RR suffered yield

losses of up to 42%. Koenning et al. (2006) estimated yield loss in nineteen cultivars in soybean fields around South Carolina afflicted with target spot to range from 20 – 40% depending on location and cultivar. In our results, our moderate susceptible cultivar (AG43X7) had greater yields on average of 8.2% compared to our highly susceptible cultivars. AG43X7 also had statistically less target spot compared to the highly susceptible cultivars. These results corroborate the maximum reported yield losses due to this disease (Koenning et al. 2006), and are probably a reflection of differences in tolerance among the cultivars. Although some cultivars may have some innate tolerance to *C. cassiicola,* information about the mechanisms associated with soybean tolerance to *C. cassiicola* is limited in the literature (Fortunato et al. 2015).

Target spot is a relatively new disease of concern in Tennessee cotton production, having first been identified in 2013 (Butler et al. 2016). Planting disease resistant or tolerant cultivars are the most cost effective way in minimizing losses associated to target spot (Stevenson et al. 2007; Hagan et al. 2018). Hagan et al. (2017) reported monetary loss of $690/ha planting the target spot susceptible cultivar PHY 499 WRF due defoliation as a result of target spot. Target spot severity and defoliation was 3x greater in 2018 and 2019 than 2020 in TN trials, which could be due to two main factors: PHY 490 WRF being more susceptible than NextGen 4936 and the environment being more conducive for target spot in 2018 and 2019 than 2020. In AL, NextGen 4936 had very low target spot severity in two locations (0-10%) in 2020, but target spot pressure

was relatively low across both of those locations (Alabama Cotton Variety Report 2020).

Fungicides are useful for suppressing target spot and defoliation in high disease pressure situations on highly susceptible cotton cultivars (Hagan et al. 2015; Mehl et al. 2017). Hagan et al. (2016) reported that a broadcast application of Headline 2.09SC protected yield up to 280 kg lint/ha. In this study, fungicide selection had little impact on target spot and defoliation outside of 2019. Miravis Top (pydiflumetofen and difenoconazole) reduced target spot and defoliation by 45% and 34% respectively. This is consistent with results from our soybean trial where target spot was reduced by 72%. None of the fungicides across all three years had a significant impact on yield. Hagan et al (2017) reported that fungicide selection has a larger impact when disease pressure is higher and in the presence of a susceptible cultivar. Mehl et al. (2017) also reported inconsistent yield gains with fungicides such as Headline, Quadris, and Priaxor. This is comparative to these results in that Headline and Priaxor had inconsistent impacts on yield although defoliation and target spot were reduced.

The goal of this study was to evaluate a range of different fungicides efficacy on target spot in TN soybean and cotton. According tothese results, soybean had a more consistent yield loss in response to target spot pressure. Moreover, when pressure was higher, yield was affected by fungicide application. Fungicides are generally an effective tool for controlling sensitive populations of pathogens. Disease pressure also correlates to yield protection with fungicides

indicated by the different locations and disease pressures in soybean. Although, planting resistant or tolerant cultivars are much more cost effective. Based on present findings, cultivar selection will be a key component of integrated management systems for target spot. Further studies should be performed to assess resistance and tolerance to target spot. With the rise in fungicide resistance, and the confirmation of resistance to strobilurins in TN and AL (Rondon and Lawrence; Smith et al. 2021) fungicide selection is becoming more important when susceptible cultivars are being used. The data reported here along with the continued use of other management strategies will help with recommendations to growers and prolong the efficacy of these fungicides.

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Appendix

Table 1. Description of fungicide mixtures tested in the soybean field.

Commercial Product	Active ingredients	Manufacturer
Domark 230 ME	Tetraconazole	Gowan Company
Quadris Top SBX	Azoxystrobin + Difenoconzole	Syngenta
Priaxor + Tilt	Pyraclostrobin + Fluxapyroxad + Propiconazole	BASF + Syngenta
Miravis Top	Pydiflumetofen + Difenconazole	Syngenta
Topsin XTR	Thiophanate methyl + Tebuconazole	UPI

Table 2. Description of fungicide mixtures tested in the cotton field trial.

Commercial Product	Active ingredients	Manufacturer	Year used
Aproach	Picoxystrobin	DuPont	2018
Priaxor	Pyraclostrobin + Fluxapyroxad	BASF	2018, 2019,2020
Headline	Pyraclostrobin	BASF	2018
Amistar Top	Azoxystrobin + Difenconazole	Syngenta	2018, 2019
Miravis Top	Pydiflumetofen + Difenconazole	Syngenta	2018, 2019,2020
Quadris	Azoxystrobin	Syngenta	2019
Provost Silver	Tebuconazole + Prothioconazole	Bayer	2019
Propulse	Fluopyram + Prothioconazole	Bayer	2020
Revytek	Fluxapyroxad + Pyraclostrobin + Mefentrifluconazole	Bayer	2020
Delaro+Luna Privilege	Prothioconazole + Trifloxystrobin + Fluopyram	Bayer	2020

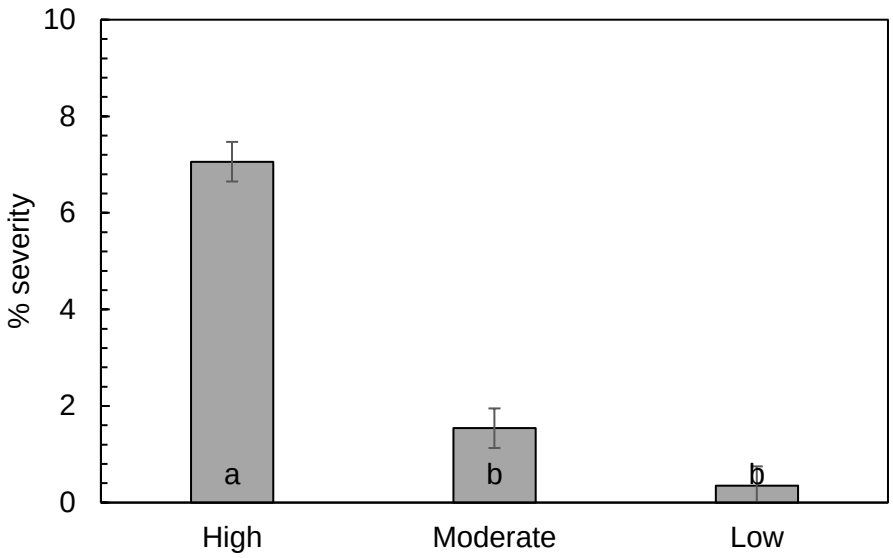

Figure 1. Target spot severity (%) based on cultivar susceptibility for MREC field location. Columns with the same letters are not statistically different based on Tukey's HSD at α=0.05.

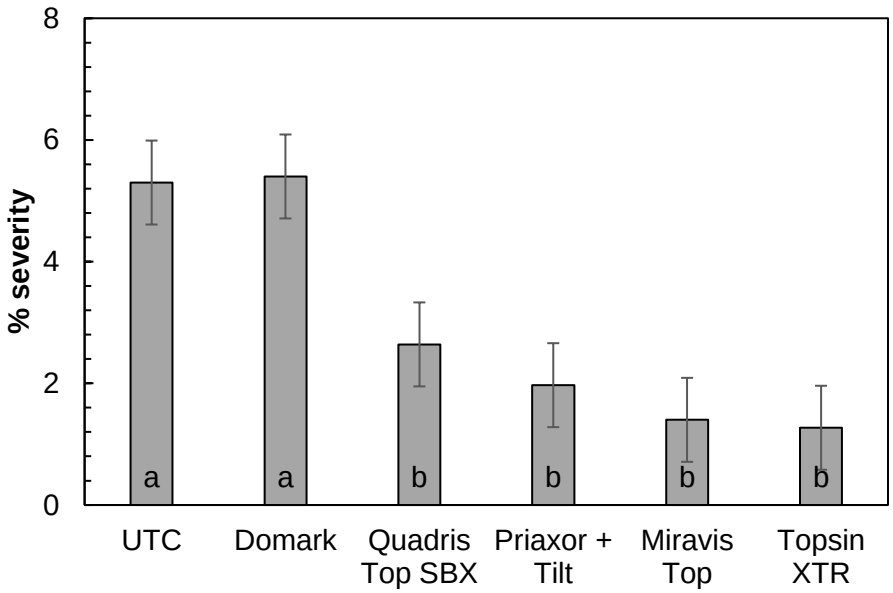

Figure 2. Target spot severity (%) based on fungicide mixture for MREC soybean field location. Columns with the same letters are not statistically different based on Tukey's HSD at α=0.05.

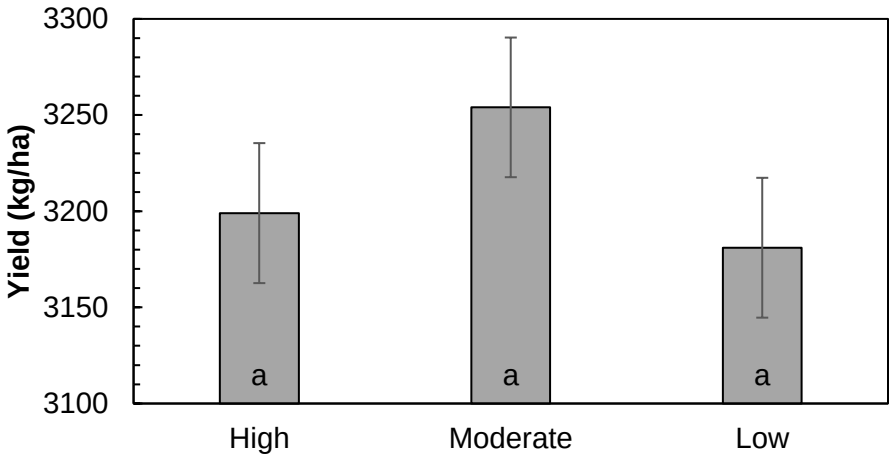

Figure 3. Yield (kg/ha) based on cultivar susceptibility for MREC field location. Columns with the same letters are not statistically different based on Tukey's HSD at α=0.05.

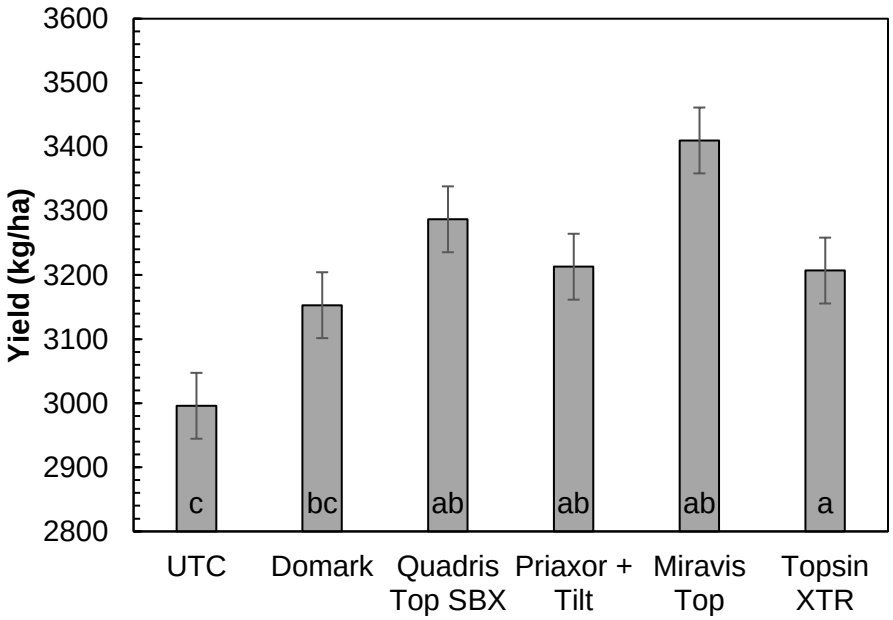

Figure 4. Yield (kg/ha) based on fungicide mixture for MREC soybean field location. Columns with the same letters are not statistically different based on Tukey's HSD at α=0.05.

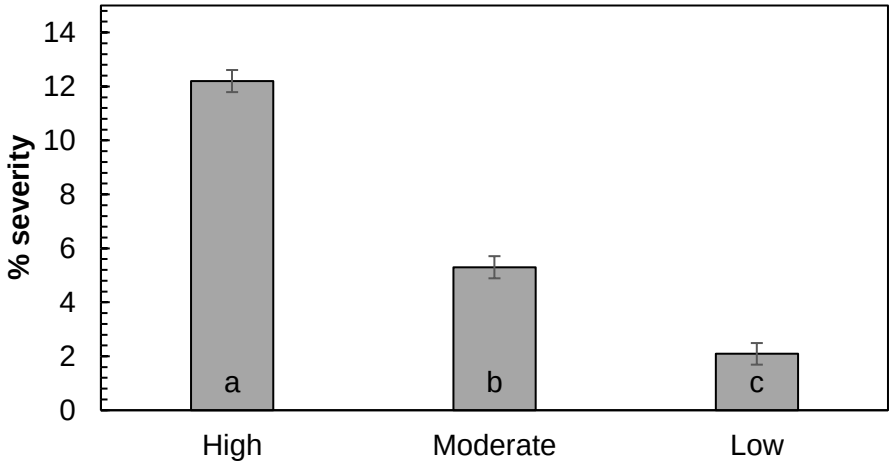

Figure 5. Target spot severity (%) based on cultivar susceptibility for On-farm field location. Columns with the same letters are not statistically different based on Tukey's HSD at α=0.05.

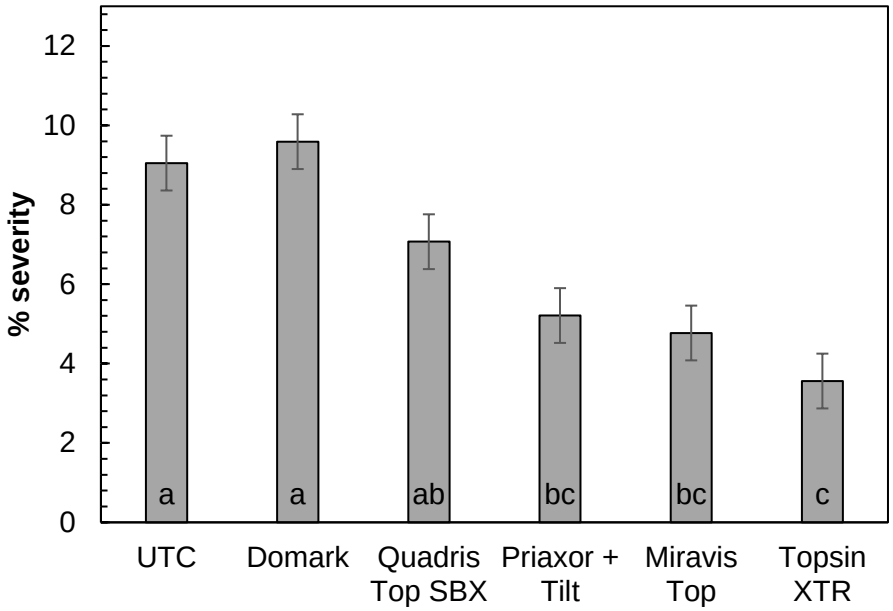

Figure 6. Target spot severity (%) based on fungicide mixture for On-farm soybean field location. Columns with the same letters are not statistically different based on Tukey's HSD at α=0.05.

