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Influence of application technology on foliar fungicide efficacy on *Cercospora sojina* infected soybean

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I am submitting herewith a thesis written by Shawn Alan Butler entitled "Influence of application technology on foliar fungicide efficacy on *Cercospora sojina* infected soybean." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.

Heather M. Kelly, Major Professor

We have read this thesis and recommend its acceptance:

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**Influence of application technology on foliar fungicide efficacy
on *Cercospora sojina* infected soybean**

**A Thesis Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville**

**Shawn Alan Butler
August 2016**

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Blessed is the one who perseveres under trial because, having stood the test, that person will receive the crown of life that the Lord has promised to those who love him. James 1:12

Abstract

Due to the constant concern with off-target contamination and application technology requirements associated with future herbicide-tolerant crops, the use of drift-reduction nozzle technology (DRT) may increase. The primary objective of this research was to evaluate the effects of coarse droplets generated by drift-reduction nozzles on foliar fungicide efficacy and residual in soybean infected by frogeye leaf spot caused by *Cercospora sojina*. No differences in disease control, soybean yield, spray retention, and residual when applying Quadris Top SB, a premix of azoxystrobin and difenoconazole, using nozzles that produce either a medium or ultra coarse droplet spectrum were determined.

Due to the challenge of timing fungicide applications targeting frogeye leaf spot infections, growers often make applications preventatively, prior to visual infection. Therefore, the second objective was to determine the EC_{50} of *C. sojina* isolates collected from trial locations to further investigate the residual control window of azoxystrobin. The effective concentration in which 50% of mycelial growth of resistant isolates tested were inhibited was determined to be $7.44638 \mu\text{g mL}^{-1}$, while the concentration for sensitive isolates was found to be $0.04789 \mu\text{g mL}^{-1}$.

The third objective was to determine the effect of droplet size on plant coverage and canopy penetration when making fungicide applications in a commercial setting with increased potential for off-target movement. These studies showed no differences between nozzles producing either medium or ultra coarse droplet spectra when considering frogeye leaf spot control or soybean yield with an application of Quadris Top SB. Coverage analyses determined that applications made with ground self-propelled sprayers deposited 43% more solution in the upper canopy than the lower canopy, but was not effected by droplet spectra.

These results indicate that DRT nozzle technology will not have a negative impact on frogeye leaf spot disease control or soybean yield when applying Quadris Top SB. Further studies should be conducted to determine the impact of DRT on other diseases of soybean or alternate crops.

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Part I. Literature Review

Soybean

Soybean (*Glycine max* L., Family Fabaceae) is one of the most valued crops produced in the world due to possessing the highest protein content and gross output of vegetable oil among all cultivated crops (LiJuan et al., 2010). Soybean was believed to be introduced to the U.S. in 1765. Originally referred to as “Chinese Vetch”, soybean cultivation has been traced back to Northern China in the eleventh century B.C.. Soybean was first grown in the U.S. as a forage, and it was not until the 1920s that soybean was cultivated for seed (Chang et al., 2015). Since World War II, the U.S. has led the world in soybean production, being grown on approximately 31 million ha in 2013 (Chang et al., 2015). Since the 1990’s, soybean production has increased rapidly primarily due to the release of genetically engineered soybean varieties containing high resistance to herbicides, as well as possessing improved yield potential from agronomic traits (Chang et al., 2015). In the U.S., 93% of soybean cultivated are genetically engineered varieties, aiding in average yield improvements of 1581 kg ha⁻¹ in 1960 to 2919 kg ha⁻¹ in 2013 (Chang et al., 2015).

Soybean is a bushy, erect, annual legume (Kumudini, 2010), with simple and opposite primary or unifoliate leaves, while all others are alternate trifoliates. Branches and flowers develop in lower leaf axils, with branches expanding from axillary buds and flowers transitioning into pods. Soybean plants retain approximately 0-5 pods per node with 1-5 seeds per pod (Hicks, 1978). Soybean may be either indeterminate or determinate in reproductive development. Flowering is generally induced by day length, in which soybean are classified as short day plants, but light intensity, temperature, and genotype may also play a factor. Indeterminate soybean types are characterized by continued vegetative growth of the apical meristem throughout the growing season, whereas vegetative growth ceases in determinate soybean types once the apical meristem develops an inflorescence (Hicks, 1978).

Growth stages of soybean are categorized by vegetation (V) and reproduction (R). Soybean may germinate at soil temperatures as low as 2° to 4° C, with the optimum range falling between 34° to 36°C (Inouye, 1953). Emergence (VE) begins when water is absorbed to equal approximately 50% of the seed’s weight. The radical emerges from the seed, followed by the hypocotyl which grows towards the

soil surface, carrying the cotyledons. The hypocotyl straightens once breaking through the soil surface and the cotyledons open, progressing to the cotyledon stage (VC). This process typically takes 5 to 10 days (McWilliams et al., 1999). The unifoliate stage is categorized as V1, as the soybean plant continues to progress in growth, vegetative stages are determined by counting nodes above the unifoliate with a leaf that has unrolled sufficiently such that two edges are no longer contacting each other (Fehr et al., 1971). The plastochrone, or time interval between generation of new leaves, in soybean is approximately 2 days (Hicks, 1978). Reproductive stages are described based on development of flowering patterns throughout the soybean plant. Initiation of floral primordia typically begins within 3 weeks after emergence, with flowers becoming noticeable after 6-8 weeks (Hicks, 1978). Reproductive stages are labeled as follows: (R1) single flower on node, (R2) flower at node below the uppermost position with a confirmed leaf, (R3) 0.5 cm pod formed at one of the four uppermost nodes with a confirmed leaf, (R4) 2 cm pod formed at one of the four uppermost nodes with a confirmed leaf, (R5) seeds developed within pod at one of the four uppermost nodes with a confirmed leaf, (R6) seeds completely filled within pod at one of the four uppermost nodes with a confirmed leaf, (R7) physiological maturity begun, pods yellowing, 50% of leaves yellowing, and (R8) harvest maturity reached, 95% of pods brown (Fehr et al., 1971). Pods are formed 10 to 14 days after flower onset, with flowering continuing for 3 to 4 weeks (Hicks, 1978).

Environmental stress, defined by Board and Kahlon (2011) as a deficiency or excess of some factor large enough to significantly reduce yield or impair crop quality, is the primary cause of non-optimal soybean yield when using an adapted variety. Environmental stresses are divided into two types, abiotic and biotic. Abiotic stresses are non-living atmospheric variables (radiation, air temperature, humidity, and rainfall) or soil variables (fertility, pH, compaction, soil structure, poor water infiltration, soil structure, etc.). Biotic stresses are living variables, such as weed, insect, disease, or nematode pests (Board & Kahlon, 2011). The final effect on soybean yield, however, is determined by total dry matter, canopy photosynthetic rate, and crop growth rate (Fageria et al., 2006; Loomis & Connor, 1992). Both soybean photosynthetic and growth rates slowly increase after VE until R1 stage, which is referred to as the exponential stage, until maximum light interception is achieved. The first accumulation of dry matter

is also initiated during the exponential stage (Carpenter and Board, 1997). Crop growth rate stabilizes during the linear growth phase until R5, and begins to reduce until reaching zero. The last phase is known as the senescent phase (Board & Kahlon, 2011). Final yield is determined during the senescent phase in which total dry matter accumulation is at its peak and is transferred to the soybean seed (Loomis and Conner, 1992).

Frogeye Leaf Spot

Frogeye leaf spot (FLS) caused by the fungal Ascomycete *Cercospora sojina* K. Hara (Swoboda & Pedersen, 2009; Mian et al., 2008) is one of the most problematic fungal diseases of soybean in the Southern and Midwestern U.S. (Mian et al., 2008; Bowers & Russin, 1998). FLS was first reported on soybean in the U.S. in 1924 (Melchers, 1925). Symptoms of FLS typically appear on the foliage of soybean, but can also occur on seeds, pods, and stems (Sinclair & Backman, 1989). Foliar lesions initially appear as brick red spots that develop into light brown spots with dark reddish-brown margins. Lesions are circular to angular with diameters between 1 and 5 mm (Grau et al., 2004) but may coalesce into larger irregular spots under ideal conditions (Phillips, 1999). Primary and secondary inocula are produced on leaf and stem residues or infested seeds in the form of hyaline conidia ranging from 5 to 7 μm \times 39 to 70 μm (Mian et al., 2008). Conidia can be viewed on the underside center of the lesion, appearing as small black hairs (Dorrance & Mills, 2010). Conidia are formed on conidiophores with size and shape varying based on the substance in which the fungus grows. Conidia may germinate on a leaf within one hour in the presence of water at temperatures between 25° to 30°C. Conidia germinate to form short germ tubes that can produce secondary conidia in culture media (Phillips, 1999).

FLS overwinters in both soybean debris and infected seed (Heatherly & Hodges, 1998). Seeds infected with FLS rarely fail to germinate, typically producing stunted seedlings with poor vigor retaining lesions on the cotyledons. Cotyledons containing sporulating lesions provide inocula to infect young leaves (Heatherly & Hodges, 1998). Lesions do not appear for 2 weeks after initial infection of the host (Mian et al., 2008) and conidia are produced within 24 to 48 hours after lesion formation (Sinclair &

Backman, 1989). FLS reproduces extensively in warm (25°-30°C) and humid (>90% relative humidity) environments, and infection can become severe in seasons with frequent rainfall and/or irrigation. Young emerging soybean leaves are more susceptible than older fully expanded leaves, but symptoms are not initially visible due to the length of time for infection. (Mian et al., 2008; Phillips & Boerma, 1981; Heatherly & Hodges, 1998). Conidia are spread relatively short distances by wind and/or splashing rain (Heatherly & Hodges, 1998). FLS is a polycyclic disease in which infection, symptom development, and reproduction are all repeated throughout the growing season (Dorrance & Mills, 2010).

Soybean yield loss from FLS is primarily induced by a reduction in photosynthetic area by lesions and/or premature defoliation which causes reductions in seed weight (Mian et al., 2008; Dashiell & Akem, 1991). Yield losses have been documented ranging from 10 to 60% (Dashiell & Akem, 1991, Akem & Dashiell, 1994; Mian et al., 1998). Disease onset occurring prior to or at flowering stages (R1-R3) allows for greater disease development, having the largest impact on soybean yield. Infection occurring at or later in the growing season (R5-harvest) has little impact on soybean yield (Dorrance & Mills, 2010). Management strategies include planting resistant soybean varieties and disease-free seed, crop rotation to a non-legume crop host, tillage to bury infected debris, treating seed with a fungicide seed treatment, and foliar applications of fungicides (Heatherly & Hodges, 1998). Genetically engineered varieties containing the single gene *Rcs3* from 'Davis' condition resistance to race 5 and all other known races of *C. sojae* in the U.S. (Dorrance & Mills, 2010; Phillips & Boerma, 1982). Fungicides applied between R1 and R5 growth stages are recommended for protection against *C. sojae* infection (Grau et al., 2004).

Strobilurin Fungicides: Azoxystrobin

The strobilurin fungicide class, since released in the U.S. in 1996, is one of the most important classes of fungicides used in crop production because of its broad-spectrum control (Bartlett et al., 2002). One of the fungicides within this class, azoxystrobin (Syngenta Crop Protection, Inc., Greensboro, NC), which is registered for use in 84 different crops in 72 countries, including 400 crop/disease systems, with

sales reaching \$415 million in 1999, reflects the importance of this class of fungicides (Bartlett et al., 2002).

The discovery of strobilurin fungicides was inspired by a group of natural derivatives of β -methoxyacrylic acid, including strobilurin A, oudemansin A and myxothiazol A, produced by basidiomycete wood-rotting-fungi. Their fungicidal activity arises from their ability to inhibit mitochondrial respiration by binding at the quinol oxidation (Q_o) site of cytochrome b, which is part of the cytochrome bc_1 complex in the inner mitochondrial membrane of fungi (Bartlett et al., 2002). Once the inhibitor binds, it blocks electron transfer between cytochrome b and cytochrome c_1 , halting the production of ATP by interrupting the energy cycle of the fungus. Due to the inhibitor binding at the Q_o site, they have been designated as Code 11, Quinone-outside Inhibitors (Q_oI) by the Fungicide Resistance Action Committee (FRAC) (FRAC, 2015). Studies of azoxystrobin have determined that conidial germination is the primary developmental stage of fungi that are sensitive to strobilurin fungicides, in which they are typically considered “preventative” fungicides (Godwin et al., 1994; Godwin et al., 1997).

Uptake of azoxystrobin into the cells of plant leaves has been shown to be dependent on formulation type, additives, crop type, and environmental factors effecting droplet drying. Uptake is typically gradual, with 25% absorbed into the leaf within 24 hours of application (cereal, suspension concentrate) (Bartlett et al. 2002). Once entering the plant, azoxystrobin possesses xylem-systemic and translaminar mobility, moving to newly emerging growth as well as through consecutive leaf layers. Godwin et al. (1999) determined 8% of azoxystrobin entering a leaf had moved above the point of contact within 8 days of application.

Q_oI -fungicides have been described by FRAC as “high risk” for fungal resistance because of their single site mode of action (FRAC, 2015). Resistance to Q_oI -fungicides has been confirmed in more than 30 species, mainly occurring as a result of single point nucleotide mutations in the *cyt b* gene (Fernández-Ortuño et al., 2008; FRAC, 2013; Standish et al., 2015). This mutation prevents the fungicide from binding to the Q_o site and has been detected in the *cyt b* region corresponding to amino acid positions 120 to 155 (Fernández-Ortuño et al., 2008; Standish et al., 2015). Complete resistance to Q_oI fungicides has

been determined in the amino acid substitution from glycine to alanine at position 143 (G143A), with reduced sensitivity noted from amino acid substitutions from phenylalanine to leucine at position 129 (F129L) and from glycine to arginine at position 137 (G137R) (Fernández-Ortuño et al., 2008). Cross resistance has been demonstrated by fungi possessing the G143A substitution, inferring that a fungus resistant to a specific QoI fungicide active ingredient will be resistant to all other QoI active ingredients (Fernández-Ortuño et al., 2008; Standish et al., 2015; FRAC, 2015). Isolates of *C. soja* resistant to QoI fungicides were first discovered in 2010 in western Tennessee (Zhang et al., 2012). Soybean leaves continued to exhibit severe FLS symptoms after multiple applications of pyraclostrobin within the same growing season. Zhang et al. (2012) determined isolates collected from this location in Tennessee possessed cross resistance to azoxystrobin, pyraclostrobin, and trifloxystrobin. Previous reports by Zhang et al. (2010) determined the concentration of azoxystrobin in which 50% of conidial germination was effectively inhibited (EC_{50}) in QoI-sensitive *C. soja* isolates ranged from 0.0029 to 0.0323 $\mu\text{g ml}^{-1}$ (mean = 0.0127 $\mu\text{g ml}^{-1}$). Of the 15 *C. soja* isolates collected from western Tennessee in 2010, EC_{50} was determined to range from 2.7826 to 4.5409 $\mu\text{g ml}^{-1}$ (mean = 3.1644 $\mu\text{g ml}^{-1}$), approximately 140 to 959-fold greater than *C. soja* baseline sensitive isolates (Zhang et al., 2010; Zhang et al., 2012), confirming high resistance to QoI fungicides. To manage resistant FLS in soybean, it has been recommended to apply an alternate mode of action fungicide, such as difenoconazole, tank-mixed or premixed with azoxystrobin (Allen; 2013; FRAC, 2015; Kelly, 2015). Difenoconazole is a Demethylation Inhibitor (DMI, FRAC Code 3) targeting demethylase of sterol biosynthesis in membranes.