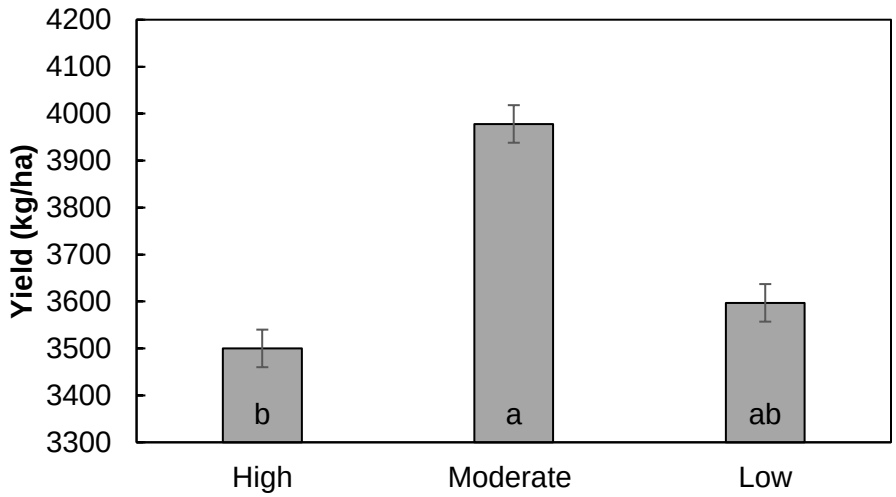

Figure 7. Yield (kg/ha) based on cultivar susceptibility for On-farm field location. Columns with the same letters are not statistically different based on Tukey's HSD at α=0.05.

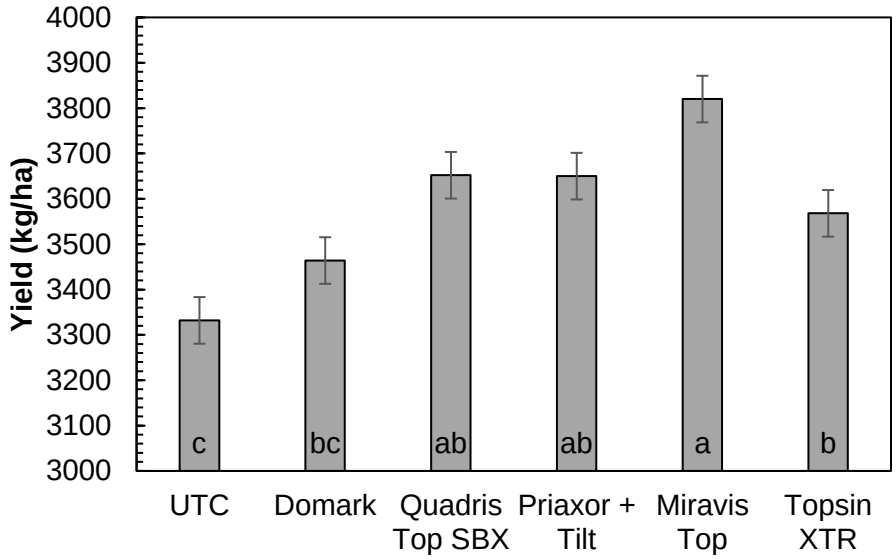

Figure 8. Yield (kg/ha) based on fungicide mixture for On-farm soybean field location. Columns with the same letters are not statistically different based on Tukey's HSD at α=0.05.

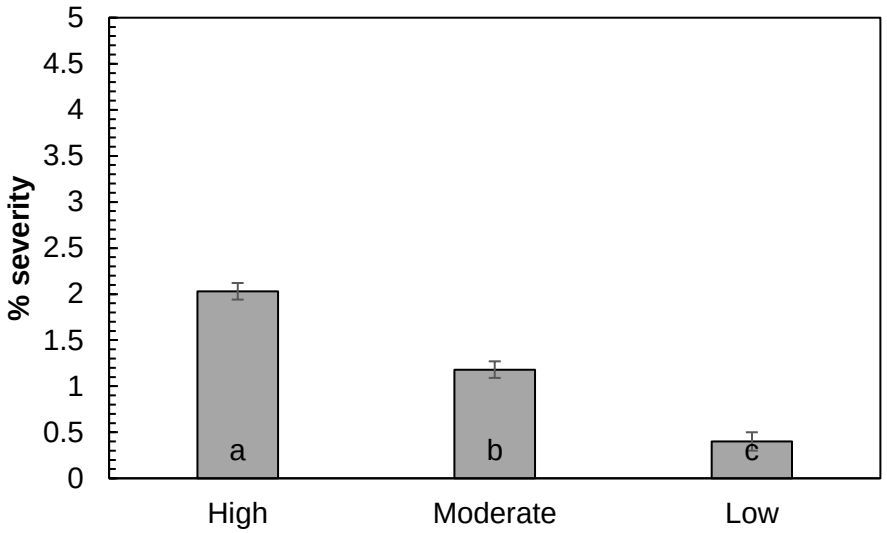

Figure 9. Target spot severity (%) based on cultivar susceptibility for wheat bean field location. Columns with the same letters are not statistically different based on Tukey's HSD at α=0.05.

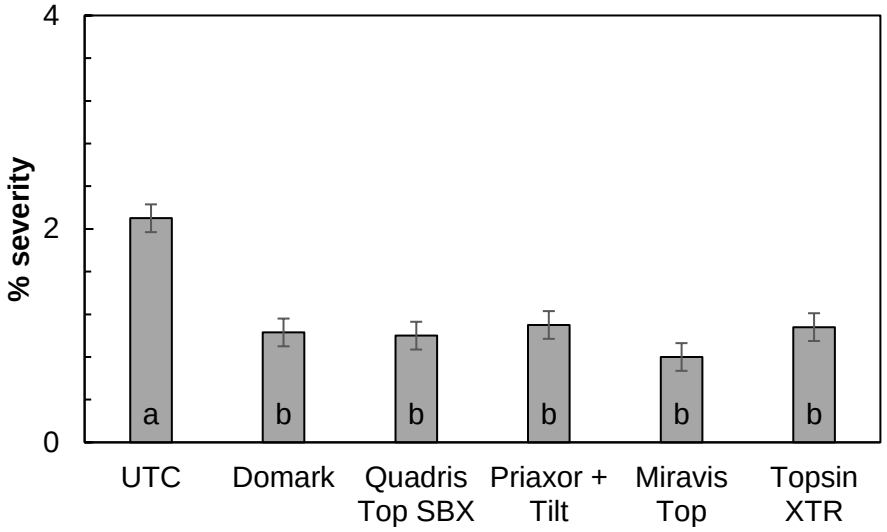

Figure 10. Target spot severity (%) based on fungicide mixture for wheat bean soybean field location. Columns with the same letters are not statistically different based on Tukey's HSD at α=0.05.

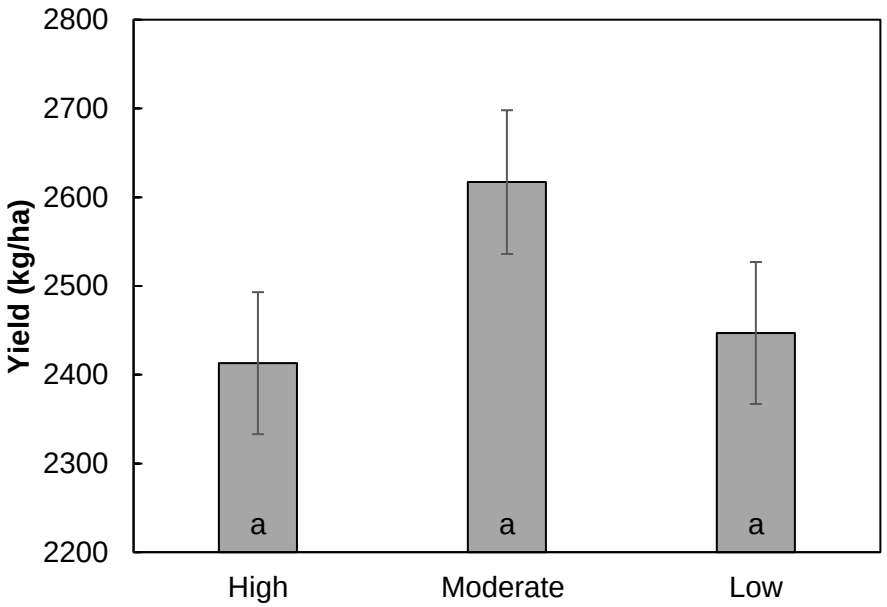

Figure 11. Yield (kg/ha) based on cultivar susceptibility for wheat bean field location. Columns with the same letters are not statistically different based on Tukey's HSD at α=0.05.

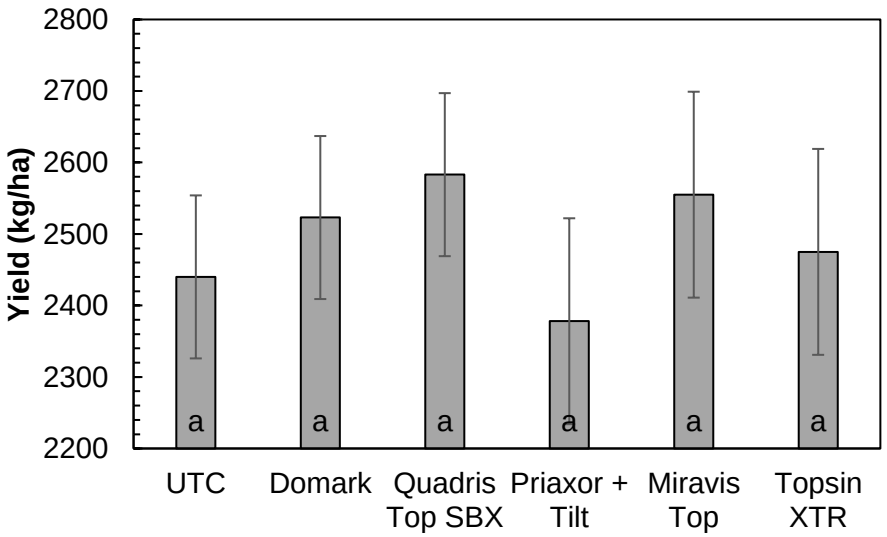

Figure 12. Yield (kg/ha) based on fungicide mixture for wheat bean soybean field location. Columns with the same letters are not statistically different based on Tukey's HSD at α=0.05.

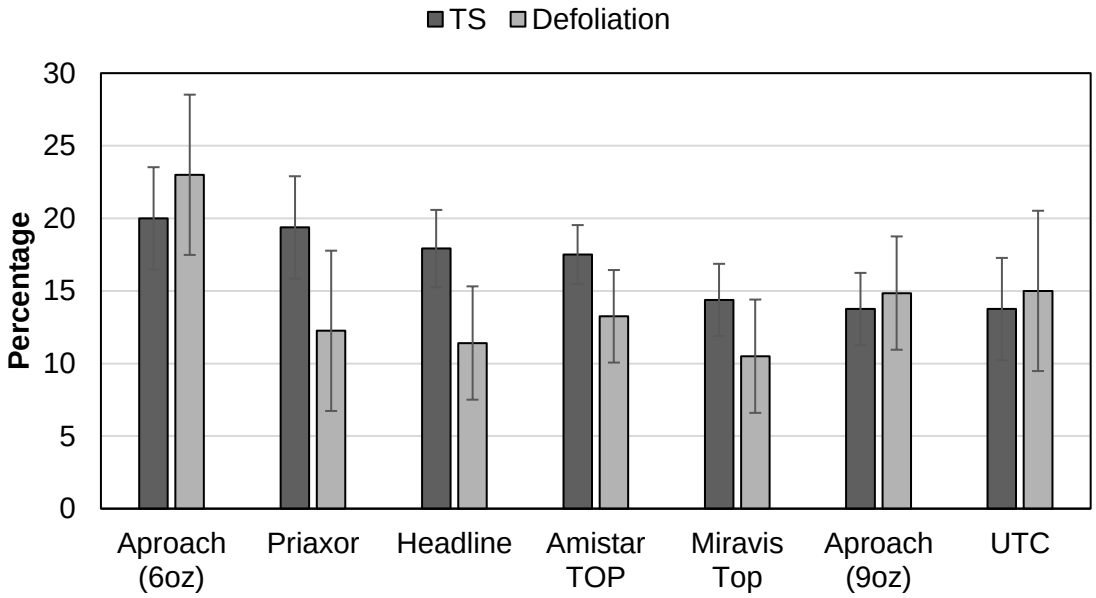

Figure 13. Target spot severity and defoliation (%) based on fungicide mixture for 2018 cotton trial. Columns with the same letters are not statistically different based on Tukey's HSD at α=0.05.

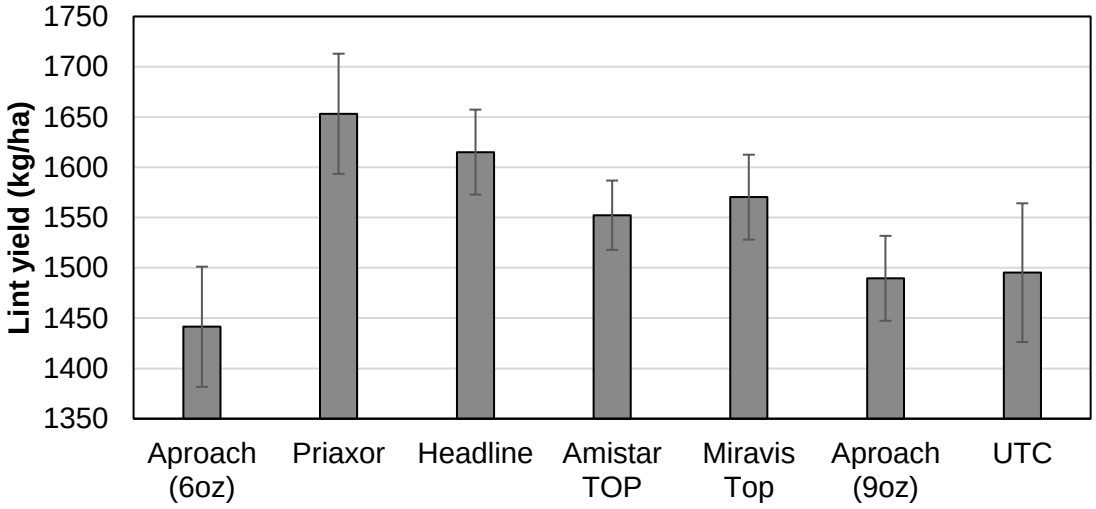

Figure 14. Lint yield (kg/ha) based on fungicide mixture for 2018 cotton trial. Columns with the same letters are not statistically different based on Tukey's HSD at α=0.05.

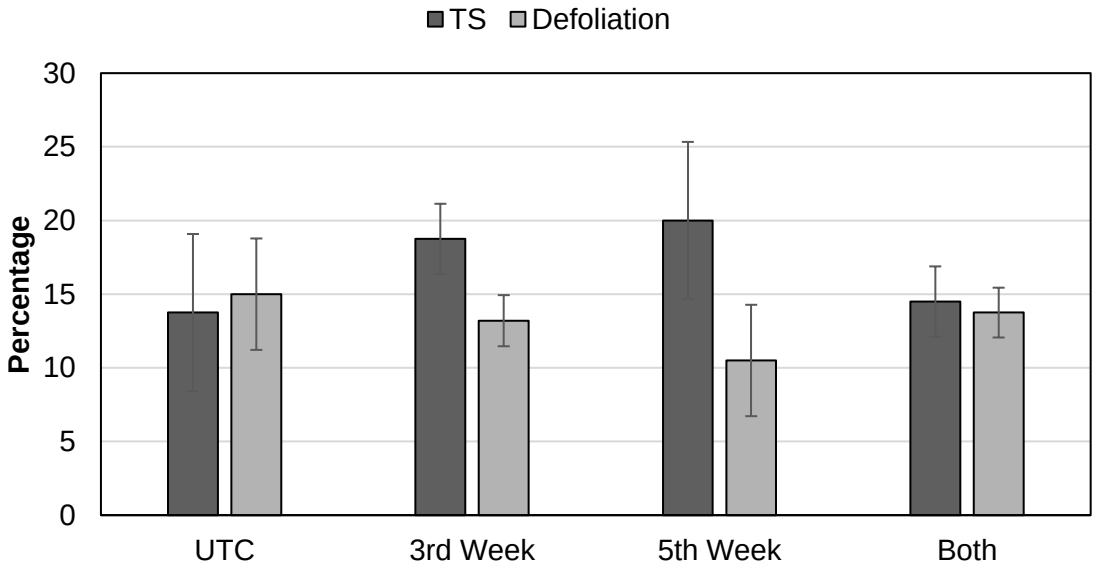

Figure 15. Target spot severity and defoliation (%) based on fungicide application timing for 2018 cotton trial. Columns with the same letters are not statistically different based on Tukey's HSD at α=0.05.

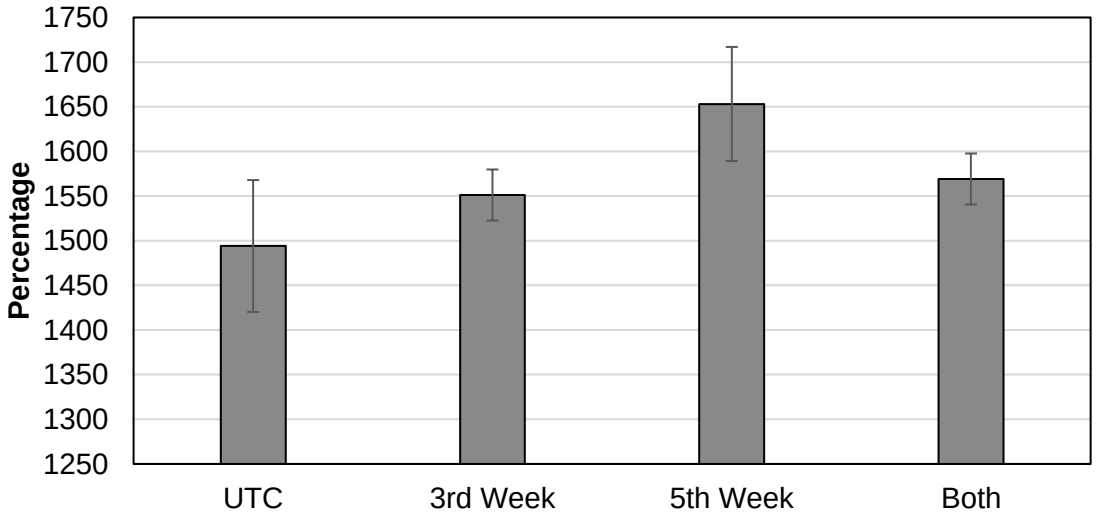

Figure 16. Lint yield (kg/ha) based on fungicide application timing for 2018 cotton trial. Columns with the same letters are not statistically different based on Tukey's HSD at α=0.05.

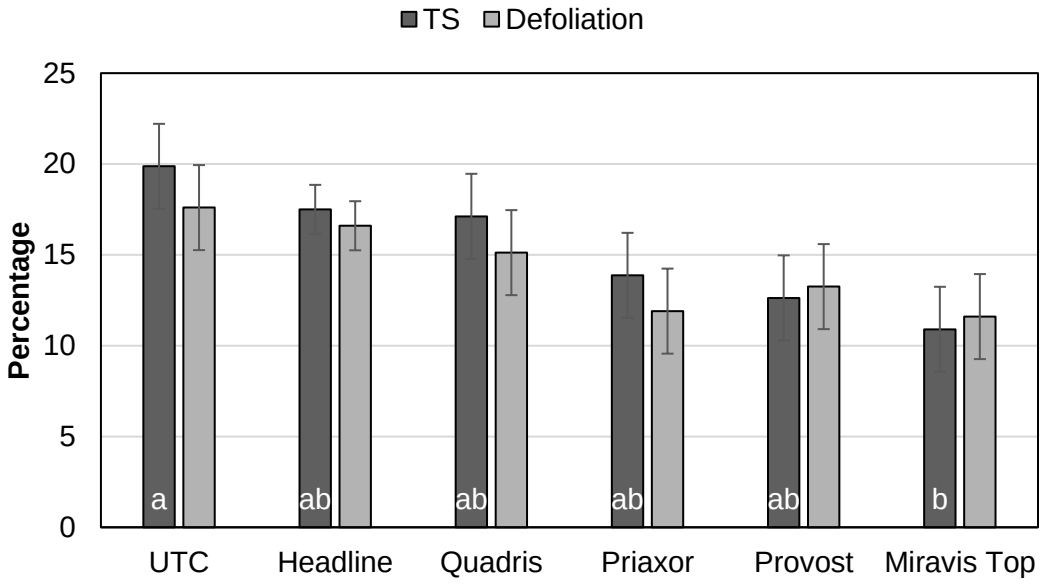

Figure 17. Target spot severity and defoliation (%) based on fungicide mixture for 2019 cotton trial. Columns with the same letters are not statistically different based on Tukey's HSD at α=0.05.

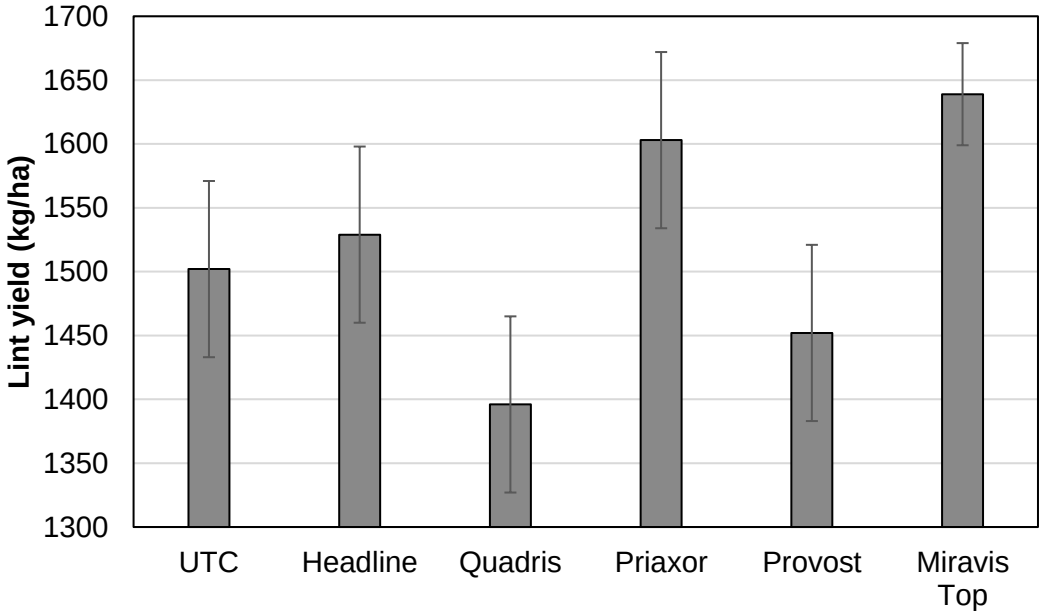

Figure 18. Lint yield (kg/ha) based on fungicide mixture for 2019 cotton trial. Columns with the same letters are not statistically different based on Tukey's HSD at α=0.05.

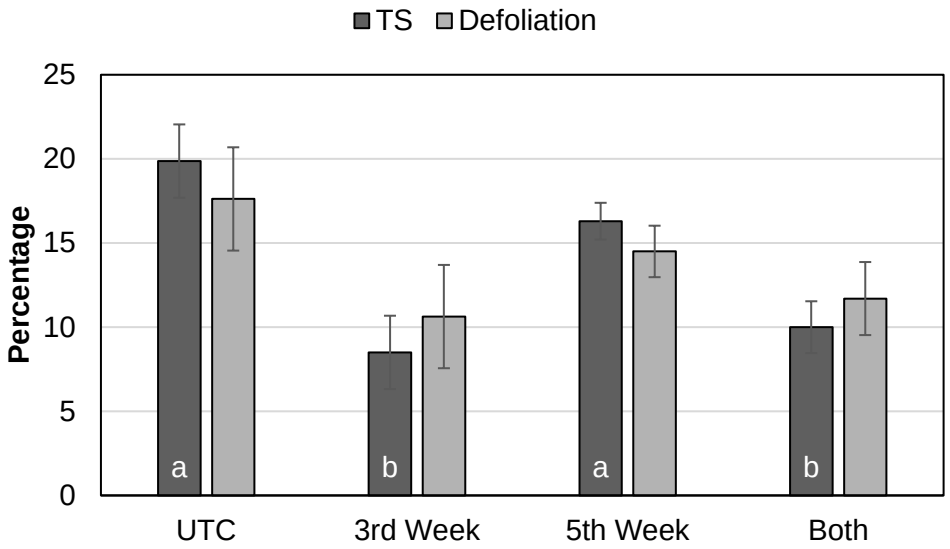

Figure 19. Target spot severity and defoliation (%) based on fungicide application timing for 2019 cotton trial. Columns with the same letters are not statistically different based on Tukey's HSD at α=0.05.

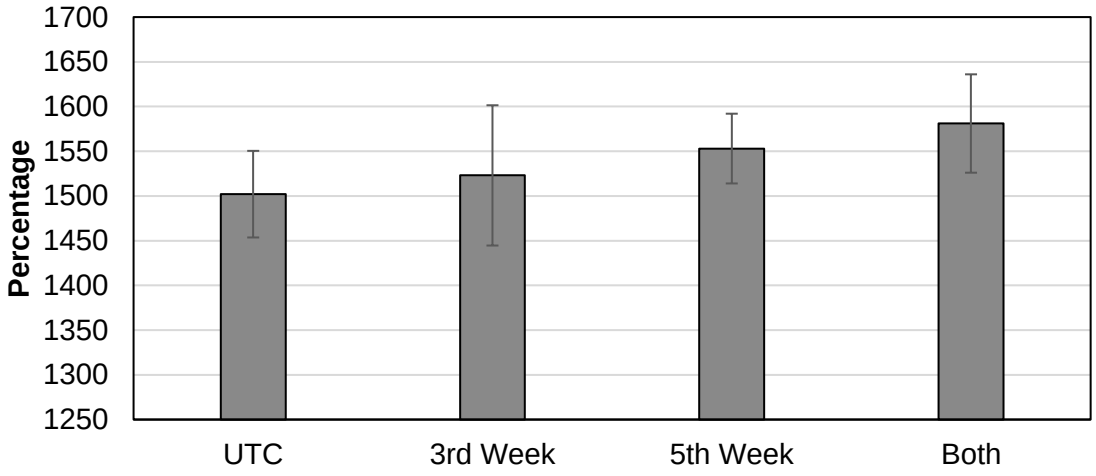

Figure 20. Lint yield (kg/ha) based on fungicide application timing for 2019 cotton trial. Columns with the same letters are not statistically different based on Tukey's HSD at α=0.05.

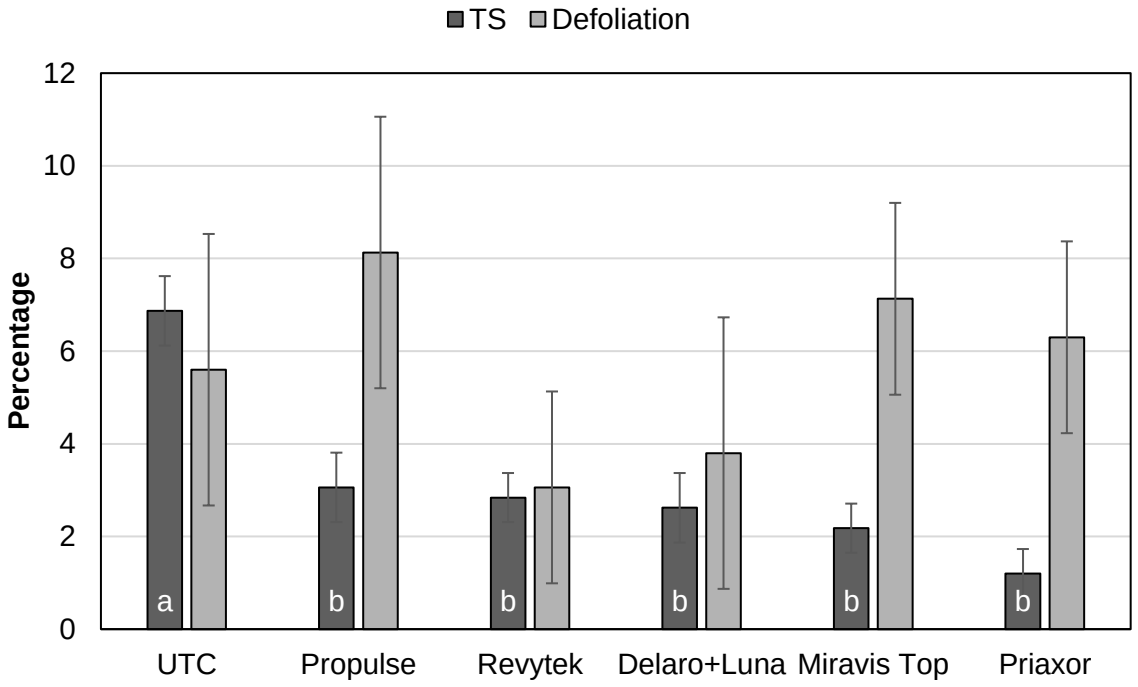

Figure 21. Target spot severity and defoliation (%) based on fungicide mixture for 2020 cotton trial. Columns with the same letters are not statistically different based on Tukey's HSD at α=0.05.

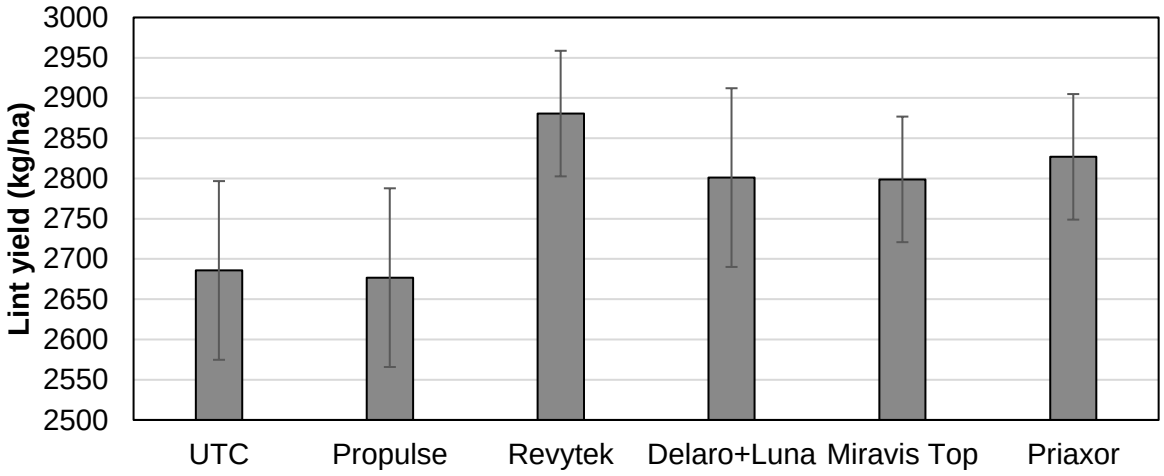

Figure 22. Lint yield (kg/ha) based on fungicide mixture for 2020 cotton trial. Columns with the same letters are not statistically different based on Tukey's HSD at α=0.05.