Fungicide Application and Mobility

The primary objective when making an application of a disease-management product is to reduce yield losses associated with pathogens (Gossen et al., 2008). Successful application of management products, such as foliar fungicides, requires that the correct product be applied at the appropriate time while optimizing spray retention, coverage, and deposition (Gossen et al., 2008). However, the majority of research conducted on application technology has been with herbicides, and equipment has been

designed to accommodate these applications (Gossen et al., 2008). Also, many currently used herbicides have some type of mobility or translocation through plant tissues they contact, in which precise deposition to the plant target is not required. Many older commercially available products, such as mancozeb and chlorothalonil, are contact fungicides that only have activity on the plant surface in which they are applied and do not penetrate the plant (Prokop & Veverka, 2006; Mueller et al., 2013). Some newer products, such as pyraclostrobin and difenoconazole, are locally systemic translaminar fungicides that are able to penetrate and redistribute within a leaf on which they are applied, but are not able to translocate through the xylem or phloem to other parts of the plants (Karadimos et al., 2005; Gossen et al., 2008; Mueller et al., 2013). Due to the limited movement of these types of fungicides, it is imperative to apply active ingredients in adequate quantities to critical sites of infection to inhibit the target pathogen (Gossen et al., 2008). However, some active ingredients in commercially available fungicides, such as azoxystrobin and thiophanate-methyl, are apoplastic and have systemic mobility, moving upwards in the transpiration stream through xylem vessels (Edgington, 1981; Mueller et al., 2013). These types of fungicides must be applied to the lower portions of the target plant to be redistributed for pathogen management (Gossen et al., 2008).

Hydraulic spray nozzles are the fundamental mechanism used to make fungicide applications. Spray nozzles are manufactured with physical components that manipulate pressurized hydraulic flow through atomization, creation of droplets, to more adequately disperse a solution. Agricultural nozzles consist of an exit orifice that forms a desired pattern, typically a tapered flat fan or hollow cone. Spray nozzles produce droplet diameters from 10 to 1,000 μm (Bouse et al., 1990). Droplet spectra are categorized based on ASABE Standard S-572.1 (Doble et al., 1985; ASABE, 2009). Droplet spectra of agricultural spray nozzles are typically associated with the volumetric median diameter (VMD) produced, defined as the diameter in which 50% of the total spray volume are in larger droplets and 50% of the total volume are in smaller droplets. Droplet spectra VMD classification categories are: extra fine ($\sim 50 \mu\text{m}$), very fine ($< 136 \mu\text{m}$), fine ($136\text{--}177 \mu\text{m}$), medium ($177\text{--}218 \mu\text{m}$), coarse ($218\text{--}349 \mu\text{m}$), very coarse ($349\text{--}428 \mu\text{m}$), extra coarse ($428\text{--}622 \mu\text{m}$), and ultra coarse ($> 622 \mu\text{m}$) (ASABE, 2009).

Spray droplet distribution has been demonstrated to be crucial to spray deposition and off-target movement (Yates et al., 1976; Whisenant et al., 1993; Taylor et al., 2004). Yates et al. (1985) stated that droplets less than 150 μm were most susceptible to drift. Although droplet size is one of the primary factors resulting in off-target movement, wind speed at application, distance from susceptible vegetation, and boom height also increase drift potential. Droplets with a 100 μm diameter have the potential to move over nine times further than droplets with a 1000 μm diameter (Akesson & Yates, 1964). To combat the risk of off-target movement, selection of proper Drift Reduction Technology (DRT) nozzles is critical (Kruger et al., 2014). DRT nozzles manipulate agricultural spray solutions to mitigate drift by reducing the percentage of driftable-fines (Yates et al., 1985; Etheridge et al., 1999) via nozzle design, such that their VMD is greater than 400 μm in diameter. DRT nozzles increase droplet diameters through different mechanisms that increase velocity through gains in kinetic energy which are balanced by internal pressure drops based upon Bernoulli's principle of fluid dynamics (Lefebvre, 1988). These mechanisms may be either flow-metering pre-orifices with reduced cross-sectional area in comparison to the exit orifice, air induction chambers that create air-infused droplets while reducing internal pressure via the Venturi effect (name coined by Giovanni Battista Venturi) (Karwatka, 2013), or turbulence chambers with deflectors (Lefebvre, 1988). The use of DRT nozzles has been shown to significantly decrease drift potential of agricultural sprays (Piggott & Matthews, 1999; Etheridge et al., 1999). Along with damaging sensitive vegetation in neighboring areas (Nordby & Skuterud, 1974), the result of off-target movement may cause decreases in herbicide efficacy in the desired application area (Johnson et al., 2006).

Droplet spectra has also been determined to be a factor in pesticide efficacy. Akesson and Yates (1986) first determined the 200 to 400 μm VMD range best for insecticide and fungicide applications. In regards to contact herbicides, Knoche (1994) detected an increase in efficacy when using finer droplet spectra in over 50% of the studies he evaluated. Enhanced plant coverage can be obtained using finer droplet spectrums (Ramsdale & Messersmith, 2001) leading to greater effectiveness of contact herbicides (Etheridge et al., 2001; Prokop & Veverka, 2003). However, no difference to improvements in efficacy have been detected when applying systemic herbicides in coarser droplets due to increased translocation

(Etheridge et al., 2001; Prokop & Veverka, 2003; Feng et al., 2003). Prokop and Veverka (2006) also determined contact fungicide efficacy increased when applied with smaller droplets, but found no effect from droplet spectra when tank-mixing a systemic and contact fungicide. Couch (1998) demonstrated a relationship of reductions of *Sclerotinia homoeocarpa* infecting bermudagrass as droplet size increased when applying a locally systemic fungicide.

Other biotic factors in soybean management systems may also effect fungicide applications. To consistently control weeds and slow development of further herbicide resistance, diverse chemistries should be applied (Diggle et al., 2003). Future soybean crops genetically engineered to possess tolerance to synthetic auxins, such as dicamba and 2,4-dichlorophenoxyacetic acid (2,4-D), and inhibitors of 4-hydroxyphenylpyruvate dioxygenase (HPPD), such as mesotrione, will provide growers postemergence options to control problematic glyphosate-resistant weeds (Riar et al., 2013), and preserve the utility of glyphosate-resistant crop technology (Duke and Powles, 2009). However, with multiple non-selective herbicides applied postemergence in soybean, the need for drift management and application stewardship will increase (Ramsdale & Messersmith, 2001). Upon release of labeled herbicides for future tolerant soybean, implemented comprehensive application stewardship practices will be required for growers to utilize this technology (EPA, 2015). These requirements include reduction of application ground speeds, utilizing buffer zones, and making applications with DRT nozzle technology.

Objectives

Once sprayers are equipped with DRT nozzles that produce coarser spray droplets to combat off-target contamination concerns and the adaptation of future herbicide-tolerant crops, fungicide applications currently recommended to be applied using smaller droplet diameters could potentially be affected negatively. Applications of azoxystrobin and difenoconazole premixes targeting FLS of soybean are one particular instance in which reductions in plant coverage from increased droplet size could potentially effect disease control and crop yield. Research studies were developed with the objectives to: 1) evaluate the effect of droplet size on foliar fungicide efficacy and residual in *C. sojae* infected soybean and 2)

evaluate the response of foliar fungicide efficacy and spray coverage to droplet size using commercial application parameters.

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Part II. Influence of Droplet Size on Foliar Fungicide Efficacy and Residual

Abstract

Field experiments were conducted in 2014 and 2015 to evaluate the influence of droplet size on foliar fungicide efficacy and residual in soybean infected with *Cercospora sojina*, the fungal agent of frogeye leaf spot. A fungicide premix of azoxystrobin and difenoconazole was applied using two spray nozzles with varying droplet spectra. No significant differences were found among treatments in regards to visual disease ratings, soybean yield, and azoxystrobin concentration at 0, 2, 7, and 14 days after application. Results suggest that the potential reduction in coverage from drift-reduction nozzle technology may not negatively affect the efficacy of a tank mix of azoxystrobin and difenoconazole on frogeye leaf spot in soybean.

Introduction

For soybean producers in the southern and mid-western U.S., frogeye leaf spot (FLS) is one of the most problematic foliar diseases, causing yield losses up to 60% (Mian et al., 2008; Bowers & Russin, 1998; Dashiell & Akem, 1991; Akem & Dashiell, 1994; Mian et al., 1998). FLS was first reported infecting soybean in the U.S. in 1924 (Melchers, 1925). The disease is caused by the fungal ascomycete *Cercospora sojina* K. Hara (Swoboda & Pedersen, 2009; Mian et al., 2008). Symptoms initially appear as spots, brick-red in color, which transition to light brown with dark reddish-brown margins. Lesions are usually circular to angular ranging from 1 to 5 mm in diameter (Grau et al., 2004). FLS overwinters in either soybean debris or infected seed (Heatherly & Hodges, 1998). The disease occurs in warm (25°-30°C) and humid (>90% relative humidity) environments, and infection can be heightened in the presence of excessive rainfall or irrigation (Heatherly & Hodges, 1998). FLS is a polycyclic disease in which infection, symptom development, and reproduction may all be repeated multiple times throughout a single growing season (Dorrance & Mills, 2010). Yield losses are typically caused by either a reduction in photosynthetic area and/or premature defoliation (Mian et al., 2008; Dashiell & Akem, 1991). Disease onset occurring prior to or during flowering stages (R1-R3) has been demonstrated to have the largest impact on soybean yield (Mian et al., 2008; Dashiell & Akem, 1991). Management strategies include

planting resistant varieties and FLS-free seed, crop rotation to a non-host, burying infected debris through tillage, and treating with fungicides (Heatherly & Hodges, 1998). When utilizing chemical control to manage FLS, applications made between R1 and R5 growth stages have been determined to be most effective in the prevention or treating of *C. sojae* infection (Grau et al., 2004).

The importance of using an integrated approach to managing FLS increased in 2010 when *C. sojae* isolates recovered from Lauderdale County, Tennessee were determined to be resistant to the QoI (strobilurin) fungicide class (Zhang et al., 2012). QoI fungicides have been described by the FRAC to be at “high risk” for fungal resistance because of their single site mode of action (FRAC, 2015). This class of fungicide’s activity arises from its ability to inhibit mitochondrial respiration by binding at the quinol oxidation site of cytochrome b, part of the cytochrome bc₁ complex in the inner mitochondrial membrane of fungi (Bartlett et al., 2002). Azoxystrobin, one of the most commonly used strobilurin fungicides because of its broad spectrum control of fungal diseases, primarily inhibits conidia germination in a “preventative” manner, but also has some “curative” properties, inhibiting mycelial growth (Godwin et al., 1994; Godwin et al., 1997). Resistance to QoI fungicides is the result of a single point nucleotide mutation in the *cyt b* gene, which prevents the fungicide molecule from binding to the Q_o site (Fernández-Ortuño et al., 2008; FRAC, 2013; Standish et al., 2015). Complete resistance to QoI fungicides has been determined in the amino acid substitution from glycine to alanine at position 143 (G143A) (Fernández-Ortuño et al., 2008). Zhang et al. (2010) previously found the concentration of azoxystrobin in which 50% of conidial germination was effectively inhibited (EC₅₀) of baseline *C. sojae* isolates ranged from 0.0029 to 0.0323 µg ml⁻¹. Of the 15 *C. sojae* isolates collected from Lauderdale County, Tennessee in 2010, EC₅₀ ranged from 2.7826 to 4.5409 µg ml⁻¹, approximately 140 to 959-fold greater than *C. sojae* baseline isolates (Zhang et al., 2010; Zhang et al., 2012). Because of the reduction in utility and efficacy of strobilurin fungicides to FLS, it is currently recommended to apply an alternate mode of action, such as a demethylation inhibitor (e.g., difenoconazole), either tank-mixed or premixed with azoxystrobin, (Allen, 2013; FRAC, 2015; Kelly, 2015).

Because of the increase in incidence of strobilurin resistant *C. sojae*, all controllable factors should be emphasized to improve foliar fungicide efficacy. Successful application of disease management products require the correct active ingredient to be applied at the appropriate time while optimizing plant coverage, spray retention, and deposition (Gossen et al., 2008). However, many of the current recommended application techniques have been based on herbicide research, and equipment has been designed primarily with these applications in mind (Gossen et al., 2008). Differing droplet spectra generated by various agricultural spray nozzle types have been determined to play a major role in pesticide efficacy. Agricultural spray nozzles are classified based on the droplet spectra produced, usually represented by the volumetric median diameter (VMD), defined as the diameter in which 50% of the total spray volume are in larger droplets and 50% of the total volume are in smaller droplets. Droplet spectra are categorized based on ASABE Standard S-572.1 (ASABE, 2009). VMD categories include: extra fine (~50), very fine (<136 μm), fine (136-177 μm), medium (177-218 μm), coarse (218-349 μm), very coarse (349-428 μm), extra coarse (428-622 μm), and ultra coarse (>622 μm) (ASABE, 2009). Akesson and Yates (1986) first determined the 200 to 400 μm VMD range to be optimum for insecticide and fungicide applications. When considering herbicide applications, Knoche (1994) found efficacy of contact herbicides can be increased by using finer droplet spectra. These findings were further supported by data suggesting enhanced plant coverage can be obtained using finer droplet spectra (Ramsdale & Messersmith, 2001), resulting in greater effectiveness of contact herbicides (Etheridge et al., 2001; Prokop & Veverka, 2003). However, when applying a systemic herbicide, no differences or improved efficacy have been seen when using coarser droplets compared to fine droplets due to an increase in translocation (Etheridge et al., 2001; Prokop & Veverka, 2003; Feng et al., 2003). When considering fungicides, Prokop and Veverka (2006) demonstrated an increase in efficacy when applying contact fungicides with fine droplets, and found no differences when tank-mixing a systemic fungicide with a contact fungicide. Azoxystrobin, one of the primary fungicides used to control FLS, is considered to be a systemic fungicide, possessing both xylem and translaminar mobility. Godwin et al. (1999) demonstrated that 8% of azoxystrobin entering a leaf moved upward above the point of retention within 8 days of

application. Uptake of azoxystrobin into plant cells is dependent on formulation type, additives, crop type, and environmental factors that affect droplet drying, and is usually gradual, with 25% being absorbed within 24 hours after application (Bartlett et al., 2002).