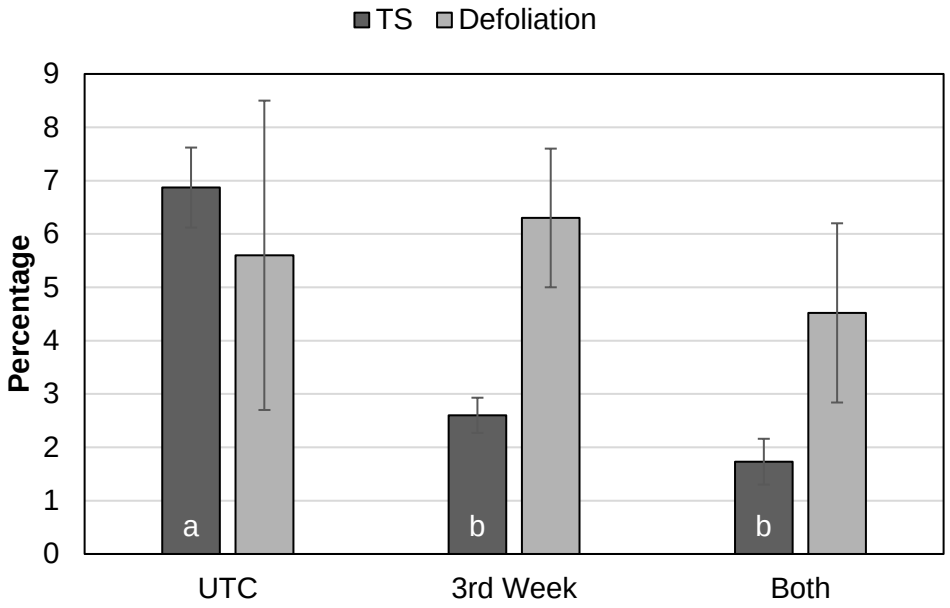

Figure 23. Target spot severity and defoliation (%) based on fungicide application timing for 2020 cotton trial. Columns with the same letters are not statistically different based on Tukey's HSD at α=0.05.

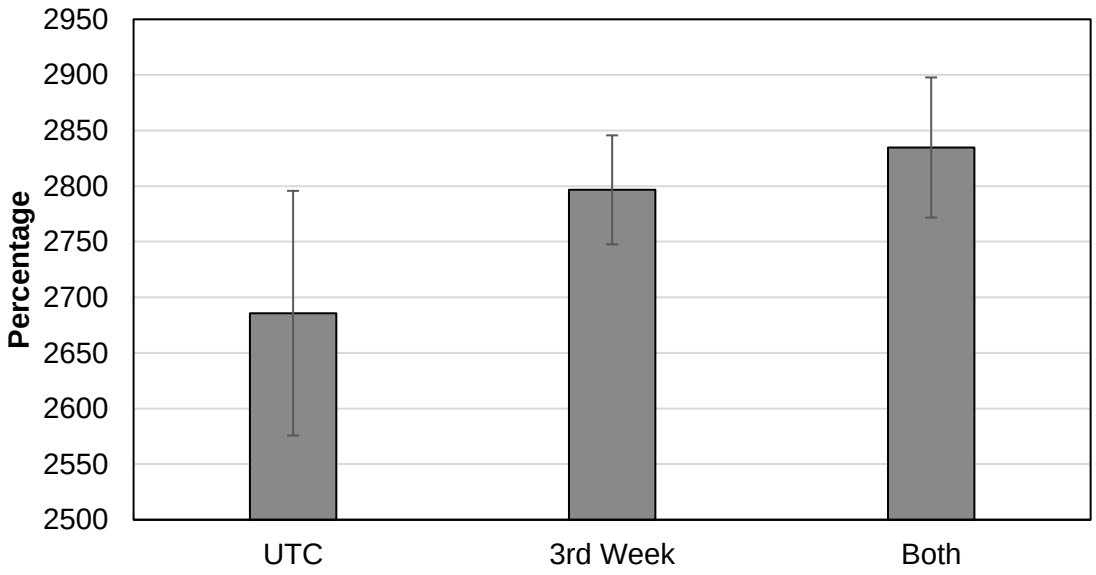

Figure 24. Lint yield (kg/ha) based on fungicide application timing for 2020 cotton trial. Columns with the same letters are not statistically different based on Tukey's HSD at α=0.05.

CHAPTER III. SENSITIVITY PROFILE OF *CORNESPORA CASSIICOLA* TO MULTIPLE FUNGICIDE GROUPS

Abstract

Corynespora cassiicola, is the causal pathogen of target spot in soybean and cotton. With target spot increasing in importance, fungicides are becoming an important tool for management of this disease. Unfortunately, there are reports of *C. cassiicola* isolates in other crops being resistant to some fungicide classes. The objectives of this study were to (i) develop baseline sensitivities for *C. cassiicola* isolates from cotton and soybean to the fungicides: azoxystrobin, pyraclostrobin, difenoconazole, propiconazole, thiophanate methyl, adepidyn, and fluxapyroxad; and (ii) determine if the sensitivity profiles are comparable across isolates taken from soybean and cotton. Thirty *C. cassiicola* isolates were used to determine baseline EC_{50} values to multiple fungicides. EC_{50} values for the tested isolates ranged from 0.448 µg/mL – 27.33 µg/mL ($\bar{x}$ = 6.04 µg/mL) for azoxystrobin; 0.024 µg/mL – 12.43 µg/mL ($\bar{x}$ = 2.11 µg/mL) for pyraclostrobin; 0.063 µg/mL – 3.94 µg/mL ($\bar{x}$ = 1.14 µg/mL) for propiconazole; 0.005 µg/mL – 2.85 µg/mL ($\bar{x}$ = 0.362 µg/mL) for difenoconazole; 0.017 µg/mL – 0.097 µg/mL ($\bar{x}$ = 0.055 µg/mL) for adepidyn; 0.031 µg/mL – 0.421 µg/mL ($\bar{x}$ = .0142 µg/mL) for fluxapyroxad; and 0.051 µg/mL – 3.41 µg/mL ($\bar{x}$ = 1.77 µg/mL) for thiophanate methyl. Fluxapyroxad was the only fungicide to exhibit statistically different EC_{50} values between isolates taken from cotton and soybean. Our findings will be useful to monitor sensitivity of U.S. populations of *C. cassiicola* from cotton and soybean, and to facilitate fungicide resistance management through detection of shifts in fungicide sensitivity.

Introduction

Corynespora cassiicola (Berk. & M.A. Curtis) C.T. Wei is a widespread pathogen that is associated with target spot disease on soybean and cotton plants. *C. cassiicola* has a large and diverse host range, infecting more than 500 plant species including the economic crops cotton (*Gossypium hirsutum*), rubber tree (*Ficus elastic*), tomato (*Solanum lycopersicum),* tobacco (*Nicotiana tabacum*), and soybean (*Glycine max)* (Ma and Michailides, 2005). Occurrence of target spot in soybean has increased in the U.S. and other countries, but recently the concern has shifted to cotton as well, as more target spot is being detected across the United States (Butler et al. 2016; Conner et al. 2013; Fulmer et al. 2012; Price et al. 2015). Disease incidence and severity has been increasing due to an increase in conservation tillage, use of susceptible varieties, lack of crop rotation, and changes in weather patterns (Avozani et al. 2014, Koenning et al. 2006). Yield losses can be significant if target spot is not managed (Fulmer et al., 2012; Faske and Kirkpatrick, 2014).

There are several strategies that can be implemented to manage field populations of *C. cassiicola* such as crop rotation and planting resistant varieties, but chemical control is the most effective method (Zhu et al., 2020). Unfortunately, the increased occurrence of fungicide resistance has been attributed to overuse of fungicides (Brent and Hollomon, 1995). According to FRAC (2020) though site-specific fungicides are generally safer for use, they can be prone to resistance development, while broad spectrum fungicides are more difficult for resistance to develop. Unfortunately, there are reported cases of *C.*

cassiicola isolates that are resistant to fungicides in crops such as soybean, tomato, rubber, and cucumber.

Quinone outside inhibitor (QoI, FRAC code 11) fungicides inhibit mitochondrial respiration by binding to the cytochrome *bc1* enzyme (complex III) at the quinone outside site (Bartlett et al., 2002). Unfortunately, soon after introduction in 1996, resistant isolates were detected in field populations of several pathogens (Bartlett et al., 2002). FRAC (2020) reports QoI fungicides as high risk to development of resistance with three major target sites in the cytochrome *b* gene. The G143A mutation has been reported in many different pathogens to confer the highest level of resistance, with F129L and G137R conferring partial resistance (Bartlett et al., 2002; Duan et al. 2019). QoI resistance in *C. cassiicola* has recently been reported in US soybean (Rondon and Lawrence, 2019). Like QoIs, succinate-dehdyrogenase inhibitors (SDHI, FRAC code 7) inhibit fungal respiration by binding to a unique site in the mitochondria (Avenot and Michailides, 2010). FRAC (2020) classifies SDHIs as medium to high risk for development of resistance with resistance becoming more widespread. *C. cassiicola* isolates from cucumber (*Cucumis sativus)* in Japan were reported to be resistant to the SDHI boscalid in 2009 (Miyamoto et al., 2010).

Demethylation inhibitor (DMI, FRAC code 3) fungicides are also known as triazoles and inhibit ergosterol production in fungi cell membranes. FRAC (2020) classifies DMIs as medium risk for fungicide resistance and these products have

continued use throughout the world. Even after widespread use for over 50 years, incidences of resistance are low and scattered (Brent and Holloman, 2007). Methyl benzimidazole carbamates (MBC, FRAC code 1) fungicides inhibit the β-tubulin assembly during mitosis causing issues with cell division in fungi (Howard and Aist, 1980). Benzimidazoles and thiophanates are among MBC fungicides which have been classified with a high risk for developing resistance (FRAC, 2020), and benzimidazole resistance was reported for *C. cassiicola* on several crops (Avozani et al., 2014; Date et al., 2004; Teramoto et al., 2017; Xavier et al., 2013). Thiophanate-methyl was very effective for controlling target spot on cucumber but a decrease in the effectiveness was observed with the emergence of *C. cassiicola* resistant isolates (Date et al., 2004).

The use of fungicides is crucial for management of diseases present in Tennessee soybean and cotton. Although important, baseline sensitivities have not been reported for *C. cassiicola* isolates taken from these crops. It is important to monitor efficacy of fungicides to determine if shifts in resistance are occurring. Therefore, the objectives of this study were to (i) develop baseline sensitivities for *C. cassiicola* isolates from cotton and soybean to the fungicides: azoxystrobin, pyraclostrobin, difenoconazole, propiconazole, thiophanate methyl, adepidyn, and fluxapyroxad; and (ii) determine if the sensitivity profiles are comparable across isolates taken from soybean and cotton.

Materials and Methods

Fungal collection and isolation

A total of 160 isolates of *C. cassiicola* were recovered from soybean and cotton leaves from 2018-2020 with symptoms of target spot in Tennessee, Mississippi, and Arkansas. Symptomatic tissue was cut from the healthy part of the leaf and surface sterilized. Leaf tissue was cleaned in sterile water and placed in 10% bleach for 1 minute and then rinsed off with sterile distilled water. Disinfected tissue was placed onto 10% water agar amended with 75 µg/mL of streptomycin. Inoculated plates were placed in darkness for 24-48 hours at 21 ± 2°C. Single mycelial strands were pulled from inoculated water agar plates and placed onto potato dextrose agar (PDA). Inoculated PDA plates were placed in 24-hour darkness and incubated at 28 ± 2°C, and pure colonies were selected to establish the *C. cassiicola* collection. Fungal identification was made based on morphological characteristics as described by Ellis and Holliday (1971). Isolates were stored on filter paper and stored at -20°C.

Mycelial growth inhibition assay

Seven technical grade fungicides (Table 3) were used to prepare stock solutions in acetone at the concentration of 100 mg/mL. Serial dilutions were prepared from stock solutions to obtain final concentrations of 0.01, 0.1, 1, and 10 µg/mL for pyraclostrobin, thiophanate methyl, propiconazole, difenoconazole, fluxapyroxad, and pydiflumetofen. Serial dilution for azoxystrobin was prepared at final concentrations of 0.1, 1, 10, and 100 µg/mL. Due to the ability of some fungi to overcome sensitivity of QoI fungicides through an alternate respiratory

pathway, salicylhydroxamic acid (SHAM) was used at a rate of 100 µg/mL for media amended with azoxystrobin and pyraclostrobin (MacKenzie et al. 2020). All fungicide dilutions were added to autoclaved PDA once cooled to 50-55°C and allowed to solidify. A control that included all ingredients except the fungicides listed (non-fungicide amended control) was included.

Thirty isolates of *C. cassiicola* from cotton (n=17) and soybean (n=13) were randomly selected to test the fungicide sensitivity in an *in vitro* bioassay. Isolates were inoculated by placing one mycelial plug (6 mm) from a 7-day-old colony at the center of a PDA plate and incubated at 28 ± 2°C under complete darkness. After 5 days, inoculated plates were removed and measured. Mycelial growth inhibition was evaluated by measuring colony diameter of each plate along two perpendicular lines. The diameter of the mycelial plugs for each plate was subtracted before calculating the average of the two measurements for each plate. The percent growth inhibition due to the fungicide treatments at different concentrations was calculated as follows: [(dc – dt) / dc] x 100, where dc = average diameter of fungal colony in control, and dt = average diameter of fungal colony in fungicide treatment (Ishii et al., 2007). The percent growth inhibition was used to calculate the EC_{50} values (fungicide concentration that inhibited 50% of the mycelial growth) for each isolate-fungicide and values were expressed in µg/mL. The experiment was a completely randomized design with two replicates of each isolate-fungicide concentration combination. A Petri dish was used as an

experimental unit and two independent experiments were conducted for each fungicide.

Data analysis

EC_{50} values were estimated by the dose response probit analysis in JMP Pro 14 (SAS Institute Inc., Cary, NC, USA). Data from two trials for each fungicide were combined for statistical analysis representing four replications per isolate-fungicide concentration. EC_{50} values were subjected to an analysis of variance using GLM in JMP Pro 14 and means were separated with Tukey's HSD at $\alpha = 0.05$. A Student's t test was used to analyze any differences between cotton and soybean isolates at $\alpha = 0.05$.

Results

EC_{50} values for the tested isolates ranged from 0.448 µg/mL – 27.33 µg/mL ($\bar{x}$ = 6.04 µg/mL) for azoxystrobin; 0.024 µg/mL – 12.43 µg/mL ($\bar{x}$ = 2.11 µg/mL) for pyraclostrobin; 0.063 µg/mL – 3.94 µg/mL ($\bar{x}$ = 1.14 µg/mL) for propiconazole; 0.005 µg/mL – 2.85 µg/mL ($\bar{x}$ = 0.362 µg/mL) for difenoconazole; 0.017 µg/mL – 0.097 µg/mL ($\bar{x}$ = 0.055 µg/mL) for adepidyn; 0.031 µg/mL – 0.421 µg/mL ($\bar{x}$ = .0142 µg/mL) for fluxapyroxad; and 0.051 µg/mL – 3.41 µg/mL ($\bar{x}$ = 1.77 µg/mL) for thiophanate methyl (Table 4 and 5).

For the fungicide azoxystrobin, no statistical difference was found for the mean EC_{50} values for isolates from cotton ($\bar{x}$ = 6.57 µg/mL) or soybean ($\bar{x}$ = 5.25 µg/mL) (*P*=0.5935) (Figure 25). Again, no statistical difference was found for the mean EC_{50} values for isolates from cotton ($\bar{x}$ = 2.38 µg/mL) or soybean ($\bar{x}$ = 1.70

µg/mL) (*P*=0.5074) for pyraclostrobin (Figure 26). There was no statistical difference found for the mean EC_{50} values for isolates from cotton ($\bar{x}$ = 1.15 µg/mL) or soybean ($\bar{x}$ = 1.01 µg/mL) (*P*=0.5742) for propiconazole. The other DMI fungicide, difenoconazole, also had no statistical difference in mean EC_{50} values for isolates from cotton ($\bar{x}$ = 0.43 µg/mL) or soybean ($\bar{x}$ = 0.29 µg/mL) (*P*=0.4208). There was no statistical difference for the fungicide adepidyn with respect to the mean EC_{50} values for isolates from cotton ($\bar{x}$ = 0.05 µg/mL) or soybean ($\bar{x}$ = 0.06 µg/mL) (*P*=0.2236). For fluxapyroxad, soybean isolates ($\bar{x}$ = 0.17 µg/mL) had statistically higher EC_{50} mean values compared to cotton isolates ($\bar{x}$ = 0.11 µg/mL) (*P*=0.0231). For the fungicide thiophanate methyl, no statistical difference was found for the mean EC_{50} values for isolates from cotton ($\bar{x}$ = 2.01 µg/mL) or soybean ($\bar{x}$ = 2.04 µg/mL) (*P*=0.8857) (Figures 1-2).