Various factors of soybean management systems can effect fungicide applications and techniques. Due to the increase in number of herbicide-resistant weeds, diverse herbicide chemistries are recommended to more consistently control weeds and prevent development of further resistance (Diggle et al., 2003). Future soybean crops genetically engineered to possess tolerance to synthetic auxins and inhibitors of 4-hydroxyphenylpyruvate dioxygenase (HPPD) will give growers new postemergence options to control problematic glyphosate-resistant weeds (Riar et al., 2013). However, with multiple non-selective herbicides applied postemergence in soybean, the need for application stewardship will increase (Ramsdale & Messersmith, 2001). Upon release of labeled herbicides for these soybean crops, application stewardship practices will be required, including the use of spray nozzles that generate coarse droplets with VMD greater than 400 μm to reduce the potential of off-target movement (EPA, 2015). The supplement label for the dicamba product, currently registered as M1691, restricts growers to only using a single nozzle type and orifice size. Along with the required nozzle, Turbo Teejet Induction (TTI) 11004 manufactured by Teejet-Spraying Systems (Springfield, IL), the label also has restrictions on the pressure range and carrier volume to be used, boom height, and wind speed at time of application (Anonymous, 2016).

Due to the increase in incidence of QoI resistant FLS, optimal application techniques should be understood and utilized. Other factors, such as requiring growers to incorporate specific nozzle types into their spray regimes for other pesticide applications, could have an overlying effect on fungicide applications. Previous data on the effect of various droplet spectra on the efficacy of pesticides are relatively limited or specific to applications other than disease management (Nuttgens et al., 2007; Creech et al., 2015). The objectives of this research were to (1) evaluate the effect of droplet size on foliar fungicide efficacy targeting FLS in soybean (2) evaluate the effect of droplet size on the residual of azoxystrobin when applied to soybean.

Materials and Methods

Field Evaluations. Field studies were conducted in 2014 and 2015 to evaluate the effect of droplet size on foliar fungicide efficacy and residual in *C. soja* infected soybean. Trials were established in four sites, either in Jackson or Milan, TN, and all within 100 km from the location of the first reported QoI-resistant *C. soja* (Zhang et al., 2012). In 2014, trials were located at the West Tennessee Research and Education Center (Jackson, TN) and the Milan Research and Education Center (Field A4-2014, Milan, TN). In 2015, trials were located at the Milan Research and Education Center (Field A8-2015, Milan, TN) and a grower's field in Jackson, TN (Cotton Grove Road). Each field site had been previously planted to soybean for at least one growing season, and had been reported to possess natural infestation of *C. soja*. Fields were planted to highly FLS susceptible, indeterminate varieties, Asgrow 4832 (Monsanto Co., St. Louis, MO) and Armor 4744 (Armor Seed, LLC, Waldenburg, AR) in 2014 and 2015, respectively, on 76.2 cm row spacing at a seeding rate of 345,800 seeds ha⁻¹. Armor 4744 was used in the second growing season due to Asgrow 4832 not being commercially available. Soybean plots were planted on 30 May 2014, 20 June 2014, 5 June 2015, and 7 June 2015 in Milan A4, Jackson, Milan A8, and Cotton Grove, respectively. A no-till production system was utilized, and with the exception of disease control, all management practices followed the University of Tennessee Extension Service recommendations. Four row by 9.14 m plots were arranged in a randomized complete block design with four replications in each location.

Field studies consisted of a single premixed fungicide applied through two spray nozzles and also a non-treated control. Quadris Top SB (Syngenta Crop Protection Inc., Greensboro, NC), a premix of azoxystrobin and difenoconazole, was applied at a rate of 0.1169 and 0.0735 kg ai ha⁻¹, respectively. Spray nozzles included: XR and TTI (Teejet Technologies, LLC, Springfield, IL) with 110° discharge angles and flow rates of 0.76 L min⁻¹ at 276 kPa. The XR11002VS nozzle was selected to represent an industry recommended standard for fungicide applications, while the TTI11002-VP was selected to represent a drift-reduction nozzle type that is required to be used on the label of dicamba-tolerant soybean

(Anonymous, 2016), although a smaller orifice size was utilized to accommodate the desired carrier volume and application speed. Treatments were applied to the two center rows once soybean reached the R3 growth stage using a CO₂-pressurized backpack sprayer adjusted to 228 kPa and a 1.5 m hand-held boom with three nozzles spaced 51 cm apart. Soybean plants had a mean height of 87 cm and canopy width of 33 cm at the time of application. Boom height was set approximately 46 cm above the soybean canopy. Applications were applied at 6.5 km hr⁻¹, resulting in a carrier volume of 140 L ha⁻¹. Air temperature, relative humidity, and wind speed at the time of application at each location can be found in Table 1. Treatment application parameters selected including boom height, ground speed, nozzle orifice flow rate, and application pressure were chosen to minimize drift between plots to decrease error in azoxystrobin concentrations.

Visual disease ratings were conducted approximately 21 days after application (DAA). FLS incidence (percentage of diseased plants within a sampling unit) and severity (percentage of disease affecting plants within a sampling unit) were recorded on a scale of 0 to 100% (Seem, 1984). Ratings were converted to a range of 1 to 12 by subjecting to the Horsfall-Barratt scale (Table 2) (Barratt & Horsfall, 1945). The Horsfall-Barratt scale is used to minimize human error when visually distinguishing differences in plant disease. To standardize ratings and form a relationship between incidence and severity for comparisons, a disease index (DI) was calculated using the formula:

$$DI = \left(\frac{\text{Incidence} \times \text{Severity}}{144} \right) \times 100$$

DI ratings ranged from 1 to 100, with 1 representing no disease and 100 representing plant death. Disease index calculated is the relationship of FLS incidence to FLS severity, normalizing assessment of plants that may have high incidence and low severity with plants that have low incidence but high severity, considering these occurrences as possessing equal levels of disease. Once soybean plots reached full physiological maturity, the two center rows were harvested using a plot combine. All yields were converted to 13% moisture content.

Data were subjected to analysis of variance using the Mixed Procedure in SAS (SAS 9.4, SAS Institute, Cary, NC). Spray nozzle type was considered the fixed main effect. Replication was analyzed as the random effect. Interactions of main effects by random effects were designated as random in the model. Means were separated using Fisher's Protected Least Significant Difference (LSD) at a significance level of 0.05.

Atomization Profiles. Atomization analyses were conducted to determine droplet spectra of each spray nozzle using a low speed wind tunnel at the West Central Research and Extension Center Pesticide Application Technology Laboratory (PAT) in North Platte, NE. The wind tunnel creates a laminar air flow at a speed of 8.0 m s^{-1} , the necessary wind required to mitigate sampling biases (Spray Drift Task Force, 1997). Droplet spectra were measured using a Sympatec HELOS-VARIO/KR laser diffraction instrument with an R7 lens (Sympatec Inc., Clausthal, Germany). The R7 lens is capable of measuring droplet diameters ranging from 18 to $3750 \text{ }\mu\text{m}$. The laser diffraction system was positioned 30 cm from the exit orifice of the nozzle (Fritz et al., 2014). Each spray nozzle was installed on a vertical actuated track, with the spray plume passing through the laser for approximately 9 sec per measurement (Henry et al., 2014). The laser was linked with WINDOX 5.7.0.0 software (Sympatec Inc., Clausthal, Germany) and is able to classify the droplet spectrum distribution. Parameters collected included the $D_{v0.1}$, $D_{v0.5}$ (VMD), and $D_{v0.9}$, representing the droplet diameter in which 10, 50, and 90% of the spray volume is contained in droplets of less than or equal values, respectively. Droplet spectra for each nozzle were measured using fungicide solution, carrier volume, and application pressure used in the field evaluations. Each nozzle treatment was replicated three times. Spray droplet classifications were derived from reference curves established from reference nozzle data at PAT as described by ASABE S572.1 (ASABE, 2009).

Data were subjected to analysis of variance using the Mixed Procedure in SAS (SAS 9.4, SAS Institute, Cary, NC). Data were analyzed using a completely randomized experimental design, with spray nozzle type as the fixed main effect. Means were separated using Fisher's Protected LSD at a significance level of 0.05.

Azoxystrobin Concentration. Soybean plant samples were collected 0, 2, 7, and 14 DAA from each plot to assess the concentration of azoxystrobin in each treatment. Ten trifoliates were randomly sampled from the 6th to 8th position, counted upwards from the cotyledon scars, on soybean plants in the two center rows of each plot. Leaves were immediately placed in polyethylene re-sealable bags on ice, and stored in a -20°C freezer.

Azoxystrobin concentration was determined using liquid chromatography – mass spectrometry (LC-MS). One frozen trifoliolate was removed from the respective re-sealable bag for each plot and thawed to room temperature. The three soybean leaflets were physically cut into smaller pieces, approximately 1 cm in size. Plant material was weighed and placed into 50 mL centrifuge tubes and extracted for 60 min in 35 mL of methanol. Tubes were placed on a reciprocating shaker operating at 60 cycles min⁻¹ during extraction. Extracts were passed through a 0.45 µm filter directly into LC-MS vials. Chemical analyses were conducted using an external standard technique with azoxystrobin standards at 0, 1, 10, and 100 parts billion⁻¹ (ppb). Analytical grade azoxystrobin standards were obtained from ChemService (Chem Service, Inc., West Chester, PA). Analytical procedures included a 1 µL injection volume, mobile phase of 60:40 acetonitrile:water (both containing 0.1% formic acid), and optimized detector parameters to perform Single Ion Monitoring. Detected concentrations were not corrected for recovery. Azoxystrobin concentration was corrected for dilution and plant sample mass.

Data were subjected to analysis of variance using the Mixed Procedure in SAS (SAS 9.4, SAS Institute, Cary, NC). Spray nozzle type, sampling date, and all interactions within were considered fixed main effects. Replication was analyzed as the random effect. Data were analyzed using a randomized complete block design and repeated measures treatment design. The first order autoregressive, AR(1), model was selected as being most appropriate for fungicide residual analyses. This model explains for decreases in correlation among concentrations as sampling dates become further apart in time (Anderson et al., 2014) and was used previously in work demonstrating the degradation of fungicide residues over time. Means were separated using Fisher's Protected LSD at a significance level of 0.05.

A single, 2 parameter expression decay regression model was run in Sigma Plot 13 (Systat Software, Inc.; Point Richmond, CA). In this model, azoxystrobin concentrations were regressed against the sampling days after application, fitting a negative slope as concentrations decreased over time, using the equation:

$$y = ae^{-bx}$$

In this model, a is the asymptote or estimated starting point of the curve (estimate of concentration at day 0), b is the rate of fungicide degradation, x represents time after application, and y equals to concentration at time x .

FLS Isolate Preparation. Soybean trifoliate possessing visible FLS symptoms were collected from non-treated control plots of each trial location for isolation of *C. sojae*. Leaf samples were immediately placed in polyethylene plastic resin re-sealable bags. Symptomatic leaves were incubated overnight under conditions of high humidity created by placing a moist paper towel into samples bags, re-sealing, and storing on a benchtop at room temperature (23°C) for ~15 hours.

Using a 20 µL pipette, conidia were dislodged by depositing approximately 10 µL of sterile deionized water onto the bottom side of lesions and pipetting repeatedly until suspended into the water. This process was conducted under a dissecting microscope to improve visibility of conidia collection and continued until an adequate quantity had been acquired from multiple lesions and trifoliate. The total resulting conidial suspension was contained in an approximately 60 µL water volume. The suspension was deposited on potato dextrose agar (PDA) media and dispersed in a circular pattern using sterile glass rods. Approximately 20 hr after plating, germinated conidia were identified under a compound light microscope. Single germinated conidia were removed from plates using a sterilized needle and re-plated on new PDA media. Cultures were also prepared by viewing conidia under a dissecting scope, removing single conidia from the bottom side of lesions with a sterilized needle, and plating on V8 media. Cultures were monitored for 7 to 10 days, and re-isolated to pure culture as needed. Pure cultures were stored in a crisper box on a laboratory benchtop at room temperature for later assessments.

Genotypic Characterization. Isolates prepared from each trial location were used to determine percentage of QoI-resistance via real-time quantitative Polymerase Chain Reaction (qPCR). One sensitive and one resistant isolate from each trial location was also identified through these analyses. Ten isolates were selected from each trial location, with the exception of Jackson due to only recovering four total isolates. Approximately one half of the mycelium from each culture was removed using a sterile pipette tip and placed in individual microfuge tubes. *C. sojae* DNA was extracted using an MP Bio FastDNA Spin Kit (MP Biomedicals, LLC, Santa Ana, CA) following manufacturer's provided methods and procedures. DNA stock solutions were stored at 4°C.

A real-time qPCR protocol developed by Zeng et al. (2014) was utilized to distinguish percentage of QoI-resistance among collected isolates. Zeng et al. (2014) developed *C. sojae* specific PCR primers to amplify the mitochondrial region in which the G143A mutation occurs. TaqMan (Life Technologies, Waltham, MA) hybridization probes specific for the QoI-sensitive and QoI-resistant alleles were also developed. TaqMan SNP genotyping assays were performed using a BioRad IQ5 thermocycler (Bio-Rad, Hercules, CA). Reactions were conducted in 25 µL volumes consisting of 10 µL TaqMan Master Mix (2x), 1.25 µL TaqMan Custom SNP genotyping assay (20x), 9.25 µL molecular grade water (Life Technologies, Waltham, MA), 0.5 µL Bovine Serum Albumin (20 µg µL⁻¹), and 4 µL of DNA stock solution. Initial denaturation was performed for 10 min at 95°C, followed by 40 amplification cycles at 95°C for 15 sec and 62°C for 1 min. The TaqMan assay consists of two probes including the VIC fluorophore hybridizing to the QoI-resistant allele and the FAM fluorophore hybridizing the QoI-sensitive allele. DNA concentrations of unknown FLS isolates were quantified by comparing cycle threshold (Ct) values to a standard curve (0.0001, 0.001, 0.01, 0.1, 1, 10 ng µL⁻¹) of known resistant and sensitive isolates.

Data were subjected to analysis of variance using the Mixed Procedure in SAS (SAS 9.4, SAS Institute, Cary, NC). Data were analyzed using a completely randomized experimental design, with resistance percentage considered the fixed main effect. Means were separated using Fisher's Protected LSD at a significance level of 0.05.