Discussion

In this study, baseline sensitivities of 30 *C. cassiicola* isolates obtained from Tennessee cotton and soybean infected leaves were established for the fungicides azoxystrobin, pyraclostrobin, propiconazole, difenoconazole, adepidyn, fluxapyroxad, and thiophanate methyl.

C. cassiicola possesses a moderate to high risk of developing resistance to the single target fungicides in the quinone outside inhibitor (QoI) class (FRAC, 2020). Development of resistance to QoIs such as pyraclostrobin and azoxystrobin has recently been discovered in *C. cassiicola* isolated from soybean

in Alabama (Rondon and Lawrence, 2020). There are numerous mutations in the cytochrome *b* gene associated with resistance to QoIs in different fungi, with the G143A mutation being the most common (Ma and Michailides, 2005). The G143A mutation is also associated with the highest levels of resistance in fungi resistant to QoI fungicides (Duan et al., 2019). Other mutations, such as F129L, G137R, and Y279, are associated with low to moderate levels of resistance (Fisher and Meunier, 2008; Duan et al., 2019). Teramoto et al. (2017) reported 10 isolates of *C.cassiicola* out of 34 to have EC_{50} < 16 µg/mL, considering them sensitive to pyraclostrobin. Only 1 isolate that was tested exhibited an EC_{50} value > 36.55 µg/mL, and it was considered non-sensitive to pyraclostrobin. Additionally, Teramoto et al. (2017) noted that 14 *C. cassiicola* had EC_{50} values > 28 µg/mL when treated with azoxystrobin, suggesting these isolates are non-sensitive to QoIs. According to FRAC (2020), QoI fungicides are at a high risk for resistance development as well as developing cross resistance within the class. Rondon and Lawrence (2019) confirmed the presence of the G143A resistance mutation in *C. cassiicola* isolates from soybean in the US. Four isolates had EC_{50} > 50 µg/mL, suggesting a complete loss of sensitivity to pyraclostrobin. In this study all 30 isolates of *C. cassiicola* had EC_{50} values < 13 µg/mL when treated with pyraclostrobin suggesting that the isolates tested are all sensitive to this active ingredient . Additionally, for azoxystrobin these isolates exhibited EC_{50} values < 28 µg/mL suggesting these isolates are sensitive to azoxystrobin as well. This agrees with to findings by Teramoto et al. (2017), where 20 of the 34

isolates tested exhibited EC_{50} values < 28 µg/mL for azoxystrobin and were considered sensitive. The large range of EC_{50} values for pyraclostrobin (0.024 µg/mL – 12.43 µg/mL) and azoxystrobin (0.448 µg/mL – 27.33 µg/mL) suggest that a shift in sensitivity for QoI fungicides is possibly occurring in Tennessee cotton and soybean fields.

Although resistance to DMI fungicides has been documented in other pathogens, there are few reports to suggest that *C. cassiicola* has developed resistance (FRAC, 2020). In this study, the efficacy of propiconazole and difenoconazole was evaluated for control of *C. cassiicola* isolates from cotton and soybean. Although there are no direct reports on the use of propiconazole and difenoconazole to control *C.cassiicola,* cross resistance can occur with DMI fungicides on the same fungi (Russell, 2005). The highest EC_{50} values for propiconazole and difenoconazole was 3.94 µg/mL and 1.43 µg/mL, respectively. Also, there were no statistical differences between isolates surveyed from cotton and soybean. Avozani et al. (2014) reported the sensitivity of 5 *C. cassiicola* isolates to multiple DMI fungicides (flutriafol, tebuconazole, cyproconazole, and epiconazole) with EC_{50} values that ranged from 0.77 to 20.32 µg/mL. Xavier et al. (2013) also studied the sensitivity of 24 isolates from soybean to the DMI prothioconazole, with EC_{50} values ranging from 0.47 to 26.44 µg/mL. Lastly, Teramoto et al. (2017) found that the EC_{50} values for 34 isolates of *C. cassiicola* isolated from soybean had EC_{50} values that ranged from 0.16 to 100 µg/mL for the DMI fungicide, cyproconazole and EC_{50} values ranging from 0.16 to 46.44

µg/mL for prothioconazole. Teramoto et al. (2017) suggests that isolates with EC_{50} values < 0.16 µg/mL be considered highly sensitive, and isolates with EC_{50} values ranging from 0.16 to 1.0 µg/mL be considered moderately sensitive. As of 2021, no reports of resistance mutations have been discovered for *C. cassiicola* in soybean and cotton for conferring resistance to DMI fungicides. This along with the results of this study suggest that DMI fungicides can continue to be used to treat target spot infestations in Tennessee soybean and cotton fields.

As with QoI fungicides, SDHI fungicides have a moderate to high risk of developing resistance due to being site-specific (FRAC 2020). Currently, few reports exist of *C. cassiicola* resistance to SDHI. In this study we the efficacy of two SDHI fungicides, fluxapyroxad and adepidyn were evaluated. The highest EC_{50} values for *C. cassiicola* was 0.42 µg/mL for fluxapyroxad and 0.09 µg/mL for adepidyn. There were no statistical differences in EC_{50} values for isolates collected from cotton or soybean when treated with adepidyn, but cotton isolates had statistically higher EC_{50} values for fluxapyroxad. According to Miyamoto et al. (2010), 200 isolates of *C. cassiicola* sampled from cucumber greenhouses in China were resistant to boscalid. Moderately resistant isolates had EC_{50} values ranging from 2.0 to 5.9 µg/mL, while very highly resistant isolates had EC_{50} values > 30 µg/mL. Along with increased EC_{50} values, Miyamota et al. (2010) also detected several mutations in the SdhB, SdhC, and SdhD subunits. Zhu et al. (2019) reported over 200 isolates, from numerous crops around China that had no exposure to SDHI fungicides, to have decreased sensitivity to boscalid

with EC_{50} values ranging from 4.20 to > 50 µg/mL. Neves and Bradley (2020) reported *C. cassiicola* isolates in the U.S. to be highly sensitive to pydiflumetofen with EC_{50} values ranging from 0.037 to 0.248 µg/mL. According to Zhu et al. (2019), cross resistance was detected between similar fungicides within the SDHI group, but this is not true for all SDHI fungicides. No reports of resistance to SDHI fungicides have been reported for *C. cassiicola* isolates from cotton or soybean. Along with results from this study, SDHI fungicides are an excellent addition to any fungicide resistance management program due to exceptional management of target spot in soybean and cotton. Care must be taken to mitigate resistance development due to the specific mode of action of SDHIs (FRAC, 2020).

MBC fungicides have been widely used across many cropping systems and cross resistance among this group has developed over time among multiple fungicides such as: thiophanate methyl, carbendazim, thiabendazole, and benomyl (FRAC, 2020). Results from this study found that all isolates of *C. cassiicola* recovered from cotton and soybean had EC_{50} values ranging from 0.89 to 2.93 µg/mL when treated with thiophanate methyl. There also were no statistical differences between isolates taken from cotton or soybean. Avonzani et al. (2014) reported sensitive isolates of *C. cassiicola* from soybean to have EC_{50} values < 1.0 µg/mL, and isolates were not considered insensitive until EC_{50} values > 40 µg/mL when treated with carbendazim. Teramota et al. (2017) also reported a loss of sensitivity for *C. cassiicola* isolates when EC_{50} values

exceeded 556 µg/mL for carbendazim and 294 µg/mL for thiophanate methyl. Duan et al. (2019) reported resistance to benzimidazoles when EC_{50} values > 192 µg/mL for carbendazim, > 78 µg/mL for benomyl, and > 18 µg/mL for thiabendazole. Duan et al. (2019) also reported the presence of “super-resistant” isolates of *C. cassiicola* due to double mutations present in the β-tubulin gene. Although the isolates from Tennessee soybean and cotton tested in this study were found to be sensitive to thiophanate methyl, it is not recommended to use MBC fungicides alone in a management system due to the likely presence of other diseases. Price et al. (2015) reported benzamidazole resistance in *Cercospora kikuchii,* a common soybean pathogen causing Cercospora leaf blight and purple seed stain, isolated from soybean in Louisiana. Duan et al. (2019) suggested that an increase in use of higher dose benzamidazoles can increase the presence of double mutations appearing in *C. cassiicola* populations, which led to suggestions to restrict the use of MBC fungicides in cucumber production in China.

Fungicides are generally an effective tool for managing sensitive populations of pathogens, but over time resistant isolates will increase as more fungicides applications are made. The rise of site-specific fungicides has increased this problem (Ma and Michailides, 2005). Fungicide resistance development cannot be stopped, but the main goal of any resistance management program should be to preserve the efficacy of fungicides and delay that resistance. There are a number of different strategies within an effective

fungicide resistance program such as: managing and using accurate application doses, using the correct number of applications, and using fungicide mixtures when needed with at-risk fungicides (Bosch et al. 2014). The use of single-target site fungicides should be applied in combination with other fungicide groups or properly rotated out from application to application (Ghini and Kimati, 2000). Using these strategies, along with other integrated pest management approaches, are imperative to slowing down the development of resistance in Tennessee cotton and soybean.

The goal of this study was to develop baseline sensitivity data for *C. cassiicola* isolated from Tennessee cotton and soybean. Russell (2002) discussed the importance of baseline sensitivity data to explain shifts in sensitivity over time, and to provide insight into resistant populations being the cause of control failures in field settings. The baseline data reported in this study along with the continued use of integrated pest management strategies such as crop rotation, resistant varieties, and possible biocontrol options, will help prolong the efficacy of these fungicides.

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Appendix

Table 3. Description of technical grade fungicides tested in this study

Fungicide classification	Active ingredient	Commercial Products	Manufacturer
Qoi (Group 11)	Azoxystrobin	Many generics	------
Qoi (Group 11)	Pyraclostrobin	Headline EC	BASF
DMI (Group 3)	Propiconazole	Tilt	Syngenta
DMI (Group 3)	Difenoconazole	Difenoconazole	Albaugh
MBC (Group 1)	Thiophanate methyl	Topsin	UPL
SDHI (Group 7)	Adepidyn	Miravis Top	Syngenta
SDHI (Group 7)	Fluxapyroxad	Priaxor	BASF

Table 4. Sensitivity of *Corynespora cassiicola* isolates obtained from soybean and cotton to QoI and DMI fungicides.

Isolate	Origin	EC_{50} (µg/mL)[x]			
		Azoxystrobin	Pyraclostrobin	Propiconazole	Difenconazole
F-501	Cotton	7.85 a-f	---	---	0.01 a
F-211	Cotton	11.68 ab	---	---	0.01 a
F-29	Cotton	5.96 a-f	5.96 c	1.15 ab	0.01 a
F-28	Cotton	10.37 abc	10.12 b	1.99 ab	0.04 a
F-27	Cotton	---	10.43 b	1.76 ab	0.02 a
F-25	Cotton	12.18 a	4.68 cd	1.64 ab	0.01 a
F-24	Cotton	7.31 a-f	5.05 b	1.16 ab	0.01 a
F-23	Cotton	---	3.20 d	1.96 ab	0.63 a
F-21	Cotton	8.06 a-f	12.43 a	2.44 ab	1.16 a
CGTS6	Soybean	8.80 a-e	6.04 c	0.99 ab	1.43 a
CGTS4	Soybean	5.64 a-f	4.63 cd	1.81 ab	---
CGTS1	Soybean	---	9.04 b	3.94 a	0.01 a
19-82750	Soybean	2.01 def	0.08 e	0.45 b	0.24 a
19-82749	Cotton	1.16 ef	0.36 e	0.65 ab	0.26 a
19-82726	Cotton	2.06 def	0.23 e	0.76 ab	0.30 a
19-82710	Cotton	0.75 f	0.16 e	0.70 ab	0.29 a
19-81638	Cotton	1.31 ef	0.24 e	0.55 b	0.23 a
19-81633	Soybean	0.61 f	0.17 e	1.18 ab	0.24 a
19-81615	Cotton	3.90 b-f	0.16 e	0.70 ab	0.15 a
19-72510	Soybean	1.10 ef	0.21 e	0.64 ab	0.29 a
19-8819	Soybean	0.93 ef	0.81 e	1.05 ab	0.81 a
19-8813	Soybean	0.45 f	0.23 e	0.44 b	0.22 a
19-8273	Cotton	0.76 f	0.14 e	0.78 ab	0.15 a
19-8221	Cotton	1.10 ef	0.12 e	0.40 b	0.51 a
19-8215	Cotton	0.57 f	0.27 e	0.89 ab	0.15 a
19-8169	Soybean	0.61 f	0.24 e	0.88 ab	0.16 a
19-887	Soybean	1.25 ef	0.12 e	0.83 ab	0.38 a
18-602	Soybean	6.44 a-f	3.09 d	2.44 ab	1.09 a
18-595	Soybean	9.35 abcd	3.03 d	1.48 ab	0.59 a
18-594	Soybean	1.63 cdef	5.21 c	2.31 ab	0.39 a
Mean		3.16	2.09	1.09	0.38
F Value		8.99	237.88	2.18	0.45

[x] Least significant mean of EC_{50} values followed by the same letter were not statistically different in Tukey's HSD (*P<0.05).*

Table 5. Sensitivity of *Corynespora cassiicola* isolates obtained from soybean and cotton to MBC and SDHI fungicides.

Isolate	Origin	EC_{50} (µg/mL)		
		Thiophanate methyl	Adepidyn	Fluxapyroxad
F-501	Cotton	---	---	---
F-211	Cotton	0.96 a	---	0.17 abcd
F-29	Cotton	1.81 a	---	---
F-28	Cotton	1.36 a	0.09 a	0.19 abcd
F-27	Cotton	2.03 a	---	0.17 abcd
F-25	Cotton	1.77 a	---	0.42 a
F-24	Cotton	1.48 a	---	0.11 ab
F-23	Cotton	1.73 a	0.06 a	0.37 ab
F-21	Cotton	0.95 a	0.07 a	0.31 abc
CGTS6	Soybean	1.74 a	0.08 a	0.2 abcd
CGTS4	Soybean	---	---	---
CGTS1	Soybean	1.21 a	---	0.09 cd
19-82750	Soybean	1.98 a	0.04 a	0.07 d
19-82749	Cotton	1.71 a	0.06 a	0.19 bcd
19-82726	Cotton	0.89 a	0.04 a	0.09 cd
19-82710	Cotton	2.25 a	0.05 a	0.05 d
19-81638	Cotton	2.60 a	0.06 a	0.09 cd
19-81633	Soybean	2.08 a	0.06 a	0.16 bcd
19-81615	Cotton	2.32 a	0.05 a	0.11 cd
19-72510	Soybean	2.83 a	0.06 a	0.15 bcd
19-8819	Soybean	1.86 a	0.09 a	0.11 cd
19-8813	Soybean	2.23 a	0.05 a	0.14 bcd
19-8273	Cotton	1.90 a	0.06 a	0.07 d
19-8221	Cotton	2.24 a	0.04 a	0.17 bcd
19-8215	Cotton	2.93 a	0.05 a	0.09 cd
19-8169	Soybean	1.81 a	0.04 a	0.08 d
19-887	Soybean	1.84 a	0.06 a	0.04 d
18-602	Soybean	2.66 a	0.06 a	0.19 abcd
18-595	Soybean	2.79 a	0.04 a	0.17 abcd
18-594	Soybean	2.17 a	0.06 a	0.19 abcd
Mean		2.02	0.05	0.14
F Value		0.93	1.56	5.71

[x] Least significant mean of EC_{50} values followed by the same letter were not statistically different in Tukey's HSD (*P<0.05).*