Phenotypic Characterization. Symptomatic soybean trifoliates collected from non-treated plots in each trial location were also used to determine percentage of QoI-resistance through germination assays. Conidia suspensions were created by using a 20 μL pipette to dislodge conidia by depositing approximately 10 μL of sterile deionized water onto the bottom side of lesions and pipetting repeatedly until conidia were suspended into the water. This process was conducted under a dissecting microscope to improve visibility of conidia collection and continued until an adequate quantity had been acquired from multiple lesions and trifoliates. The total resulting conidial suspension was contained in an approximately 60 μL water volume. The suspensions were equally divided and deposited to non-amended and amended PDA media. Both plates were supplemented with salicylhydroxamic acid (SHAM) which prevents the alternative oxidase respiratory pathway of QoI-sensitive conidia from overcoming the mitochondrial inhibitory activity of azoxystrobin *in vitro*. Amended media plates contained a discriminatory dose of azoxystrobin (0.1 $\mu\text{g } \mu\text{L}^{-1}$). Plates were stored on a benchtop at room temperature for assessment ~15 hr after depositing. A minimum of 100 conidia were counted for each plate and characterized as germinated or non-germinated if the respective germ tube exceeded half the length of the conidium. To account for non-viable conidia, the germination percentage of amended plates was adjusted using the following formula:

$$\% \text{ Germinated} = \left(\frac{GA/TA}{GN/TN} \right) \times 100$$

In this formula, GA represents the number of conidia germinated on amended plates, TA represents total number of conidia counted on amended plate, GN represents number of conidia, and TN represents total number of conidia counted on non-amended plates.

Azoxystrobin Dosage Response. Selected isolates were subjected to varying levels of azoxystrobin amended media to develop response curves for the determination of effective concentration in which 50% (EC_{50}) of *C. sojae* mycelia growth is inhibited of isolates collected from each trial location. One QoI-resistant and one QoI-sensitive isolate, determined by real-time qPCR analysis, was selected from each

location. No isolates recovered from Jackson in 2014 and Milan A8 in 2015 were determined to possess any sensitivity to QoI fungicides, and therefore were not represented in the present data set. Six isolates (four resistant, two sensitive) were re-isolated on non-amended V8 media supplemented with calcium carbonate to increase culture sporulation. Isolates were stored in the dark at 25°C. Cultures were grown until adequate radial mycelial growth was present for transfer.

To assess the inhibition of mycelial growth of the selected isolates, PDA plates were prepared amended with varying dosages of technical grade azoxystrobin based on previous research (Bradley & Pederson, 2011). Plugs were taken from selected isolates and deposited on PDA media amended with 0, 0.001, 0.01, 0.1, 1, and 10 $\mu\text{g mL}^{-1}$ of azoxystrobin. Plugs were taken from the culture periphery using a hollow cylinder punch with an inside diameter of 5 mm. Each isolate was replicated ten times for each concentration. All amended PDA media were supplemented with SHAM to prevent the alternative oxidase respiratory pathway from allowing QoI-sensitive cultures to overcome the inhibitory effect of azoxystrobin in the media. Approximately 15 days after isolates were plated, radial growth was measured in two perpendicular locations. The average diameter was recorded in mm for each isolate and concentration. The 5 mm diameter of the original plug was subtracted from the mean radial growth. Radial growth of each concentration was then compared to the unamended plates to determine the percent of mycelial growth inhibited.

Data were analyzed using the nonlinear curve fitting model in Origin 9.1 (OriginLab Corp., Northampton, MA). Curves were fit using the Dose Response function macro interfaced within the software. Concentrations were transformed to Logarithmic scale prior to analysis, with 0.00 $\mu\text{g mL}^{-1}$ represented as -5. Once determining the center point of the hill slope curve, the anti-LOG was taken to determine EC_{50} values using the formula:

$$y = A_1 + \frac{A_2 - A_1}{1 + 10^{(\text{LOG}x_0 - x)p}}$$

The formula was solved for y, which represents 50 % inhibition of mycelial growth. A_1 represents the minimum value plotted on the curve, while A_2 represents the maximum value. P is the unit-less symmetry

parameter, while x is the unit-less slope factor or Hill slope. $\text{LOG}x_0$ represents the LOG concentration in which 50 % of mycelial growth is inhibited.

Results

Field Evaluations. Twenty-one days after application, FLS disease index means ranged from 13.6 to 66.7 (Figure 1) when treated with Quadris Top SB across all locations and years using XR11002VS and TTI11002-VP nozzles, respectively. Disease index from treated plots was significantly lower than untreated plots (Milan A4 ($p = 0.0036$), Jackson ($p < 0.0001$), Milan A8 ($p < 0.0001$), Cotton Grove ($p < 0.0001$)) at all locations, however, no differences in disease index were detected between the two varying spray nozzle types evaluated at any of the four locations. Yield loss from non-treated soybean plots with greater levels of FLS was evident in Milan (Field A4-2014) in 2014 and in 2015, Milan (Field A8-2015) and Cotton Grove, (Milan A4 ($p = 0.0495$), Jackson ($p = 0.464$), Milan A8 ($p = 0.00289$), Cotton Grove ($p = 0.0226$)), however no difference was found between treated and untreated plots in Jackson. No differences were found between the two spray nozzles evaluated in any of the four trial locations, however, the soybean yield from plots treated with XR11002VS nozzles were greater than the non-treated in Milan (Field A8-2015), and yield from plots treated with TTI11002-VP were greater than the non-treated in Milan (Field A4-2015).

Atomization Profiles. Droplet size distribution determined by laser diffraction was significantly different (Table 3) among two Teejet-Spraying Systems nozzle types when applying Quadris Top SB using field trial parameters. Reference nozzles and curves were used to define droplet spectrum quality as described by ASABE (2009). Droplet size classification of nozzles consisted of medium and ultra coarse for the XR11002VS and TTI11002-VP, respectively, as described by ASABE S572.1 (ASABE, 2009). Droplet spectra from the TTI nozzle, consisting of a turbulence chamber and venturi, were larger than those from the XR nozzle ($p < 0.0001$) with a VMD of 838. The XR, a simple flat fan nozzle type, had a medium droplet spectra with a VMD of 247.

Azoxystrobin Concentration. Treatment differences based on the concentration of azoxystrobin detected at days 0, 2, 7, and 14 days after application varied based on sampling date and location (Figure 2). No differences were detected between azoxystrobin concentrations influenced by XR11002VS and TTI11002-VP nozzles 0 and 2 days after application in any of the four locations (Milan A4-2014 ($p = 0.0029$), Jackson ($p = 0.0252$), Milan A8-2015 ($p = 0.0343$), and Cotton Grove ($p = 0.0381$)); however, all treatments were greater than the non-treated control. Differences in concentrations between treatments and sampling dates were variable for the four locations 7 and 14 days after application. In Milan (Field A4-2014), concentrations from the TTI11002-VP decayed lower at day 7, but were not different at day 14. Concentrations from the XR11002VS were not lower until day 14. Concentrations from TTI11002-VP were greater than XR11002VS at day 14, while both treatments were greater than the non-treated. In Jackson, concentrations from the TTI11002-VP nozzle were lower at day 7 and again lower at day 14, while concentrations from the XR11002VS were lower at day 7 but not 14. The TTI11002-VP's concentrations were greater than the XR11002VS at day 7, but not different at day 14. Both nozzle treatment concentrations were greater than the non-treated. In Milan (Field A8-2015), concentrations from the TTI11002-VP and XR11002VS were lower at day 7 and again lower at day 14. The XR11002VS's concentrations were greater than the TTI11002-VP at day 14, but not different at day 7. Neither spray nozzle concentration was different than the non-treated at day 7 or day 14. In Cotton Grove, concentrations from the TTI11002-VP and XR11002VS were not lower at day 7, but were lower at day 14. No differences were found between the two spray nozzle treatment concentrations, however, both treatments were greater than the non-treated.

Azoxystrobin dissipation was associated with increase of time after application. The model chosen compared the concentration of azoxystrobin with the amount of time after application for each spray nozzle evaluated. The exponential decay model explained the relationship well for both nozzles evaluated (TTI11002VS: $r^2 = 0.53, 0.48, 0.64$, and 0.49 ; XR11002VS: $r^2 = 0.80, 0.82, 0.50$, and 0.37 for Milan (Field A4-2014), Jackson, Milan (Field A8-2015), and Cotton Grove, respectively). A negative slope represents the degradation of fungicide residual after application. The model predicted the half-life

of azoxystrobin to occur between 2.54 (Figure 4) and 3.99 days after application for the XR11002VS, and between 2.69 (Figure 3) and 4.82 days after application for the TTI11002-VP.

FLS Isolate Characterization. Germination assays were used to determine phenotypic characterization of QoI-resistant *C. sojae* populations in each field site and year (Table 6). Milan (Field A4-2014) in 2014 was determined to have the lowest mean percentage of QoI-resistant *C. sojae* population to azoxystrobin at 22%, followed by Cotton Grove at 55% in 2015. Jackson in 2014 and Milan (Field A8-2015) were determined to have the greatest population of QoI-resistant *C. sojae* at 95 and 99%, respectively.

Real-time qPCR analyses were conducted to determine genotypic characterization of QoI-resistant isolates from each field site and year. Ten isolates were evaluated from each location with the exception of Jackson due to only recovering four viable isolates (Table 4). Mean resistance percentages were calculated for each field site and year. Cotton Grove was determined to have lower percentage of QoI-resistant isolates at 40% (Table 5) when compared to Jackson and Milan (Field A8-2015), however, was not significantly different than Milan (Field A4-2014) ($p=0.0041$). Milan (Field A4-2014) was also not different than Jackson or Milan A8 at 71, 100, and 100%, respectively.

Germination assays evaluate resistance percentage based on the number of conidia germinated on fungicide amended media in comparison to un-amended media. Conidia are collected from multiple leaves and symptomatic lesions, representing a general population. Real-time qPCR analyses evaluate the percentage of the G143A allele in a single isolate. Because of the differences in populations being evaluated, it could be expected to see varying results. Interestingly, Jackson and Milan (Field A8-2015) possessed the greatest percentage of resistance, but also had the lowest disease incidence at the time of application at 37.5 and 25%, respectively. Cotton Grove and Milan (Field A4-2014) possessed lower percentages of resistance, but had greater percentages of disease incidence at the time of application, at 95 and 75%, respectively.

Azoxystrobin Response Curve. QoI-resistant (Figure 5) and sensitive (Figure 6) *C. soja* isolate mycelial inhibition responded significantly to increasing concentrations of azoxystrobin. The dosage response model selected explained this relationship well (resistant isolate $r^2 = 0.84, 0.78, 0.77$, and 0.48 for Milan (Field A4-2014), Jackson, Milan (Field A8-2015), and Cotton Grove, respectively; sensitive isolate $r^2 = 0.93$, and 0.99 for Milan (Field A4-2014), and Cotton Grove, respectively). QoI-resistant isolate EC_{50} values ranged from 6.20 to $13.88 \mu\text{g mL}^{-1}$, while QoI-sensitive isolate EC_{50} values were equal to 0.02 and $0.11 \mu\text{g mL}^{-1}$. When applying these results to the Exponential Decay model used to regress azoxystrobin concentration with days after application as effected by droplet size generated from the two nozzles evaluated, concentrations were not high enough to inhibit 50% of mycelial growth in Milan (Field A4-2014), Jackson, or Cotton Grove. Concentrations were predicted to be adequate in Milan (Field A8-2015) to control 50% of *C. soja* mycelial growth up to 13.3 hours after azoxystrobin application by XR11002VS spray nozzles, and up to 27.4 hours after application by TTI11002-VP. However, these results may be somewhat misleading based upon the methodology used to detect azoxystrobin from soybean samples in this study as well as the previous work stating that only 25% of azoxystrobin is absorbed to possess activity on a pathogen within 24 hours (Bartlett et al. 2002). In Milan (Field A4-2014) and Cotton Grove in which QoI-sensitive isolates were recovered, concentrations were predicted to be adequate in Milan (Field A4-2014) to control 50% of *C. soja* mycelial growth up to 16.9 and 29.2 days after azoxystrobin application by XR11002VS spray nozzles, and up to 8.6 and 28.3 days after application by TTI11002-VP, respectively.

Discussion

Field Evaluations. Differences in FLS disease index and soybean yield as effected by applications of Quadris Top SB with varying droplet size ranges have not previously been reported. Reductions in FLS disease index and protection of soybean yield when applying Quadris Top SB compared to non-treated controls were consistent with previous research (Allen et al., 2015; Cochran et al., 2015). In a study

evaluating droplet size effects on soybean rust using a systemic fungicide, no differences were determined amongst eight spray nozzle types with varying droplet size classifications (Mueller, 2007). Not distinguishing differences in disease index and soybean yield between XR11002VS and TTI11002-VP nozzles generating varying droplet spectra was consistent with previous work (Mueller, 2007).

Atomization Profiles. Droplet spectra differences among XR11002VS and TTI11002-VP nozzle types when applying Quadris Top SB have not been previously reported. Etheridge et al. (1999) demonstrated air induction, venturi-type nozzles significantly increase droplet spectra in comparison to flat fan nozzles when applying herbicides. Gil et al. (2013) demonstrated that a spray nozzle possessing both air induction and a turbulence chamber generates coarser droplets than an air induction or flat fan nozzle. Differences among droplet spectra generated by flat fan and air induction nozzles are consistent with previous work (Etheridge et al., 1999; Gil et al., 2013).

Azoxystrobin Concentration. The effect of droplet spectra generated by XR11002VS and TTI11002-VP nozzles on azoxystrobin concentrations has not been previously reported. Feng et al. (2003) studied the differences in retention, absorption, and translocation of a systemic herbicide, glyphosate, using three droplet spectra including fine, medium, and coarse. Spray retention was greatest with fine droplets, however, absorption and translocation was greatest when glyphosate was applied with coarse droplets. No differences were found based on concentrations of azoxystrobin at the time of application, suggesting no differences in the spray retention of a systemic fungicide from two varying droplet spectra. No consistent results indicated that the absorption rates, translocation, and/or residual of a systemic fungicide, azoxystrobin, are influenced by droplet size deposition.

Azoxystrobin Response Curve. QoI-resistant and sensitive *C. sojae* isolate mycelial response to increasing doses of azoxystrobin has not previously been reported. Previous reports by Zhang et al. (2010) determined the concentration of azoxystrobin in which 50% of conidial germination was effectively inhibited in QoI-sensitive *C. sojae* isolates ranged from 0.0029 to 0.0323 $\mu\text{g ml}^{-1}$ (mean = 0.0127 $\mu\text{g ml}^{-1}$). Of the 15 *C. sojae* isolates collected from western Tennessee in 2010, EC₅₀ was

determined to range from 2.7826 to 4.5409 $\mu\text{g ml}^{-1}$ (mean = 3.1644 $\mu\text{g ml}^{-1}$), approximately 140 to 959-fold greater than *C. soja* baseline sensitive isolates (Zhang et al., 2010; Zhang et al., 2012), confirming high resistance to QoI fungicides. These results suggest that mean concentrations required to inhibit 50% of mycelial growth of QoI-sensitive *C. soja* isolates are 377% greater than that required to inhibit conidial germination, and 235% higher than that required to inhibit QoI-resistant *C. soja* conidial germination.

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Part III. Response of Fungicide Efficacy and Coverage to Droplet Size in Commercial Application Setting

Abstract

Field experiments were conducted in 2015 to evaluate the influence of droplet size on foliar fungicide efficacy and plant coverage on frogeye leaf spot in soybean in a commercial application setting with high potential for off-target movement. A premix of azoxystrobin and difenoconazole was applied using two spray nozzle types with varying droplet spectra. No differences were found among treatments in regards to visual disease index and soybean yield. Coverage was greater in the upper canopy than in the lower canopy, however, no differences were found in coverage based on nozzle type. Results suggest that the potential reduction in coverage from drift-reduction nozzle technology may not negatively affect the efficacy of a tank mix of azoxystrobin and difenoconazole on frogeye leaf spot in soybean in a commercial application setting.

Introduction

In today's soybean production, growers face a constant "revolving door" of pest management issues. When treating for disease, as well as weeds and insects, growers are challenged with selecting the correct active ingredient to be applied at the appropriate time while optimizing plant coverage, spray retention, and deposition (Gossen et al., 2008). Due to these challenges, especially strict application timings, growers are tasked with trying to treat large areas at a rapid pace. To combat this task, machinery ground speeds tend to increase and weather conditions are sometimes neglected. Both of these parameters may increase the potential of off-target movement and reduce plant coverage and/or canopy penetration (Ramsdale & Messersmith, 2001; Gilbert & Bell, 1988; Bode et al., 1976; Nuttyens et al., 2007). While wind speeds and boom height at application, as well as distance from susceptible vegetation, are all key factors contributing to off-target movement of agricultural sprays, droplet size is one of the primary factors. Yates et al. (1986) stated that droplets smaller than 150 μm in diameter were most susceptible to drift. Droplets 100 μm in diameter have the potential to move over nine times further off-target than droplets with 1000 μm in diameter (Akesson & Yates, 1964). To minimize the risk of off-target movement, selecting proper Drift Reduction Technology (DRT) nozzles is critical (Kruger et al., 2014).

DRT nozzles manipulate spray solutions to decrease drift potential by reducing the percentage of driftable-fines (Yates et al., 1986; Etheridge et al., 1999) through hydraulic mechanics within the nozzle design. DRT nozzles typically generate droplet spectra with volumetric median diameters (VMD) greater than 400 μm in diameter. The VMD is the value in which 50% of total spray volume is in droplets larger than the median diameter and 50% of the total spray volume is smaller droplets. The use of DRT nozzles has been shown to significantly decrease drift potential of agricultural sprays (Piggott & Matthews, 1999; Etheridge et al., 1999). Along with contaminating sensitive vegetation in neighboring areas (Nordby & Skuterud, 1974), the result of off-target movement may cause decreases in pesticide efficacy in the desired application area (Johnson et al., 2006).

One instance in which applicators could be negatively affected by reductions in canopy penetration, plant coverage, and fungicide efficacy occurs when treating soybean foliage infected with *Cercospora sojina*, the causal agent of frogeye leaf spot (FLS) (Swoboda & Pedersen, 2009; Mian et al., 1998). FLS is one of the most problematic foliar diseases of soybean in the southern and mid-western U.S.. This disease has been found to cause yield losses up to 60% (Mian et al., 2008; Bowers & Russin, 1998; Dashiell & Akem; 1991, Akem & Dashiell, 1994; Mian et al., 1998) through reductions in photosynthetic area and/or premature defoliation (Mian et al., 2008; Dashiell & Akem, 1991), and disease onset occurring prior to or during flowering stages (R1-R3) is understood to have the largest impact. Symptoms begin as brick-red spots, which transition to light brown with dark reddish-brown margins. Lesions are usually circular to angular ranging from 1 to 5 mm in diameter (Grau et al., 2004). FLS survives in both soybean debris and infected seed (Heatherly & Hodges, 1998). Infected cotyledons containing sporulating lesions provide inoculum to infect young leaves (Heatherly & Hodges, 1998). Lesions usually do not appear for 2 weeks after disease onset in the host (Mian et al., 2008), however, conidia may be produced within 24 to 48 hours after formation of the lesion (Sinclair & Backman, 1989). New, emerging soybean leaves are most susceptible, but lesions are not visible initially due to the length of time for infection (Mian et al., 2008; Phillips & Boerma, 1999; Heatherly & Hodges, 1998). It is

important to note the area of infection such that application strategies can be devised targeting this specific site.

When growers select nozzles to equip their sprayers, many parameters must be considered. Applicators must first determine the type of sprayer system they are operating, and narrow selection to the compatible nozzle types. Many sprayers are currently equipped with nozzle turrets, allowing growers to install multiple nozzles allowing for easy transition to the desired nozzle. Based on preference, applicators may select a single nozzle type with varying orifice sizes, allowing them to spray differing carrier volumes without altering ground speed and application pressure, or they may look to select nozzles that provide either the greatest efficacy or drift management. However, because of the multitude of pests with overlaying control windows, applicators may decide to make tank-mixed applications. With these types of applications, the various pesticide products may have different levels of efficacy with different droplet spectra. Although relatively understudied, disease management applications are currently recommended to be made with finer droplet spectra (Gossen et al., 2008) due to providing greater plant coverage in comparison to coarse droplets (Ramsdale & Messersmith, 2001). However, future regulatory requirements brought forth by other pest management systems could force applicators to choose from a narrow selection of nozzle types (EPA, 2015). Due to the increase in incidence of herbicide-resistant weeds, herbicide chemistries with different modes of action are recommended to more consistently control weeds and prevent development of further resistance (Diggle et al., 2003). Future soybean crops engineered with tolerance to synthetic auxins and inhibitors of 4-hydroxyphenylpyruvate dioxygenase (HPPD) will provide growers new postemergence options to control problematic glyphosate-resistant weeds (Riar et al., 2013). Consequently, with multiple non-selective herbicides applied postemergence in soybean, the need for application stewardship will increase (Ramsdale & Messersmith, 2001). Upon release of labeled herbicides for these soybean crops, application stewardship practices will be required, including the use of DRT spray nozzles that generate coarse droplets with VMD greater than 400 μm to reduce the potential of off-target movement (EPA, 2015). In the occurrence of a tank-mix application including these types of herbicides and a fungicide, or an applicator that does not look to

switch nozzles for a fungicide application because of cost and/or time, coarser droplets generated by DRT could negatively affect fungicide applications (Prokop & Veverka, 2006).

Research studying variable spray droplet spectra of agricultural nozzles to better understand the effects of off-target movement is limited, generally focusing on a detailed application scenario (Nuttgens et al., 2007; Creech et al., 2015). Much of previous work on application technology targeting plant diseases has also been conducted in highly controlled environments. Thus, the objectives of this study were to (1) evaluate the influence of droplet size on foliar fungicide efficacy in a commercial application setting and (2) determine plant coverage and canopy penetration between two differing droplet sizes in a commercial application setting.

Materials and Methods

Field Evaluations. Field studies were conducted in 2015 to evaluate the effect of droplet size on foliar fungicide efficacy and coverage in *C. sojae* infected soybean under commercial application parameters. Trials were located at the West Tennessee Research and Education Center (Jackson, TN) and the Milan Research and Education Center (Milan, TN). Each field site had been previously planted to soybean for at least one growing season, and had been reported to possess natural infestation of FLS. Field sites were planted as a double crop system following wheat. Asgrow 4835 (Monsanto Co., St. Louis, MO), a mildly FLS susceptible variety, was planted on 76.2 cm and 38.1 cm row spacings in Milan and Jackson, respectively, at a seeding rate of 345,800 seeds ha⁻¹. Soybean plots were planted on 15 June 2015, and 20 June 2015 in Jackson and Milan, respectively. A no-till production system was utilized, and with the exception of disease control, all management practices followed the University of Tennessee Extension Service recommendations. Plots were randomly arranged throughout the trial area. In Jackson, plots were twelve rows wide by 60 m in length. In Milan, plots were six rows wide, ranging from 245 to 305 m in length.

Field studies consisted of a single fungicide applied with two spray nozzle treatments and also a non-treated control. Quadris Top SB (Syngenta Crop Protection Inc., Greensboro, NC), a premix of

azoxystrobin and difenoconazole, was applied at an application rate of 0.1169 and 0.0735 kg ai ha⁻¹, respectively. Additionally, 1, 3, 6, 8-pyrene tetra sulfonic acid tetra sodium salt (PTSA) (Spectra Colors Corp., Kearny, NJ) was added to the spray solution to serve as a tracer dye at a rate of 0.6 mg mL⁻¹ based on recommendations for agricultural sprays (Hoffman et al., 2014). Spray nozzles included: extended range (XR) and turbo teejet induction (TTI) (Teejet Technologies, LLC, Springfield, IL) with 110° discharge angles and flow rates of 1.52 L min⁻¹ at 276 kPa. The XR11004VS flat-fan nozzle was selected to represent an industry recommended standard for fungicide applications, while the TTI11004-VP air induction, turbulence chamber nozzle was selected to represent the only current spray nozzle labeled for use with dicamba-tolerant soybean (Anonymous, 2016). Treatments were applied once soybean reached the R3 growth stage using a John Deere 6500 self-propelled sprayer with an 18.3 m boom (Deere & Company, Moline, IL) adjusted to 276 kPa. Soybean plants had a mean height of 91 cm and canopy width of 28 cm at the time of application. Nine spray nozzles of each type were installed on opposites ends of the spray boom, spaced 51 cm apart. The resulting 9.2 m section of boom between nozzle sets were blanked off and served as a buffer area. Boom height was set approximately 76 cm above the crop canopy. Applications were made at a ground speed of 17 km hr⁻¹, resulting in a carrier volume of 140 L ha⁻¹. Application parameters selected including ground speed and boom height were chosen to simulate a commercial application setting with increased drift potential. Air temperature, relative humidity, and wind speed at the time of application at each location are listed in Table 7.

Visual evaluations were conducted approximately 21 days after application (DAA). Visual control rating followed methodology described in the previous chapter. All ratings were subjected to the Horsfall-Barratt scale and converted to a disease index, ranging from 1 to 100. Once soybean plots reached full physiological maturity, the entire plots were harvested using a commercial combine. Prior to harvest, global position system (GPS) coordinates were obtained from the corners of each plot using a hand-held GPS unit (Garmin Corporation, Olathe, KS). Coordinates were mapped in ArcMap software (Esri, Redlands, CA) and plots were converted to shape files. After harvest, yield data files were retrieved from the combine and geo-referenced in ArcMap. Yield data were overlaid with plot shape files, and

average mean yield across the entire area of the plot was calculated. All yields were converted to 13% moisture content.

Data were analyzed using a randomized complete block design. Spray nozzle type was considered the fixed main effect. Replication within location was analyzed as the random effect. Data were subjected to analysis of variance using the Mixed Procedure in SAS (SAS 9.4, SAS Institute, Cary, NC). Means were separated using Fisher's Protected Least Significant Difference (LSD) at a significance level of 0.05.

Atomization Profiles. Atomization analyses were conducted to determine droplet spectra of each spray nozzle using a low speed wind tunnel at the West Central Research and Extension Center Pesticide Application Technology Laboratory (PAT) in North Platte, NE. Atomization analysis followed methodology described extensively in the previous chapter. Parameters collected included $D_{v0.1}$, $D_{v0.5}$ (VMD), and $D_{v0.9}$. Droplet spectra for each nozzle were calculated using fungicide solution, carrier volume, and application pressure used in the field evaluations. Spray droplet classifications were derived from reference curves established from reference nozzle data at PAT as described by ASABE S572.1 (ASABE, 2009).

Data were subjected to analysis of variance using the Mixed Procedure in SAS (SAS 9.4, SAS Institute, Cary, NC). Data were analyzed using a completely randomized experimental design, with spray nozzle type considered the fixed main effect. Means were separated using Fisher's Protected LSD at a significance level of 0.05.

Coverage Analysis. Field assays to compare differences in canopy penetration and plant coverage between XR11004VS and TTI11004-VP spray nozzles were conducted following modified methods and procedures developed by Hoffman et al. (2014) where Mylar cards were utilized to catch spray deposits and were stored in re-sealable plastic bags. In the current study, round solid-white polypropylene jars (United States Plastic Corp., Lima, OH) with an inside diameter of 1000 mm were utilized. The jars possessed threaded lids which were selected to improve the efficiency of securely containing the deposited sample during storage. The inside of the containers was 38 mm deep, and the height of the jar

assisted in reducing sample placement and collection time, allowing for stands to be constructed holding jars freely, without having to mechanically fasten. Stands were created using 107 cm long pieces of rebar with foot pegs attached 15 cm from the base. Screws were welded to the stands 46 cm and 92 cm from the foot peg, slightly offset such that jars would not overlap one another. Polyvinyl chloride (PVC) pipe with a 10 cm diameter was cut into 4 cm strips. Holes were drilled approximately 0.5 cm from the base of the PVC rings. The hole was threaded onto the screw welded to the rebar, and was repeated for the upper and lower screw on each stand. Stands were physically pressed into the soil using the foot pegs among the soybean canopy in each plot, simulating the position of a soybean plant. The results were stands able to position jars approximately 20° perpendicular to the soil surface, simulating soybean leaf position and angle, at heights of 46 and 92 cm, representing the lower and upper canopy, respectively. Four stands were randomly placed within soybean rows in each plot. Prior to application, labeled jars were placed in position on each stand. One plot for each respective nozzle treatment was sprayed simultaneously, with a buffer zone incorporated between treated plots. Once the sprayer passed through each plot, jars were immediately collected, lids were installed, and sealed jars were placed in a dark container at room temperature to reduce the potential of photodegradation.