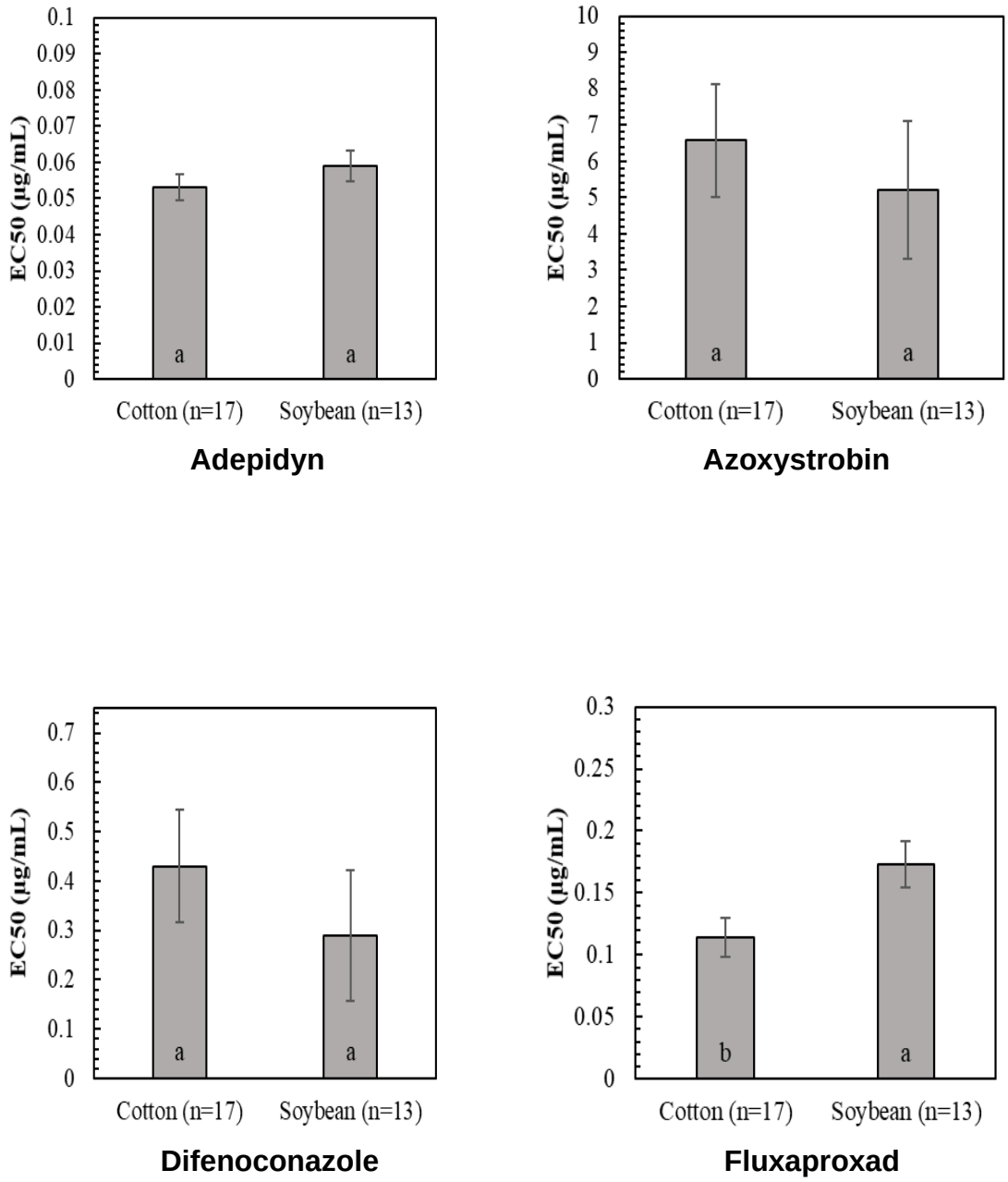

Figure 25. EC_{50} values of *Corynespora cassiicola* isolates for the fungicides; adepidyn, azoxystrobin, difenoconazole, and fluxapyroxad, separated by origin of isolation (soybean or cotton). Bars labeled with different letters are statistically different according to Student's t-test (α=0.05).

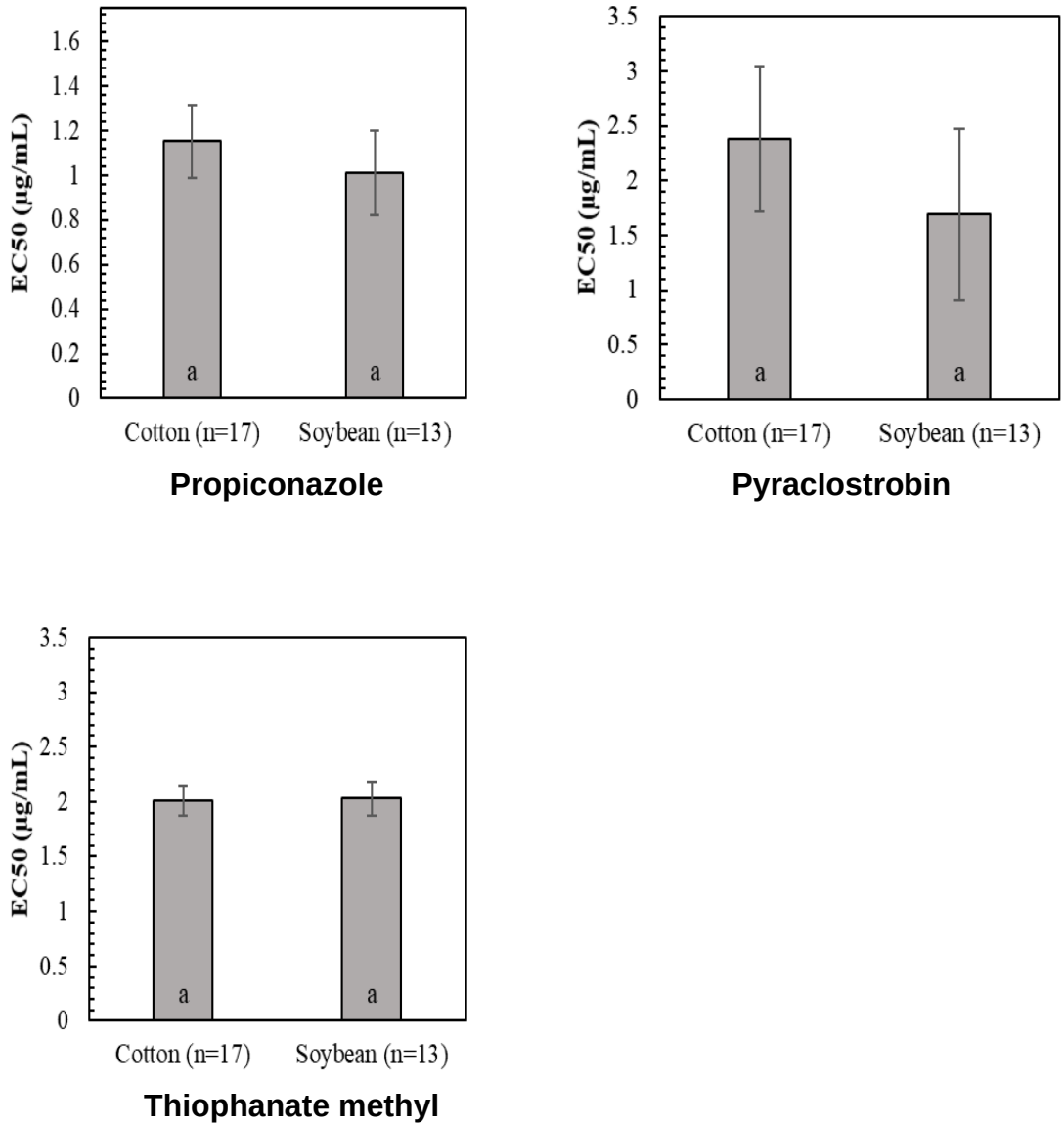

Figure 26. EC_{50} values of *Corynespora cassiicola* isolates for each fungicide: propiconazole, pyraclostrobin, and thiophanate methyl, separated by origin of isolation (soybean or cotton). Bars labeled with different letters are statistically different according to Student's t-test (α=0.05).

CHAPTER IV. INVESTIGATING THE EFFECTS OF DEMETHYLATION INHIBITOR FUNGICIDES AND THE INSECTICIDE MALATHION ON *CORYNESPORA CASSIICOLA* MANAGEMENT

Abstract

Target spot of soybean is a foliar disease caused by the ascomycete, *Corynespora cassiicola*. Over recent years, target spot has become an increasing concern to soybean for its ability to decrease yields and the development of resistance to the strobilurin (FRAC group 11) fungicides. Previous research into characterizing herbicide resistance in the ALS inhibitors (HRAC group 2) and microtubule inhibitors (HRAC group 3) has shown that the insecticide malathion can help combat a resistant plant's ability to metabolize herbicides belonging to the aforementioned classes due to sites of action in the cytochrome P450s being similar. Similar to the herbicides mentioned, the site of action of demethylation inhibitor fungicides (DMI) is located in P450 sites. The purpose of this study was to investigate if malathion had an effect on the ability of DMI fungicides to manage *C. cassiicola.* This experiment was composed of three parts: a small plot field trial, a detached leaf assay, and an *in vitro* mycelial growth assay. Two fungicides, Tilt and Domark 230ME, of varying effectiveness against target spot of soybean were used along with labeled rates of Malathion 57%. Across all trials/assays malathion alone did not statistically reduce the amount of target spot compared to non-treated checks. In the small plot trial, Domark 230ME nor Tilt did not reduce the amount of target spot in the field compared to the non-treated plots. In the detached leaf assay, the treatments Tilt and Tilt + Malathion statistically reduced the amount of target spot compared to the non-treated checks. In the mycelial growth assay, the colony size of *C. cassiicola* was not statistically reduced when malathion was added with Tilt or

Domark 230ME. Although malathion had little effect when combined with Tilt or Domark 230EC, it should be further studied in the presence of isolates of *C. cassiicola* that are insensitive to DMI fungicides.

Introduction

Corynespora cassiicola (Berk. & M.A. Curtis) C.T. Wei is a widespread pathogen that is associated with target spot disease on soybean and cotton plants. *C. cassiicola* has a large and diverse host range, infecting more than 500 plant species. Economic crops that are infected by *C. cassiicola* include: cotton, rubber tree, tomato, tobacco, and soybean (Ma and Michailides 2005). Occurrence of target spot in soybean has increased in the U.S. and other countries, but recently the concern has shifted to cotton as well, as more target spot is being detected across the U.S. (Fulmer et al. 2012; Price et al. 2015; Conner et al. 2013; Butler et al. 2016). Disease incidence and severity has been increasing due to an increase in conservation tillage, use of susceptible varieties, lack of crop rotation, and changes in weather patterns (Avozani et al. 2014; Koenning et al. 2006). Yield losses can be significant if target spot is not managed correctly (Fulmer et al. 2012).

There are several strategies that can be implemented to control field populations of *C.cassiicola* such as crop rotation and planting resistant varieties, but chemical control is the most effective method (Zhu et al. 2020). Unfortunately, the increased occurrence of fungicide resistance has been attributed to overuse of fungicides (Brent and Hollomon 1995). According to

FRAC (2021) site-specific fungicides are generally safer for use, but they can be prone to resistance development, while broad spectrum fungicides are more difficult for resistance to develop. Unfortunately, there are reported cases *C. cassiicola* isolates that are resistant to fungicides in crops such as soybean, tomato, rubber, and cucumber.

Demethylation inhibitor fungicides (DMI, FRAC code 3) are also known as triazoles and inhibit ergosterol production in fungal cell membranes. FRAC (2021) classifies DMIs as medium risk for resistance and are widely used today. Two methods of resistance have been identified in fungi in response to DMIs: mutations in the CYP51 gene or an overexpression of the CYP51 gene (Ma and Michailides 2005). The CYP51 is a family of proteins that belong to the cytochrome P450 superfamily found in all biological kingdoms. CYP51 are the target areas for DMI fungicides and assist in biodefense, such as, detoxification and production of sterols (Lepesheva et al. 2008).

Malathion is an organophosphate first introduced in the 1950s, and has been widely used in the control of cotton boll weevil and control of mosquitoes in urban areas for West Nile Virus prevention (Cook et al. 2005). Organophosphate insecticides are known to have a synergistic interaction with ALS inhibitor herbicides (HRAC, Group 2) that include the sulfonylureas (Kruez and Fonne-Pfister 1992; Targif and Powles 1999). This action is caused by competitive inhibition of cytochrome P450 enzymes present in different organisms. Christopher et al. (1994) reported that a population of rigid ryegrass (*Lolium*

rigidum) with an application of the organophosphate malathion and the herbicide chlorsulfuron decreased the rate of resistance in the ryegrass population. It is possible that the enzymatic system responsible for this interaction is present in fungi that contain similar cytochrome P450s.

With the interaction of malathion as a possible inhibitor in ALS inhibitor herbicides, the purpose of this study was to evaluate the possible interaction between malathion and the DMI fungicide class. The objectives of this study were (i) to evaluate the effects on management of target spot in soybean when treated with the DMI fungicides, Domark and Tilt, and the insecticide malathion in a field environment; (ii) evaluate the interaction between DMI fungicides, Domark and Tilt, and the insecticide malathion on reducing lesion size of *C. cassiicola* infected soybean leaves; and (iii) evaluate the efficacy of fungicides Domark and Tilt, and the insecticide malathion on reducing colony size of individual *C. cassiicola* isolates from soybean and cotton.

Materials and Methods

Field Setup

This experiment was conducted at a grower field 13.4 km east of Jackson, TN to evaluate the effects of tank mixed Domark, Tilt, and Malathion on the control of target spot in soybean. This field was in a multi-year rotation between corn and soybean and had moderate to high pressure of target spot and frogeye leaf spot. This trial was planted with a target spot susceptible variety of AG53X0 (Asgrow, Bayer Crop Science, Research Triangle Park, NC, USA) on May 21,

2020. The experiment was arranged in a randomized complete block design with a 3x2 factorial arrangement of treatments. Treatments consisted of the fungicides, Tilt (Syngenta Crop Protection) and Domark 230 ME (Gowan Company), and the insecticide Malathion 57 (Loveland Products, Inc). Malathion was applied as a standalone application, as a tank mix with another fungicide, or applied 30 minutes before fungicide application. Soybeans were planted on 76 cm rows with a plant density of 24 seeds per meter and plots were 9 m long by 3 m wide and had 4 replications at each location. Weed and insect control was based on recommendations from the University of Tennessee Extension Service. The center two rows of each plot were harvested with an Almaco two-row combine and yield was adjusted based on 13 percent moisture in ARM 2020 (Gylling Data Management, Brookings, SD, USA). Fungicide applications were made with a Lee Spider Sprayer (LeeAgra Inc, Lubbock, TX, USA) fitted with TeeJet 8002 flat fan spray tips during the R3 growth stage as described by Fehr et al. (1971). Applications were made at 15 GPA, at a pressure of 35 PSI, and at a speed of 5.6 km/h. Visual disease ratings for target spot were taken twenty-one days after fungicide application and again at twenty-eight days after application on a 0-100% scale with 0 = no disease present and 100 = total infection of the canopy. Percent target spot, defoliation, and yield were measured responses analyzed with a mixed model using JMP 9.4. Timing, insecticide, and fungicide applied were treated as fixed effects; whereas random effects were block, block x

timing, block x timing x insecticide, and block x timing x insecticide x fungicide. Means were separated using Tukey's least significant difference test (P≤0.05).

Detached leaf assay

Pots were filled with sterilized soil and soybean seeds from AG53X0 were sown 3 to a pot and allowed to grow for 4-6 weeks until plants reached V3-V4. Leaves from trifoliates were excised from the fourth node from the top of each plant and then over sprayed with Tilt (Syngenta Crop Protection), Domark 230 ME (Gowan Company), or Malathion 57 (Loveland Products, Inc.) or a mixture of each fungicide with the based on the highest labeled volume for use in Tennessee soybean. Controls were over sprayed with sterile deionized water. Once leaves were dried, leaves were placed face-up, and petioles were submerged in 1% water agar plates. Plates were placed in humidity chambers set at 27°C on 12-hour light to dark cycles. The chamber contained 5 untreated leaves and 25 treated leaves. Each leaf was inoculated by an isolate chosen based on least sensitive to DMI fungicides as described in chapter 3. 6 mm plugs from each isolate were placed mycelium side down on each individual leaf. Each experiment was conducted four times over a 4-week period. Disease severity was calculated based on lesion diameter after 1 week in the humidity chamber.