Samples were sent to the PAT Lab in North Platte, NE for analysis. Jars were rinsed with 40 mL of a 9:1 distilled water to isopropyl alcohol solution using a bottle top dispenser (LabSciences Inc., Reno, NV). Hoffman et al. (2014) determined this solution to result in the maximum recovery of PTSA deposits from agricultural sprays. Jars were agitated by hand, and a two mL sample was drawn via a pipette and deposited into a glass cuvette. The cuvette was placed into a PTSA module (0.1 ppb - 10,000 ppb linear range) within a fluorometer (Trilogy Laboratory Fluorometer, Turner Designs, Sunnyvale, CA). Fluorometric readings were conducted and recorded in relative fluorescent units (RFU).

Data were subjected to analysis of variance using the Mixed Procedure in SAS (SAS 9.4, SAS Institute, Cary, NC). Data were analyzed using a randomized complete block experimental design, with spray nozzle type, jar position, and the interaction of nozzle type and position considered the fixed main

effects. Location was analyzed as the random effect. Means were separated using Fisher's Protected LSD at a significance level of 0.05.

Results

Field Evaluations. Twenty-one days after application, FLS disease index means equaled 11 and 15 (Table 8) in plots treated with Quadris Top SB across both locations. However, the only treatment that significantly reduced FLS disease index compared to the untreated control was Quadris Top SB applied using the XR11004-VP nozzle ($p=0.0007$). The differences in disease index were not reflective to soybean yield. Yield loss in non-treated soybean plots with greater levels of FLS was evident ($p=0.0223$). An application of Quadris Top SB protected yield over non-treated plots infected with FLS up to 14.2%. Yields in treated plot means equaled 3383 to 3420 kg ha⁻¹ for the XR11004VS and TTI11004-VP nozzles, respectively, however, no differences in yields were detected between spray nozzle types evaluated. No difference was detected between either location based on disease index or soybean yield, suggesting differences in row spacing did not effect spray application.

Atomization Profiles. Droplet size distribution determined using a laser diffraction system varied (Table 9) between two Teejet-Spraying Systems nozzle types when applying Quadris Top SB using field trial parameters. Reference nozzles and curves were used to define droplet spectrum quality as described by ASABE (2009). Droplet size classification of nozzles consisted of medium and ultra coarse for the XR11004VS and TTI11004-VP, respectively, as described by ASABE S572.1 (ASABE, 2009). The droplet spectra generated by the TTI nozzle, consisting of a turbulence chamber and venturi, was larger than the XR nozzle ($p<0.0001$) with a VMD of 734. The XR, a simple flat fan nozzle type, had a droplet spectra with a VMD of 263.

Coverage Analysis. Retention, plant coverage, and canopy penetration were assessed by catching spray solutions in jars in both the upper and lower canopy of the soybean plot. Treatments were compared by measuring relative fluorescence of solution caught. When assessing RFU differences between the upper and lower canopy across both nozzle types, fluorescence was greater from solutions caught in the upper

canopy ($p=0.0051$), suggesting that 43% more of the solution was retained at the top of the plant (Table 10). When comparing the XR11004VS and TTI11004-VP nozzles based on overall coverage across both the upper and lower canopy, no significant differences were detected. Assessments of the interaction of jar position in the canopy and nozzle type also demonstrated no differences between nozzle treatments, with differences only found based on jar position.

Discussion

Field Evaluations. Differences in FLS disease index and soybean yield as effected by applications of Quadris Top SB with varying droplet size ranges have not previously been reported. Reductions in FLS disease index and protection of soybean yield when applying Quadris Top SB compared to non-treated controls were consistent with previous research (Allen et al., 2015; Cochran et al., 2015). In a study conducted by Mueller (2007) evaluating droplet size effects on soybean rust using a systemic fungicide, no differences were determined among eight spray nozzle types with varying droplet size classifications.. No differences between XR11004VS and TTI1104-VP nozzles when considering soybean yield was consistent with previous reports (Mueller, 2007).

Atomization Profiles. Droplet spectra differences between XR11004VS and TTI11004-VP nozzle types when applying Quadris Top SB has not been previously reported. Etheridge et al. (1999) demonstrated air induction, venturi-type nozzles increase droplet spectra in comparison to flat fan nozzles when applying herbicides. Gil et al. (2013) demonstrated that a spray nozzle possessing both air induction and a turbulence chamber generates coarser droplets than an air induction or flat fan nozzle. Differences among droplet spectra generated by flat fan and air induction nozzles are consistent with previous work (Etheridge et al., 1999; Gil et al., 2013).

Coverage Analysis. Differences in spray retention when applying Quadris Top SB using XR11004VS and TTI11004-VP nozzle have not previously been reported. Bradley et al. (2007) evaluated coverage of flat fan nozzles and air induction nozzles with differing droplet spectra in soybean using tebuconazole. Differences were not found among spray nozzles with different droplet spectra in the upper or lower

canopy. A positive correlation was also found suggesting that as droplet size increased, coverage in the lower canopy increased. No differences between XR11004VS and TTI1104-VP nozzles when considering retention, canopy penetration, and plant coverage were consistent with previous reports (Bradley et al., 2007). These data suggest that although finer droplet spectra possess a larger number of droplets with the potential to have greater plant coverage, applying fungicide solutions with coarse droplets in a commercial application setting with the potential for off-target movement compensate for the lower number of droplets. Due to the site of FLS infection occurring in the upper portion of the soybean canopy where young leaves are emerging, using either a standard flat fan nozzle or DRT nozzle with current application techniques may not have a significant impact on disease control or soybean yield.

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Part IV. Conclusions

Conclusions

Once growers equip their sprayers with DRT nozzles that produce coarser spray droplets to combat for off-target contamination concerns and the adaptation of future herbicide-tolerant crops, fungicide applications currently recommended to be applied using finer droplet diameters could potentially be negatively affected. Applications of azoxystrobin and difenoconazole premixes targeting frogeye leaf spot (FLS) of soybean are one particular instance in which reductions in plant coverage from increased droplet size could potentially effect disease control and yield. The overall objective of this research was to determine if drift-reduction nozzles that have the potential to decrease coverage would have a negative impact on an application of a foliar fungicide with systemic mobility. The first part of this research focused on the efficacy and residual of Quadris Top SB when using two nozzle types producing different droplet volumetric median diameters. Within this study, isolates in each location were determined to be either resistant or sensitive to strobilurin fungicides through phenotypic and genotypic characterization. Isolates were then subjected to response curves produced an EC_{50} value to demonstrate the point that azoxystrobin concentration is degraded where it no longer controls 50% of *Cercospora sojina* mycelial growth. The second part of this research was to further evaluate the selected nozzle types in a commercial application setting with the potential for off-target movement. Within this study, canopy penetration was evaluated to distinguish differences in spray retention in the upper and lower canopy as well as between droplet spectra.

Part II

Droplet size did not have an effect on the control of frogeye leaf spot in soybean. This indication was also confirmed through soybean yield, in that no differences were found among applications. However, considering both disease control and soybean yield, an application of Quadris Top SB decreased FLS disease index as well as protected yield over the non-treated control. To further explain this occurrence, azoxystrobin was detected from each plot to understand if differences were present amongst the two varying droplet spectra and a non-treated. No differences were found between the two

varying droplet spectra at 0, 2, 7, and 14 days after application, however, each date was greater than the non-treated control, suggesting that a quantity of azoxystrobin was still present to have activity on the disease. Mean azoxystrobin concentrations required to inhibit 50% of mycelial growth from trial locations was determined to equal $7.44638 \mu\text{g mL}^{-1}$ and $0.04789 \mu\text{g mL}^{-1}$ for resistant and sensitive isolates of *C. sojae*, respectively.

These data support the integration of drift-reduction nozzle technology into soybean pest management application systems both because of the concern with off-target contamination as well as the future label requirements of herbicide-tolerant soybean crops.

Part III

Droplet size did not have an effect on the control of FLS in soybean in a commercial application environment. This indication was also confirmed through soybean yield, in that no significant differences were found among applications. However, considering both disease control and soybean yield, an application of Quadris Top SB significantly decreased FLS disease index as well as protected yield over the non-treated control. To further explain these occurrences, spray solutions were collected in the upper and lower canopy of each soybean plot. Solutions were determined to be greater in the upper canopy than the lower canopy, however, no differences in coverage were found between the two droplet spectra. Due to the infection of FLS occurring in the upper canopy with new emerging soybean leaves, applications that deposit more solution in the upper canopy than the lower are sufficient.

These data also support the integration of drift-reduction nozzle technology into soybean pest management application systems. However, because higher concentrations were deposited in the upper canopy, diseases that occur in the lower canopy may be negatively impacted. Since no difference was noted in nozzle type on canopy penetration, proper nozzle selection may not help to improve fungicide efficacy on diseases in the lower canopy. Alternate application techniques and methods should be evaluated for these types of diseases.

Appendix. Tables & Figures

Tables

Table 1. Environmental conditions recorded at the time of application for studies evaluating the influence of droplet size on foliar fungicide efficacy and residual. (Part II.)

Location	Date	Time	Air Temperature ^a °C	Relative Humidity %	Wind Speed km hr ⁻¹
Jackson	25 Aug 2014	9:32:00	27	82	9.7
Milan A4	25 Jul 2014	10:53:00	26	62	1.6
Milan A8	11 Aug 2015	11:30:00	32	65	6.4
Cotton Grove	11 Aug 2015	15:00:00	34	52	12.9

^aEnvironmental data measured using Kestrel 3000 wind meter (Loftopia, LLC., Birmingham, MI)

Table 2. Horsfall-Barratt (H-B) Scale, percentage range for scale, and size interval of each scale used to correct for human visual error when rating plant disease. FLS visual disease ratings including incidence and severity were taken 21 days after fungicide application using a 0 to 100% scale. Ratings were adjusted to form a scale following guidelines below.

H-B Scale	Range %	Size of Interval
1	0	0
2	0 ⁺ -3	3
3	3 ⁺ -6	3
4	6 ⁺ -12	6
5	12 ⁺ -25	13
6	25 ⁺ -50	25
7	50 ⁺ -75	25
8	75 ⁺ -87	13
9	87 ⁺ -94	6
10	94 ⁺ -97	3
11	97 ⁺ -100	3
12	100	0

Table 3. Atomization analysis of XR11002VS and TTI11002-VP nozzles with Quadris Top SB. Volumetric distribution of diameters in which droplets of equal or smaller size delineate 10, 50, and 90% ($D_{v0.1}$, $D_{v0.5}$, and $D_{v0.9}$) of the total spray volume. Spray classification determined in accordance with ASABE S572.1 standards from curves generated using reference nozzles.

Nozzle	Droplet Diameter ^a			Spray Classification ^b
	$D_{v0.1}$	$D_{v0.5}$	$D_{v0.9}$	
		μm		
XR11002VS	124 b ^b	247 b	403 b	Medium
TTI11002-VP	465 a	838 a	1197 a	Ultra Coarse

^a Droplet sizes determined through atomization analysis using laser diffraction in a low-speed wind tunnel, applying a rate of 0.1169 and 0.0735 kg ai ha⁻¹ of azoxystrobin and difenoconazole, respectively, at 228 kPa application pressure resulting in 140 L ha⁻¹ carrier volume

^b Spray classification based on ASABE S572.1

^c Means within a column followed by the same letter are not different according to Fisher's Protected LSD ($p < 0.05$).

Table 4. Genotypic characterization of *C. sojina* isolates.

Year	Location	Isolate ^a	% Resistant ^b
2014	Jackson	J1	100
2014	Jackson	J3	100
2014	Jackson	J4	100
2014	Jackson	J5	100
2014	Milan A4	A4-3	100
2014	Milan A4	A4-6	2.92
2014	Milan A4	A4-7	100
2014	Milan A4	A4-8	5.37
2014	Milan A4	A4-15	100
2014	Milan A4	A4-21	100
2014	Milan A4	A4-22	100
2014	Milan A4	A4-26	100
2014	Milan A4	A4-4	100
2014	Milan A4	A4-33	1
2015	Cotton Grove	CG-5	100
2015	Cotton Grove	CG-8	2.5
2015	Cotton Grove	CG-10	17.2
2015	Cotton Grove	CG-24	100
2015	Cotton Grove	CG-25	2.95
2015	Cotton Grove	CG-26	70.3
2015	Cotton Grove	CG-2	1
2015	Cotton Grove	CG-3	1
2015	Cotton Grove	CG-9	1
2015	Cotton Grove	CG-12	100
2015	Milan A8	A8-2	100
2015	Milan A8	A8-3	100
2015	Milan A8	A8-4	100
2015	Milan A8	A8-5	100
2015	Milan A8	A8-7	100
2015	Milan A8	A8-8	100
2015	Milan A8	A8-11	100
2015	Milan A8	A8-13	100
2015	Milan A8	A8-10	100
2015	Milan A8	A8-12	100

^a Isolates screened for QoI sensitivity using real time qPCR methods. Isolates established by plating single conidia on PDA media obtained by single spore isolation. Conidia were collected from symptomatic leaves sampled from untreated plots within field trial locations.

^b Resistance determined by comparison to standard curves generated from the DNA of known resistant and wild-type *C. sojina* isolates

Table 5. Mean resistance of *C. sojae* isolates by location determined through genotypic characterization

Year	Location	% Resistant ^a
2014	Jackson	100 a ^b
2014	Milan A4	71 ab
2015	Cotton Grove	40 b
2015	Milan A8	100 a

^a Isolates screened for QoI sensitivity using real time qPCR methods. Isolates established by plating single conidia on PDA media obtained by single spore isolation. Conidia were collected from symptomatic leaves sampled from untreated plots within field trial locations. Resistance determined by comparison to standard curves generated from the DNA of known resistant and wild-type *C. sojae* isolates

^b Means within a column followed by the same letter are not different according to Fisher's Protected LSD ($p < 0.05$).

Table 6. Mean resistance of *C. sojae* isolates by location determined through phenotypic characterization

Year	Location	% Resistant ^a
2014	Jackson	95
2014	Milan A4	22
2015	Cotton Grove	55
2015	Milan A8	99

^a Isolates established by plating conidia suspensions on PDA media obtained from symptomatic lesions on multiple leaves sampled from untreated plots from each field trial location. Conidia plated on unamended and amended (discriminatory dose of azoxystrobin) media. Conidia germination inhibition assessed ~15 hours after plating.

Table 7. Environmental conditions recorded at the time of application for studies evaluating the response of foliar fungicides to droplet size in a commercial application setting. (Part III.)

Location	Date	Time	Air Temperature	Relative Humidity	Wind Speed
			(°C)	%	km hr ⁻¹
Jackson	21 Aug 2015	9:56:00	27	56	16.1
Milan	21 Aug 2015	2:23:00	33	52	3.2

^aEnvironmental data measured using Kestrel 3000 wind meter (Loftopia, LLC., Birmingham, MI)

Table 8. Disease index and soybean yield from studies evaluating the response of foliar fungicides to droplet size in a commercial application setting. Spray nozzles were determined to produce medium to ultra coarse VMD for XR11004VS and TTI11004-VP, respectively.

Spray Nozzle	Disease Index ^a	Soybean Yield ^b
	1-100	kg ha ⁻¹
XR11004VS	15 a ^c	3420 a
TTI11004-VP	11 b	3383 a
Untreated Check	18 a	2933 b

^a FLS disease index calculated from visual ratings 21 days after application

^b Soybean yield collected from entire plot and adjusted to 13% moisture content

^c Means within a column followed by the same letter are not different according to Fisher's Protected LSD ($p < 0.05$).

Table 9. Atomization analysis of XR11004VS and TTI11004-VP nozzles with Quadris Top SB. Volumetric distribution of diameters in which droplets of equal or smaller size delineate 10, 50, and 90% ($D_{v0.1}$, $D_{v0.5}$, and $D_{v0.9}$) of the total spray volume. Spray classification determined in accordance with ASABE S572.1 standards from curves generated using reference nozzles.

Nozzle	Droplet Diameter ^a			Spray Classification ^b
	$D_{v0.1}$	$D_{v0.5}$	$D_{v0.9}$	
		μm		
XR11004VS	131 b ^b	263 b	428 b	Medium
TTI11004-VP	386 a	734 a	1070 a	Ultra Coarse

^a Droplet sizes determined through atomization analysis using laser diffraction in a low-speed wind tunnel, applying a rate of 0.1169 and 0.0735 kg ai ha⁻¹ of azoxystrobin and difenoconazole, respectively, at 276 kPa application pressure resulting in 140 L ha⁻¹ carrier volume

^b Spray classification based on ASABE S572.1

^c Means within a column followed by the same letter are not different according to Fisher's Protected LSD ($p < 0.05$).

Table 10. Interaction effect of jar position x nozzle type on spray coverage from studies evaluating the response of foliar fungicides to droplet size in a commercial application setting. Spray nozzles were determined to produce medium to ultra coarse VMD for XR11004VS and TTI11004-VP, respectively.

Nozzle Type	Jar Position	Fluorescence Intensity
		RFU ^a
XR11004VS	Upper Canopy ^b	212967 a ^c
	Lower Canopy ^d	126105 b
TTI11004-VP	Upper Canopy	220695 a
	Lower Canopy	123863 b

^a Relative fluorescent units of PTSA tracer dye quantified by fluorimeter

^b 100 mm diameter jars arranged in the upper canopy were 90 cm above the soil surface

^c Means followed by the same letter are not different according to Fisher's Protected LSD at $p < 0.05$.

^d 100 mm diameter jars arranged in the lower canopy were 45 cm above the soil surface

Figures

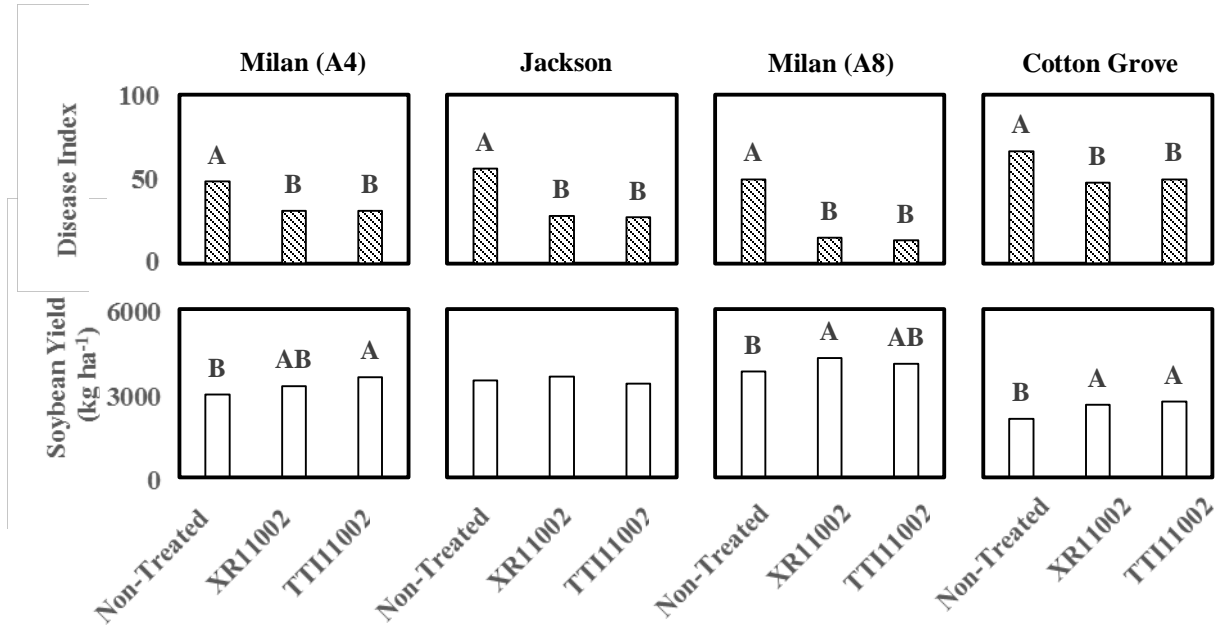


Figure 1. Effect of spray nozzle on Quadris Top SB efficacy. FLS disease index and soybean yield for two spray nozzles applying Quadris Top SB and non-treated control. Fungicide was applied at the R3 growth stage to soybean naturally infested with *C. sojae*. XR11002VS generates a medium VMD, while TTI11002-VP generates an ultra coarse VMD. Visual disease index ratings were assessed 21 days after application with a scale of 1 to 100, with 1 representing no disease and 100 representing total plant failure. The two center rows of each plot were harvested with a plot combine and all yields were adjusted to 13% moisture content, reported in kg ha⁻¹. Each bar represents four replicates within each location. Bars labeled with the same letter are not different according to Fisher's Protected LSD at $p < 0.05$.

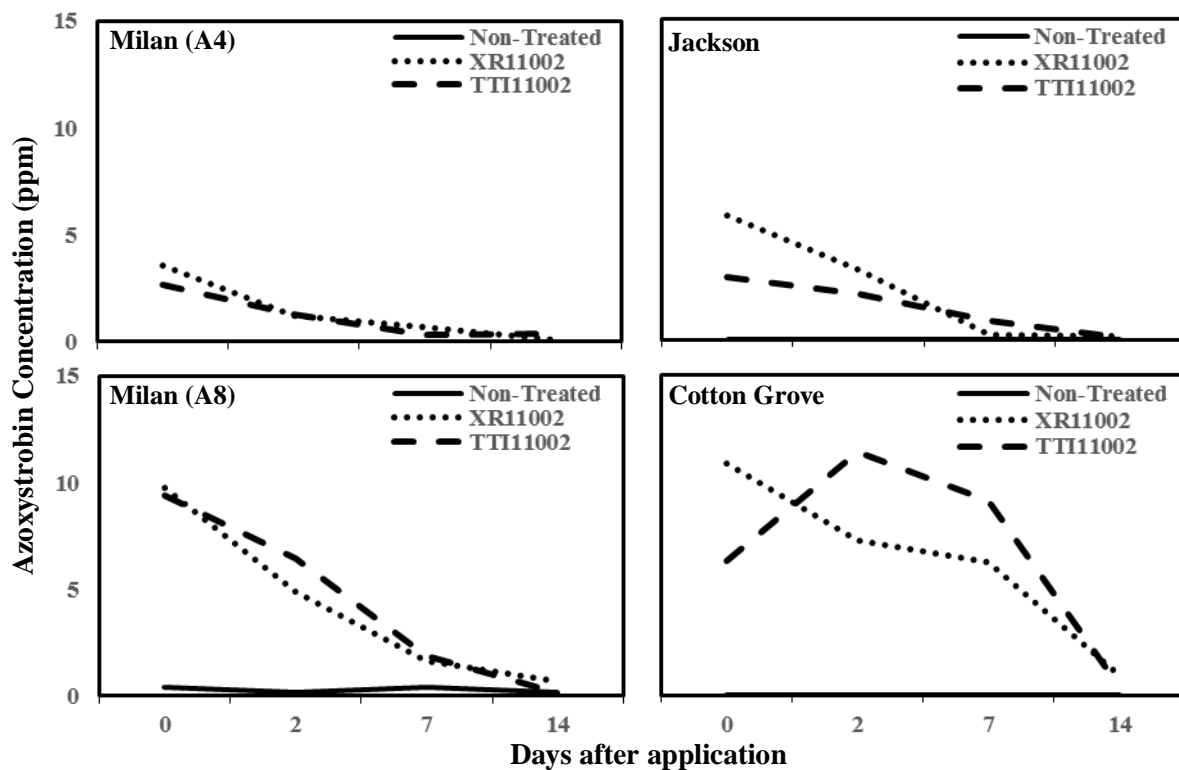


Figure 2. Effect of spray nozzle on azoxystrobin concentration. Azoxystrobin concentration for two spray nozzles and non-treated control. Fungicide was applied at the R3 growth stage to soybean naturally infested with *C. sojae*. XR11002VS generates a medium VMD, while TTI11002-VP generates an ultra coarse VMD. Concentrations were quantified using LC-MS methods. Soybean trifoliates were collected from each plot 0, 2, 7, and 14 days after application. Plotted are back-transformed predicted concentrations for each treatment assessed using repeated measures analyses.

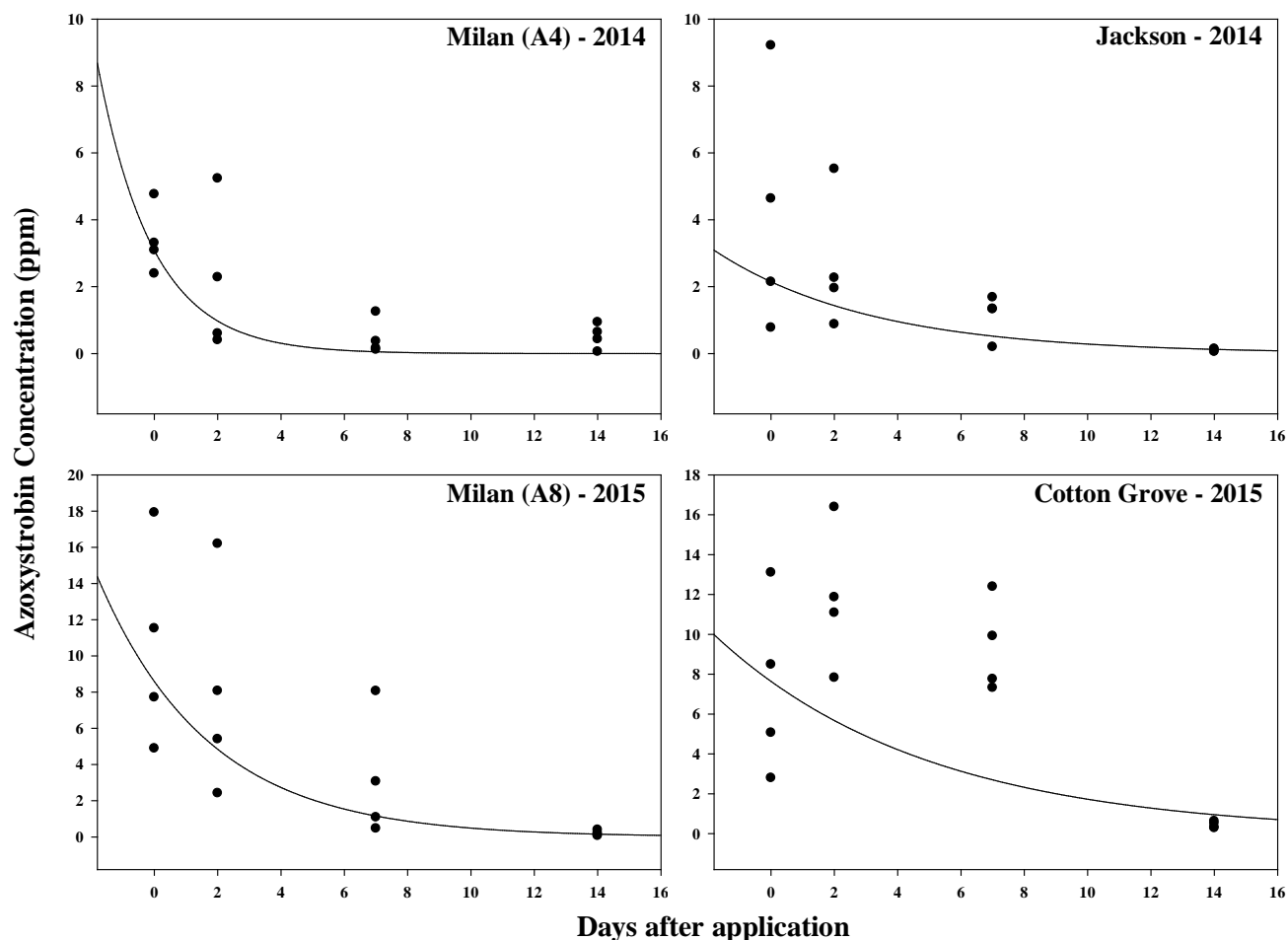


Figure 3. Effect of TTI11002-VP on azoxystrobin concentration residual. Fungicide was applied at the R3 growth stage to soybean naturally infested with *C. sojae*. TTI11002-VP generates an ultra coarse VMD. Concentrations were quantified using LC-MS methods. Soybean trifoliates were collected from each plot 0, 2, 7, and 14 days after application. Dots represent raw concentrations (ppm) for each replication assessed regressed against sampling date after application. $r^2 = 0.53, 0.45, 0.64$, and 0.49 for Milan (Field A4-2014), Jackson, Milan (Field A8-2015), and Cotton Grove, respectively. Fitted lines are calculated from regression models $y = 3.0797 * \exp(-0.5757 * x)$, $y = 2.1487 * \exp(-0.202 * x)$, $y = 8.5877 * \exp(-0.2862 * x)$, $y = 7.6417 * \exp(-0.1487 * x)$, for Milan (Field A4-2014), Jackson, Milan (Field A8-2015), and Cotton Grove, respectively.