Mycelial growth inhibition assay

Three isolates of *C. cassiicola* were selected from a large set of isolates for mycelial growth inhibition assays testing the possible antagonism or synergism of Malathion, Domark, and Tilt. Three isolates were selected based on

least sensitivity to DMI fungicides tested in Chapter 3. Isolates were inoculated on PDA amended with 10 µg/mL of commercial grade formulations of Tilt (41.8% a.i.), Domark (20.5% a.i.), and Malathion (57% a.i.). The formulations were amended by themselves or in conjunction with Malathion. Stock solutions of 10,000 µg/mL were prepared in acetone and diluted down to 10 µg/mL. Fungicide stocks were added to the media at 55°C, poured at approximately 20 mL per Petri dish, and allowed to cool before transfers.

Isolate plugs were recovered from sterile filter paper and grown on PDA for 5-7 days at 25°C under complete darkness. Plugs (6 mm) were taken from the growing edge of each isolate colony and placed mycelium-side down on fungicide-amended media. Each Petri plate was grown out for 5 days, and colony diameter measurements were taken. Plug size was subtracted from the final number and percent inhibition of the colony size was calculated. A discriminatory dose of 10 µg/mL was used to differentiate possible sensitivities among isolates tested based on mycelial growth inhibition.

Data analysis

Discriminatory dose data are observed as mean percentage inhibition relative to the control. Individual experiments were assessed for homogeneity of variance using a mixed model analysis. For the field test and detached leaf assay, mean separations were performed to determine significant differences amongst treatments using Tukey's LSD (α=0.05). All data was analyzed using JMP Pro 14.

Results

Field Trial

The interaction fungicide x insecticide was not statistically significant for target spot ($p = 0.66$), defoliation ($p = 0.06$), or for yield ($p = 0.75$). There were no statistical effects on target spot percentage for fungicide ($p = 0.18$), insecticide ($p = 0.24$), or timing ($p = 0.48$). Likewise, there were no statistical effects on defoliation for fungicide ($p = 0.99$), insecticide ($p = 0.18$), or timing ($p = 0.78$). Yield was not statistically affected by fungicide ($p = 0.15$), insecticide ($p = 0.32$), or timing ($p = 0.48$).

Fungicide treatments did not significantly affect target spot percentage. Target spot percentage was highest in untreated plots ($\bar{x} = 19.1\%$) followed by Domark ($\bar{x} = 16.5\%$), and finally Tilt ($\bar{x} = 14.2\%$) (Figure 3). The effects of malathion added alone or in a tank mix ($\bar{x} = 17.9\%$) had no statistical difference when malathion was not added to plots to reduce target spot ($\bar{x} = 15.2\%$) (Figure 4). There was no reduction of target spot percentage when waiting 1 hour after malathion was added ($\bar{x} = 17.1\%$) versus not waiting ($\bar{x} = 16.1\%$) (Figure 5). Fungicide treatment also did not impact yield ($\bar{x} = 3192$-3310 kg/ha) (Figure 6). Malathion had no statistical effect on soybean yield with yields ranging from $\bar{x} = 3227$-3279 kg/ha (Figure 7). Timing of fungicide application also had no impact on soybean yield ranging from $\bar{x} = 3228$-3278 kg/ha (Figure 8).

Detached leaf assay

In the detached leaf assay, all experiments were combined due to no statistical differences amongst experiments ($p = 0.2625$). There was a statistical

effect regarding the fungicide or insecticide used ($p < 0.001$). Tilt when applied by itself ($\bar{x}$ = 50%) and Tilt applied with Malathion ($\bar{x}$ = 54.22%) inhibited target spot the most of all treatments tested. Domark by itself ($\bar{x}$ = 26.54%) and Domark and Malathion mixed together ($\bar{x}$ =28.85%) reduced target spot percentage more than the solo Malathion treatment ($\bar{x}$ = 12.91%) (Figure 9).

Mycelial growth assay

The sensitivity of 3 isolates of *C. cassiicola* were subjected to *in vitro* mycelial growth assays containing the fungicides Tilt (propiconazole) and Domark (tetraconazole), and the insecticide malathion to determine if any interaction between insecticides and DMI fungicides exist in inhibiting colony size of *C. cassiicola*. For the fungicide Tilt, both Tilt by itself ($\bar{x}$ = 46.07%) and Tilt with Malathion ($\bar{x}$ = 46.40%) statistically reduced colony size of *C. cassiicola* compared to the check of Malathion by itself ($\bar{x}$ = 4.53%) ($p < 0.0001$). Domark ($\bar{x}$ = 24.33%) and Malathion plus Domark ($\bar{x}$ = 25.59%) statistically reduced colony size of *C. cassiicola* compared to the Malathion tratment but underperformed compared to the treatments containing Tilt ($p < 0.0001$) (Figure 10).

Discussion

In this study, the DMI fungicides Tilt and Domark 230ME were evaluated on effectiveness to manage *C. cassiicola* with and without the inclusion of the insecticide malathion. Experiments were performed at the field level in small plots, with a detached leaf assay, and *in vitro* using a mycelial growth assay.

In the field study, we evaluated the efficacy of Domark 230ME and Tilt with the addition of malathion either in a tank mix or applied 30-min before fungicide application.

Although resistance to DMI fungicides have been documented in other pathogens, there are few observations that *C. cassiicola* has developed any resistance to this class of fungicides (FRAC 2021). In this study, we did not have significant reduction of target spot in soybean, but Domark and Tilt did reduce target spot percentage by 13% and 25 %, respectively. Malathion only reduced target spot by 6%. Teramoto et al. (2017) reported an 87% reduction in target spot in soybean with the fungicide cyproconazole and a 79% reduction with the fungicide prothioconazole. Busi et al. (2016) reported that the organophosphate, malathion, can inhibit metabolic resistance mechanisms in a number of herbicide-resistant plant species, specifically in a range of plant cytochrome P450s. Our experiment tested this interaction in fungi, that also possess similar cytochrome P450 enzymes. The field population of *C. cassiicola* tested in this study was sensitive to the DMI fungicides used. The results of this study confirm that there is no interaction between DMI fungicides and the organophosphate malathion when managing susceptible populations of *C. cassiicola*.

Malathion did reduce target spot lesion sizes in the detached leaf assay by an average of 13%, suggesting that malathion may have some efficacy on *C. cassiicola*. Although it is uncommon, there have been reports of phylum-specific pesticides with efficacy outside of its intended purpose. Malathion has been

reported to inhibit metabolic resistance in some plant species (Tardif and Powles 1999; Kruez and Fonne-Pfister 1992). Kutlesa and Caveney (2001) reported the insecticide, glufosinate, had insecticidal activity on the larval stage of *Calpodes ethlius.*

Media only amended with malathion had little to no effect on colony size of *C. cassiicola* in the mycelial growth assay, reducing colony size by less than 5%. Tilt with and without malathion inhibited colony size better than Domark with and without malathion. There were no differences between media amended with just Domark and Tilt versus being amended with the fungicides and malathion. This suggests that malathion had no effect on increasing efficacy of Domark and Tilt in a mycelial growth assay. Media amended with Tilt reduced colony size of *C. casiicola* by roughly 50%, while Domark reduced colony size by roughly 25%. Teramoto et al. (2017) reported an 87% reduction in target spot in soybean with the fungicide cyproconazole and a 79% reduction with the fungicide prothioconazole. These results along with findings from the field trial suggest that Tilt is a more effective DMI product for managing *C. casiicola.* Both Tilt and Domark should only be used in a tank mix with a more effective fungicide from a different class.

The goal of this study was to investigate the interaction of DMI fungicides and the organophosphate insecticide, malathion, on the management of *C. cassiicola*. With a rise in fungicide resistance in plant pathogens, looking at alternative ways to help manage that resistance is important. Although there are

reports of resistance to DMI fungicides, it has not been reported in *C. cassiicola*. The data shown here can be used as a basis for comparison for future testing of the interaction of DMI fungicides and malathion when used on populations no longer susceptible to DMIs.

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Appendix

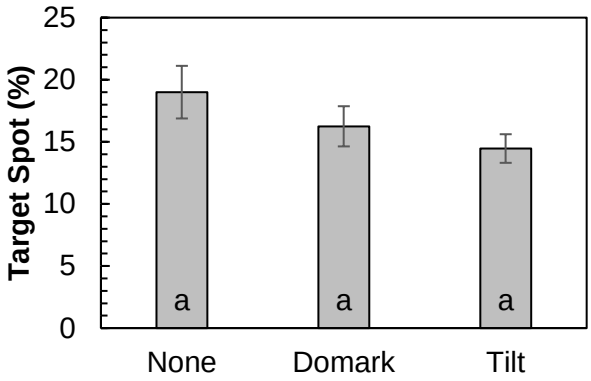

Figure 27. Target spot severity (%) of two DMI fungicides, Tilt and Domark, in a soybean field trial. Bars with different letters are statistically different at α=0.05.

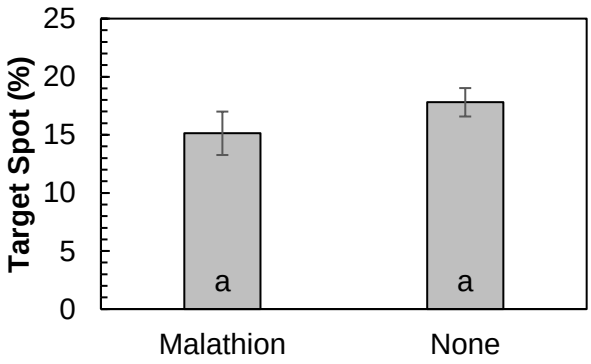

Figure 28. Target spot severity (%) for soybean plots treated with and without the addition of malathion. Bars with different letters are statistically different at α=0.05.

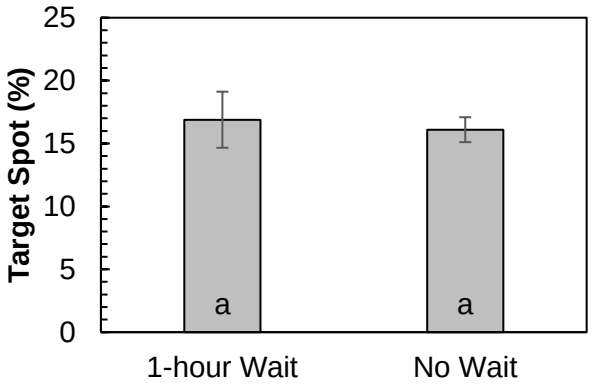

Figure 29. Target spot severity (%) for soybean plots treated with tank mixes including malathion or by applying malathion 1-hour before fungicide application. Bars with different letters are statistically different at α=0.05.

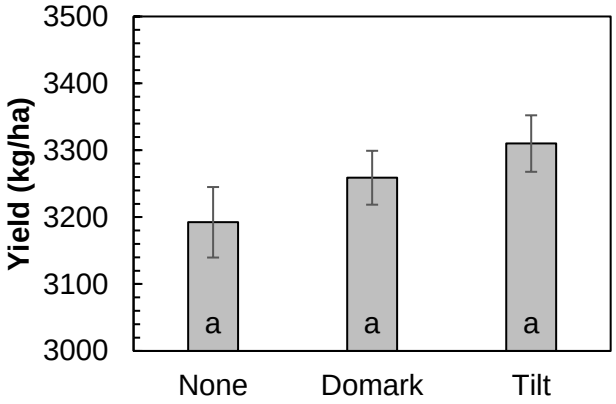

Figure 30. Yield (kg/ha) of two DMI fungicides, Tilt and Domark, in a soybean field trial. Bars with different letters are statistically different at α=0.05.

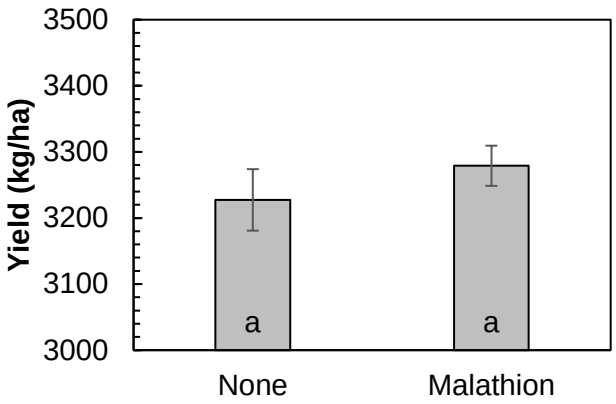

Figure 31. Yield (kg/ha) for soybean plots treated with and without the addition of malathion. Bars with different letters are statistically different at α=0.05.

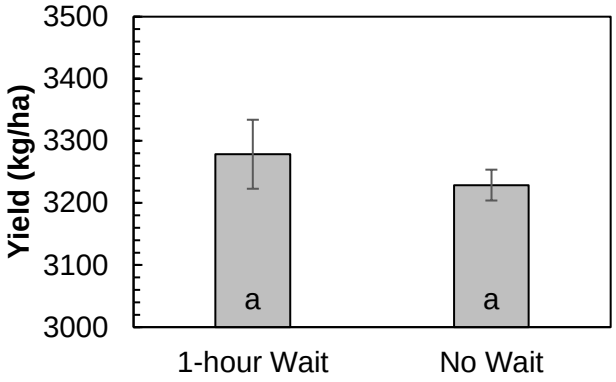

Figure 32. Yield (kg/ha) for soybean plots treated with tank mixes including malathion or by applying malathion 1-hour before fungicide application. Bars with different letters are statistically different at α=0.05.

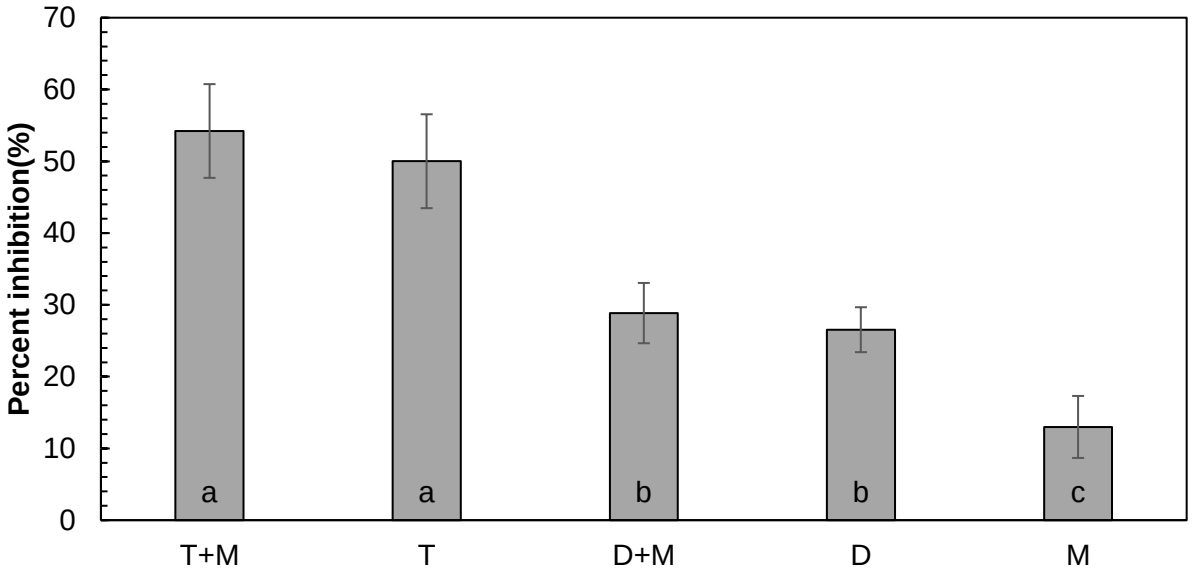

Figure 33. Inhibition of *C. cassiicola* lesion size on infected soybean leaves across treatments: Tilt + malathion (T+M), Tilt (T), Domark + malathion (D+M), Domark (D), and malathion (M). Bars with different letters are statistically different at α=0.05.