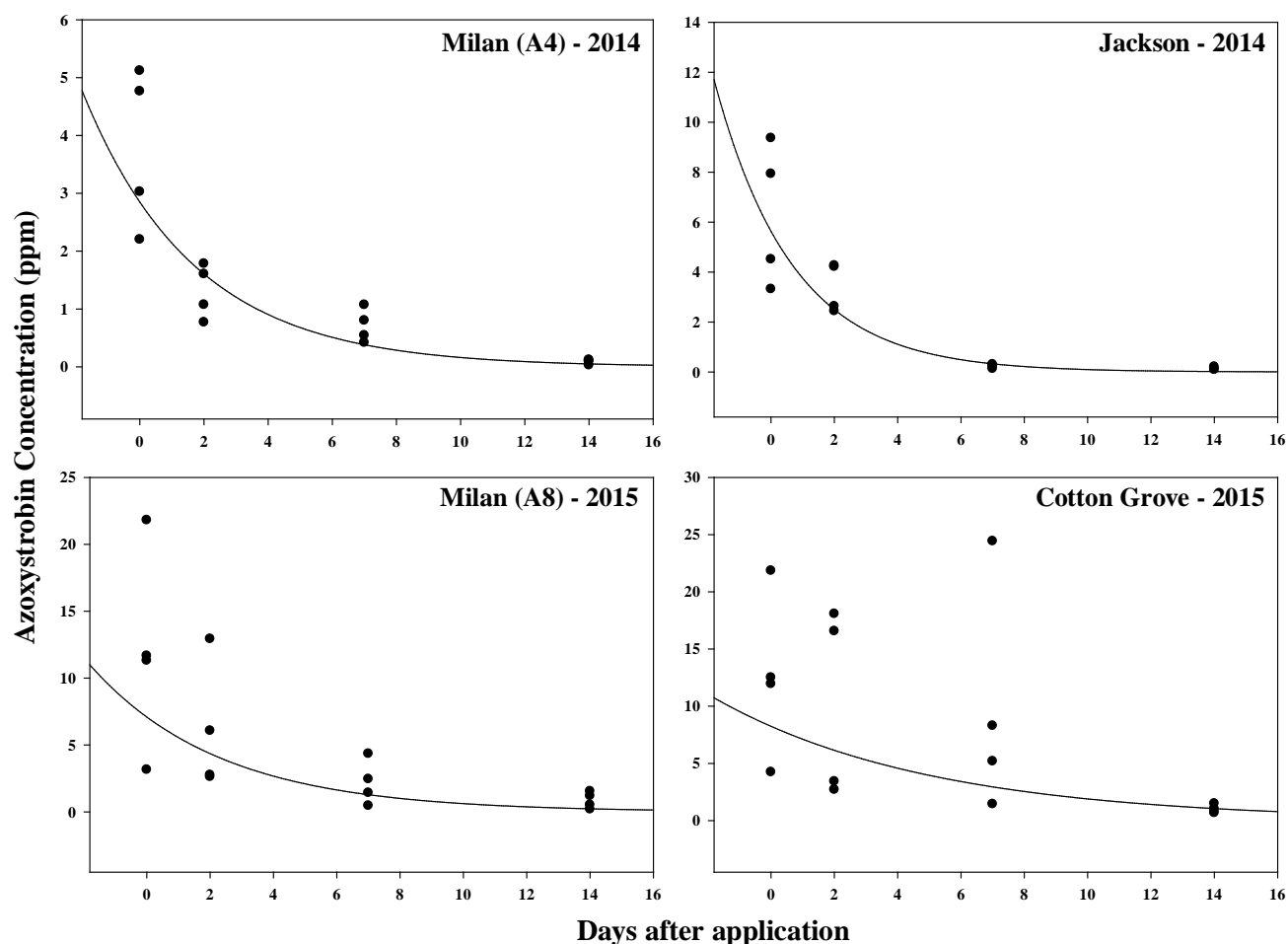


Figure 4. Effect of XR11002-VS on azoxystrobin concentration residual. Fungicide was applied at the R3 growth stage to soybean naturally infested with *C. sojae*. XR11002VS generates and medium VMD. Concentrations were quantified using LC-MS methods. Soybean trifoliates were collected from each plot 0, 2, 7, and 14 days after application. Dots represent raw concentrations (ppm) for each replication assessed regressed against sampling date after application. $r^2 = 0.80, 0.82, 0.50$, and 0.37 for Milan (Field A4-2014), Jackson, Milan (Field A8-2015), and Cotton Grove, respectively. Fitted lines are calculated from regression models $y = 2.8495 * \exp(-0.2867 * x)$, $y = 5.6345 * \exp(-0.4061 * x)$, $y = 7.0942 * \exp(-0.2436 * x)$, $y = 8.2460 * \exp(-0.1467 * x)$ for Milan (Field A4-2014), Jackson, Milan (Field A8-2015), and Cotton Grove, respectively.

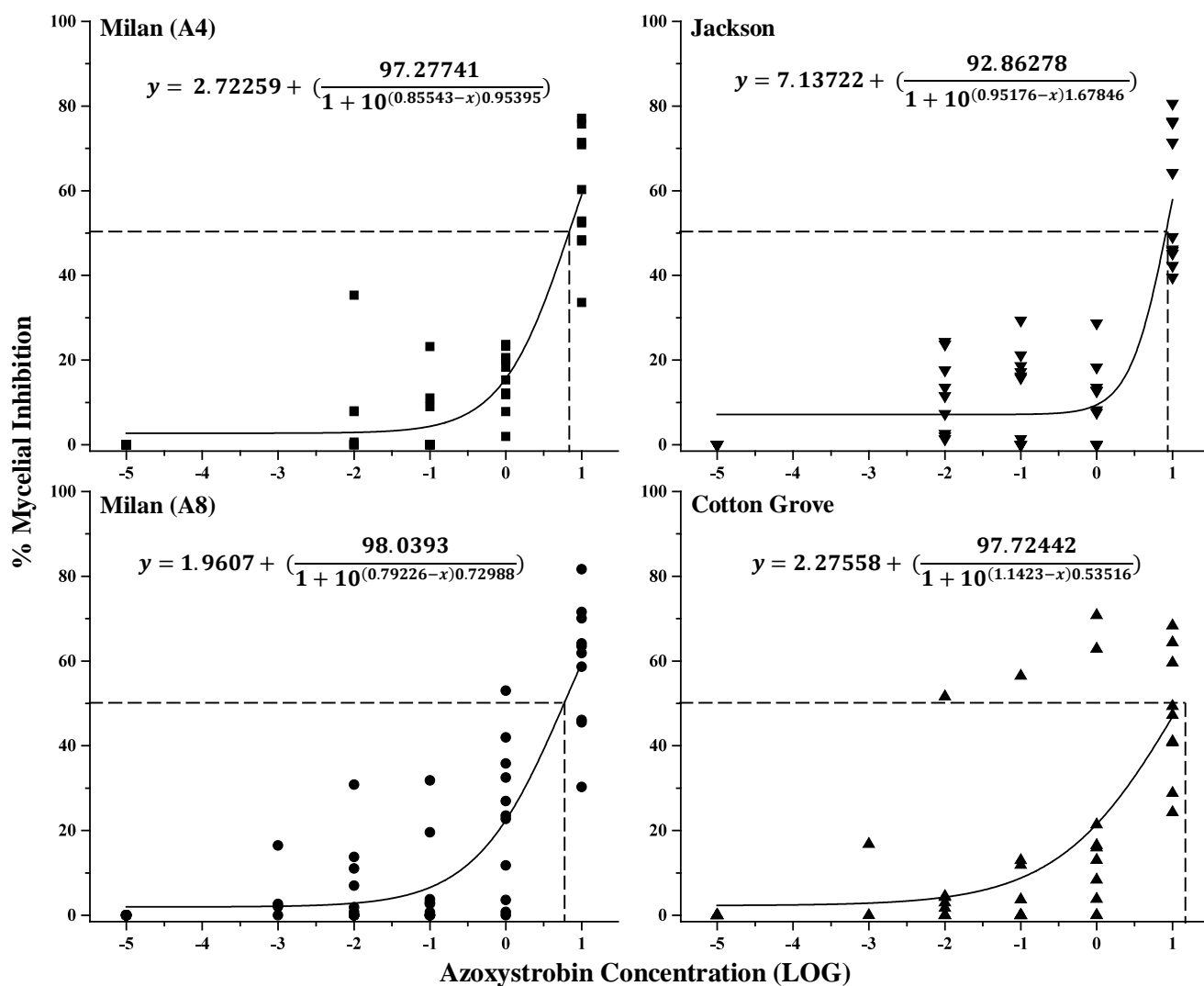


Figure 5. *C. sojae* azoxystrobin dosage response curves. Q_oI-resistant *C. sojae* isolates, distinguished by real-time qPCR methods for 4 locations in West TN, were selected to determine the effective concentration in which 50% of mycelial growth was inhibited (EC₅₀) by azoxystrobin. Mycelial plugs were taken from each isolate and subjected to 6 concentrations including 0, 0.001, 0.01, 0.1, 1, and 10 $\mu\text{g mL}^{-1}$, reported in LOG scale. Each concentration was replicated 10 times per isolate. Inhibition was recorded from 0 to 100%. $r^2 = 0.84, 0.78, 0.77$, and 0.48 for Milan (Field A4-2014), Jackson, Milan (Field A8-2015), and Cotton Grove, respectively. Dotted lines indicate EC₅₀ values determined to equal 7.17, 8.95, 6.20, and 13.88 $\mu\text{g mL}^{-1}$ for Milan (Field A4-2014), Jackson, Milan (Field A8-2015), and Cotton Grove, respectively.

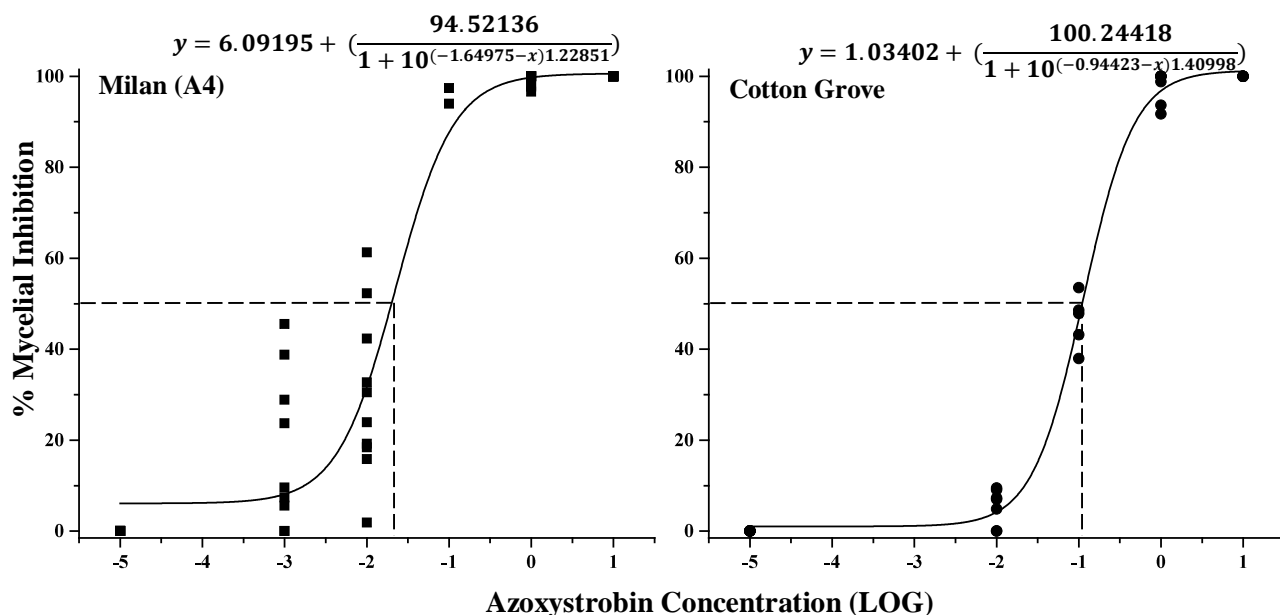


Figure 6. *C. sojae* azoxystrobin dosage response curves. QoI-sensitive *C. sojae* isolates, distinguished by real-time qPCR methods for 2 locations in West TN, were selected to determine the effective concentration in which 50% of mycelial growth was inhibited (EC_{50}) by azoxystrobin. Mycelial plugs were taken from each isolate and subjected to 6 concentrations including 0, 0.001, 0.01, 0.1, 1, and 10 $\mu\text{g mL}^{-1}$, reported in LOG scale. Each concentration was replicated 10 times per isolate. Inhibition was recorded from 0 to 100%. $r^2 = 0.93$, and 0.99 for Milan (Field A4-2014), and Cotton Grove, respectively. Dotted lines indicate EC_{50} values determined to equal 0.02, and 0.11 $\mu\text{g mL}^{-1}$ for Milan (Field A4-2014), and Cotton Grove, respectively.

Vita

Shawn Alan Butler was born June 26, 1988, in Jackson, TN. He is the son of Jimmy Butler and Debbie Myrick of Jackson, TN. Shawn attended North Side High School where he lettered in football, baseball, and cross country, graduating in May of 2006. He then continued his education at Jackson State Community College, majoring in Pre-Engineering, in the fall of 2006. After his first semester, Shawn was forced to go back to work, where he held positions in car audio and video installation, as well as Engineering Lab Technician with Black and Decker for 3 years. During this time, Shawn got into production agriculture, growing corn, cotton, soybean, and wheat with his uncle. Shawn later landed a student assistantship working for Dr. Larry Steckel, University of Tennessee Extension Weed Specialist, at the West Tennessee Research and Education Center in Jackson, TN. Shawn held this position for four years. In the fall of 2010, Shawn began working on his education again, re-enrolling at Jackson State Community College, majoring in Agriculture Sciences. Shawn completed his Associate of Science in the spring of 2012, and in that same semester also began working on his Bachelor of Science at the University of Tennessee at Martin. In the summer of 2013, while continuing research projects with Dr. Steckel, Shawn held a Sales internship with Sanders Inc. in Ripley, TN. He completed his degree in the spring of 2014, majoring in Agricultural Sciences with a concentration in Row Crop Production. Upon graduation, Shawn continued his education at the University of Tennessee at Knoxville, joining Dr. Heather Kelly's program to pursue his Master of Science degree in Plant Pathology. In the spring of 2015, Shawn formed Farm Specific Technology, LLC., a company started to create innovative solutions to improve food production systems, in which he serves as President and CEO. Shawn has worked diligently within his company to commercialize his patent pending technology the Flex Roller Crimper. Shawn has been honored with numerous awards during his undergraduate and graduate career, including Outstanding Agricultural Science Student, Elmer Counce Memorial Award, and Ed and Gladys Seigel Agriculture Award at the University of Tennessee at Martin, and Charles Wheeler Outstanding M.S. Student Award and Glyn and Lynda Newton Research and Development Award at the University of Tennessee at Knoxville. He has also presented at numerous professional and extension meetings and won awards at the Southern Weed Science Society annual meeting. To date, Shawn is the author of 1 peer reviewed journal

article and 3 non peer reviewed publications. Following the completion of his M.S. degree, Shawn will begin working on his Ph.D. at the University of Tennessee at Knoxville in Dr. Tyson Raper's program majoring in Plant, Soils, and Insects.