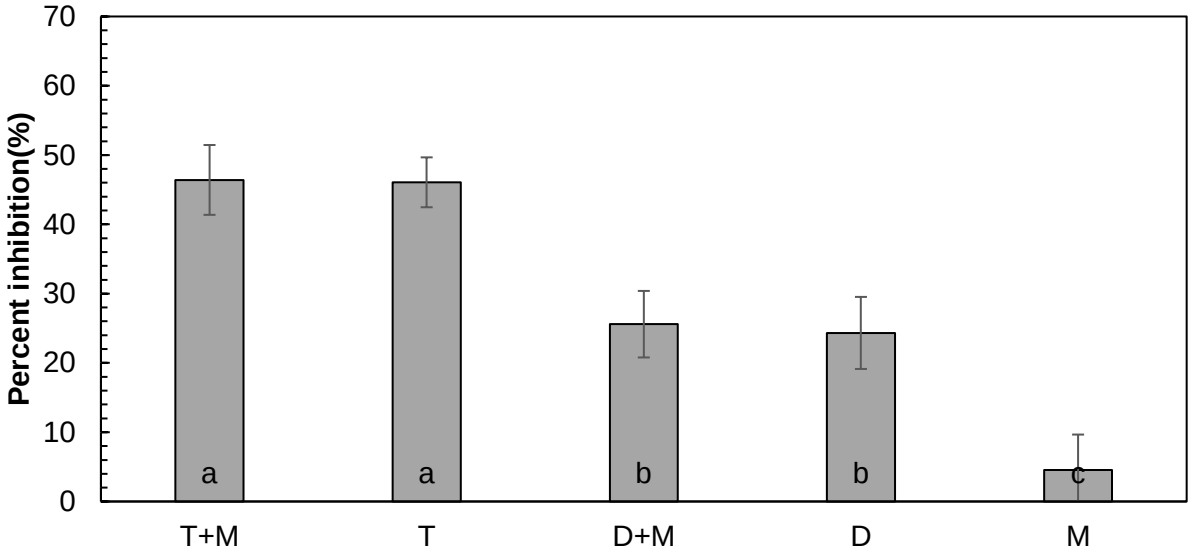

Figure 34. Inhibition of *C. cassiicola* colony size on potato dextrose agar across treatments: Tilt + malathion (T+M), Tilt (T), Domark + malathion (D+M), Domark (D), and malathion (M). Bars with different letters are statistically different at α=0.05.

CHAPTER V. DETECTION OF THE G143A MUTATION IN THE *CYTOCHROME B* GENE OF *CORYNESPORA CASSIICOLA* ISOLATES FROM SOYBEAN IN TENNESSEE

This chapter is in prep for submission into *Plant Health Progress* as a brief.

Corynespora cassiicola (Berk. & M.A. Curtis) C.T. Wei is a widespread pathogen with a large and diverse host range and is the causal pathogen of target spot in cotton and soybean (Ma and Michailides 2005). Occurrence of target spot has increased in US soybeans, but recently concerns over target spot in cotton have arisen with it being more widespread (Butler et al. 2016; Shrestha et al. 2017).

FRAC (2021) considers *C. cassiicola* a high risk pathogen for development of fungicide resistance, and mutations associated with quinone outside inhibitor (QoI) fungicides have already been detected in the *cytochrome b* gene. Three mutations (G143A, G137R, and F129L) have already been detected in *C. cassiicola* in cucumber and tomato (Duan et al. 2019; MacKenzie et al. 2020). Rondon and Lawrence (2019) reported that soybean isolates out of Alabama were confirmed to have the G143A mutation in the *cyt b* gene in *C. cassiicola*. Due to the presence of QoI resistant populations of *C. cassiicola* in Alabama, the purpose of this study was to assess the occurrence of the G143A point mutation of *C. cassiicola* from isolates resistant to QoI from Tennessee soybean fields.

Isolates of *C. cassiicola* were recovered from soybean and cotton leaves with target spot symptoms. Symptomatic lesions were cut and sterilized in a 10% bleach solution for 1 minute and rinsed with sterile water. Lesions were placed in 10% water agar for 24 hours. Single mycelial strands were taken from inoculated

plates and put onto potato dextrose agar (PDA) and incubated at room temperature (25±2°C). *C. cassiicola* isolates were identified based on colony growth and conidial morphology. Genomic DNA of six *C. cassiicola* isolates was extracted using the MagMAX kit (Thermo) according to manufacturer's directions. Four isolates from soybean were used along with two isolates recovered from tomato shared from Florida that were previously identified as resistant (MacKenzie et al. 2020). The DNA was processed using kits from KAPA to produce PCR-free Illumina libraries and sequenced on a HiSeqX device running a 2x150bp paired-end configuration. The raw data was trimmed, aligned and SNPs genotyped using CLC Genomics Workbench (Qiagen). Sequences were deposited into GenBank within a BioProject (accession number SUB9052403).

A mycelial growth assay was conducted to determine the effective concentration to inhibit 50% (EC_{50}) of each *C. cassiicola* colony. Six isolates of *C. cassiicola* were selected based on results from genetic sequencing mentioned above. The QoI fungicide, pyraclstrobin, was prepared in acetone in a 100,000 µg/mL stock solution. Serial dilutions were prepared from the stock solution to obtain final concentrations of .1, 1, 10, and 100 µg/mL. Due to the ability of some fungi to overcome sensitivity of QoI fungicides through an alternate respiratory pathway, salicylhydroxamic acid (SHAM) was used at a rate of 100 µg/mL. Isolates were placed in complete darkness at 28±2°C for 5 days. Two perpendicular measurements were made and the center of the mycelial plug was subtracted to determine mycelial growth inhibition. Percent inhibition was used to

calculate EC_{50} (effective concentrations to inhibit 50% of a population) values using a probit analysis in JMP Pro 14.

According to SNPs generated, four of six isolates of *C. cassiicola* that were sequenced were found to have mutation in the *cyt b* gene that changes the amino acid glycine to alanine (G143A). This mutation is a result of the codon change at the 143 amino acid from GGT to GCT. Two of the *C. cassiicola* isolates were from soybean (19-7203 and 19-8814) located in Madison County and Gibson County, Tennessee. The other two isolates of *C. cassiicola* were taken from tomato and were already established to have the G143A mutation and were included as positive checks. No other point mutations were present.

The 2 isolates of *C. cassiicola* (19-7203 and 19-8814) from soybean exhibited EC_{50} values of 27.2 µg/mL and 121.8 µg/mL, respectively, to pyraclostrobin. This is comparable to the 2 resistant isolates (GEV 3125 and GEV 3183) from tomato that were provided by MacKenzie et al. (2020) with values of 20.6 and 22.5 µg/mL, respectively, to azoxystrobin. Isolates 82710 and 942020-5 did not have the G143A mutation and had EC_{50} values of 5.47 and 3.17 µg/mL, respectively. FRAC (2021) reports that cross resistance is possible across all members of the Group 11 fungicides.

The G143A mutation has been reported in many different pathogens to confer the highest level of resistance with the F129L and G137R mutations conferring partial resistance (Duan at el. 2019; Bartlett et al. 2002). *C. cassiicola* has a high risk of developing fungicide resistance and has now been confirmed

to have resistance to the Group 11 fungicides in 2 soybean producing states in the US, Alabama (Rondon and Lawrence 2019) and now Tennessee. With the increase in fungicide-resistant pathogen populations in soybean in Tennessee, managing for fungicide resistance is becoming more of a challenge.

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Ma, Z. and Michailides, T.J. 2005. Advances in understanding molecular mechanisms of fungicide resistance and molecular detection of resistant genotypes in phytopathogenic fungi. Crop Prot. 24: 853-863.

Rondon, M. N., & Lawrence, K. S. (2019). Corynespora cassiicola isolates from soybean in Alabama detected with G143A mutation in the cytochrome b gene. *Plant Health Progress*, *20*(4), 247-249.

Appendix

Figure 35. *Corynespora cassiicola,* causal pathogen of target spot. Symptomatic leaves of cotton and soybean. Top and bottom of pure culture of *C. cassiicola* isolated from soybean.

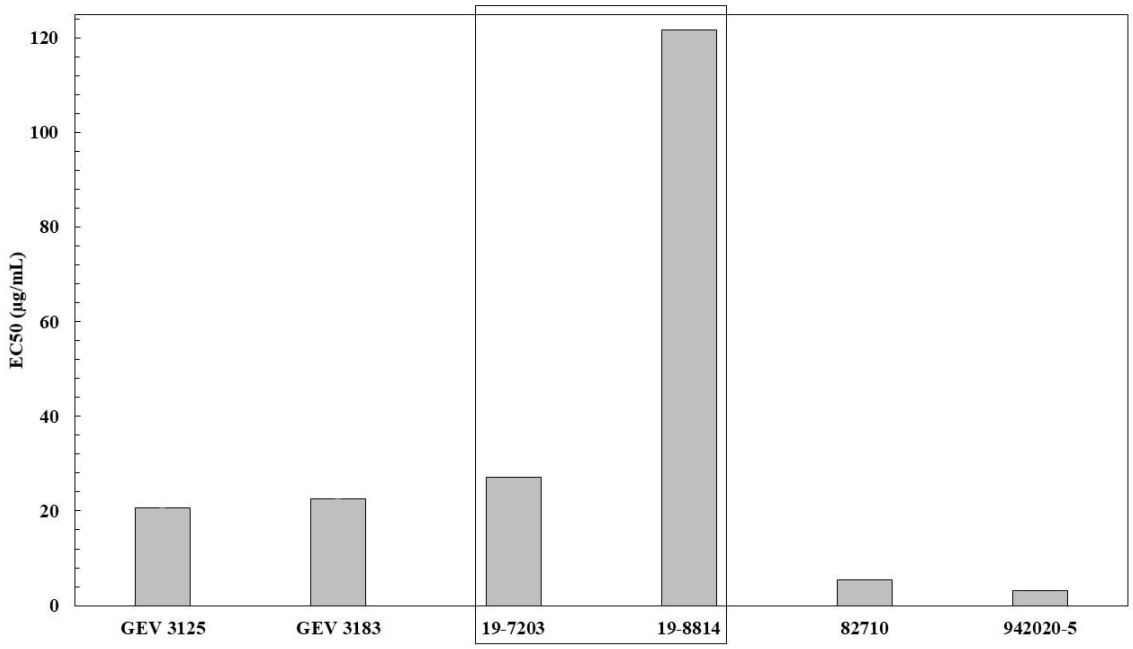

Figure 36. Effective concentrations (EC_{50}) of pyraclostrobin to inhibit 50% of colony growth of *Corynespora cassiicola* isolates from tomato, soybean, and cotton. QoI- resistant isolates (GEV 3125 and GEV 3183) from MacKenzie et al. (2020) used as resistant checks. Boxed area represents isolates (19-7203) and (19-8814) from Tennessee resistant to QoIs.

CHAPTER VI. CONCLUSION

The first objective was to evaluate a range of different fungicides and mixes on the management of target spot in cotton and soybean in Tennessee. This information should help in the development of fungicide recommendations for growers in TN across different locations and cultivars. Target spot was more common in the 2018 and 2019 growing seasons for both soybean and cotton. Moderately susceptible cultivars tended to keep target spot severity to a minimal level. They also yielded as high if not higher in some instances than the most tolerant cultivars. This suggests that there is some inherent tolerance to target spot in some cotton and soybean cultivars, but true resistance has yet to be confirmed. The on-farm location that practices a regular crop rotation of soybean and corn had the highest target spot severity, while the trial planted after wheat had the least amount of target spot. At all locations, products containing an SDHI component, such Miravis Top and Priaxor + Tilt, tended to reduce target spot the most while also enhancing yield. A single active ingredient product, such as Domark, had little impact on reducing target spot severity and protecting yield. This suggests that multiple active ingredient products should be used if you are treating target spot in a susceptible soybean cultivar. In cotton, Miravis Top reduced target spot severity and defoliation more than any other fungicide tested. This occurred in the 2019 and 2020 growing season, although target spot severity was lower in 2020 compared with the other two growing seasons. Application timing had a small impact in 2019, with target spot severity being reduced when fungicides were applied at 3rd week of bloom or at 3rd + 5th week of

bloom. Fungicide choice nor timing impacted lint yields across all 3 years. Other research suggests that at higher levels of target spot severity and defoliation, yield impacts may occur. This data suggests that fungicide applications are much more beneficial in soybean rather than cotton. Applications in cotton may not be needed if a semi-tolerant cultivar is planted, or target spot severity is not expected to be severe.

The second objective was to evaluate a number of technical grade fungicides on the management of *Corynespora cassiicola* isolates from cotton and soybean, and to develop baseline sensitivities for these products. SDHI fungicides, such as adepidyn and fluxapyroxad, had more intrinsic efficacy on isolates of *C. cassiicola*. Strobilurin fungicides, azoxystrobin and pyraclastrobin, had the least amount of intrinsic efficacy at managing *C. cassiicola* isolates. The MBC fungicide, thiophanate methyl, had moderate efficacy on *C. cassiicola* isolates. When comparing isolates from cotton and soybean, only fluxapyroxad-treated isolates exhibited differences in EC_{50} values across each host. This suggests that the sensitivity profiles for isolates from cotton and soybean are similar in Tennessee. Caution should be taken when applying strobilurins in soybean though, as resistance to them has been confirmed in Alabama and Tennessee.

The third objective was to determine if malathion had any positive or negative interactions with demethylation inhibitor fungicides when managing target spot or the causal pathogen, *C cassiicola.* Across all three experiments,

the addition of malathion did not impact the efficacy of either fungicide product that was tested. There were no differences amongst treatments in the field trial on target spot management or yield. Treatments containing Tilt reduced target spot lesion size in the detached leaf assay, but when malathion was added there was no impact. Malathion did have a small impact on target spot lesion size when applied by itself reducing it around 10%. The same was true for the *in vitro* assay. Tilt treatments performed better than Domark and Malathion, but there was no difference when malathion was added. The impact of malathion was much smaller with only a 5% reduction in *C. cassiicola* colony size.

The final objective was to screen isolates of *C. cassiicola* for the presence of the G143A mutation that confers resistance to strobilurin fungicides. Two isolates of *C. cassiicola* that were pulled from soybean had increased EC_{50} values when exposed to pyraclostrobin. These isolates had values consistent with positive check isolates from Florida tomato. These two TN isolates also were genetically sequenced and were confirmed to have the G143A mutation. This data confirms that resistance to strobilurins has been observed in Tennessee soybean isolates of *C. cassiicola.*

Target spot in soybean and cotton is of growing concern for producers in Tennessee and across the Mid-South. Due to a lack of host plant resistance, fungicides are relied on heavily for control of target spot. Although fungicides are effective, the concern over resistance development is ever present. Based on results from this study, isolates of *C. cassiicola* from soybean have been

confirmed to be resistant to strobilurin fungicides. Although resistance is present, how prevalent it is in the field is not yet known. For that reason, fungicide applications using alternative classes such as SDHIs and DMIs should be used in rotation with strobilurins to mitigate the spread of resistance.

VITA

Ty Smith was born in Indianola, MS. From a young age, he had an interest in agriculture working on a farm with his father. In 2016, he obtained a B. S. in Agricultural Science from Mississippi State University. He then acquired his M.S. in Entomology from Mississippi State University under the supervision of Dr. Angus Catchot and Dr. Jeff Harris. Lastly, he has obtained his Ph. D. in Plant Pathology from the University of Tennessee in the Department of Entomology and Plant Pathology under the tutelage of Dr. Heather Kelly.