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Influence of droplet size and azoxystrobin insensitivity on frogeye leaf spot management in soybean

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ABSTRACT

Field experiments were conducted in 2014 and 2015 to evaluate the influence of droplet size on foliar fungicide efficacy and leaf residue 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 leaf residue 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.

1. 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., 1998, 2008; Bowers and Russin, 1999; Dashiell and Akem, 1991; Akem and Dashiell, 1994). The disease is caused by the fungal ascomycete *Cercospora sojina* K. Hara (Swoboda and Pedersen, 2009; Mian et al., 2008). FLS is a polycyclic disease in which infection, symptom development, and reproduction may all be repeated multiple times throughout a single growing season (Dorrance and Mills, 2010). Yield losses are typically caused by either a reduction in photosynthetic area and/or premature defoliation (Mian et al., 2008; Dashiell and 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 and Akem, 1991). Management strategies include an integrated approach of planting resistant varieties and FLS-free seed, crop rotation to a non-host, burying infected debris through tillage, and treating with fungicides (Heatherly and Hodges, 1998). When utilizing chemical control to manage FLS, applications made between R1 and R5 growth stages have been determined to be the most effective in the prevention or treating of *C. sojina* infection (Grau et al., 2004).

The importance of using an integrated approach to managing FLS increased in 2010 when *C. sojina* isolates recovered from Lauderdale County, Tennessee were determined to be resistant to the Q_OI (strobilurin) fungicide class (Zhang et al., 2012). Q_OI 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 bc_1 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, 1997). Resistance to Q_OI 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, 2015; 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) (Fernández-Ortuño et al., 2008). Zhang et al. (2010) previously found the concentration of azoxystrobin in which 50% of conidial germination was effectively inhibited (EC_{50}) of baseline *C. sojina* isolates ranged from 0.0029 to 0.0323 $\mu g\, mL^{-1}$. Of the 15 *C. sojina* isolates collected from Lauderdale County, Tennessee in 2010, EC_{50} ranged from 2.7826 to 4.5409 $\mu g\ mL^{-}$, approximately 140–959-fold greater than *C. sojina* baseline isolates (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).

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Because of the increase in incidence of strobilurin resistant *C. sojina*, all controllable factors should be emphasized to improve foliar fungicide efficacy. Successful application of disease management products requires 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 (Akesson and Yates, 1986; Etheridge et al., 2001; Feng et al., 2003; Knoche, 1994; Prokop and Veverka, 2003; Ramsdale and Messersmith, 2001). Spray classifications include very fine, fine, medium, coarse, very coarse, extremely coarse, and ultra-coarse as determined from reference nozzles in accordance with ASABE Standard S-572.1 (ASABE, 2009). Akesson and Yates (1986) first determined the 200–400 μm 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 and Messersmith, 2001), resulting in greater effectiveness of contact herbicides (Etheridge et al., 2001; Prokop and 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 and 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. In orchard application with air blast sprayers, Doruchowski et al. (2017) found low-drift nozzles possessed equal biological efficacy in comparison to standard flat-fan nozzles. 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 h 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 and 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 very coarse to reduce the potential of off-target movement (BASF Corporation, 2018; Dow AgroSciences, 2018; Monsanto Company, 2018). The current label for Engenia (BASF), Xtendimax (Monsanto), and Enlist Duo (Dow AgroSciences) restricts growers to using only a short list of drift reducing nozzle types, orifice sizes, and pressure ranges. Along with these application requirements, additional requirements are placed on sprayer ground speeds, wind speed at application, downwind buffers, and the completion of specialized training courses.

Due to the increase in incidence of Q_OI 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. 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.

2. Materials and methods

2.1. 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. sojina* 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 Q_OI-resistant *C. sojina* (Zhang et al., 2012). In 2014, trials were located at the West Tennessee Research and Education Center (Jackson, TN – N 35.630231° W −88.850273°) and the Milan Research and Education Center (Milan, TN – N 35.938454° W −88.715574°). In 2015, trials were located at the Milan Research and Education Center (Milan, TN – N 35.93736° W −88.708795°) and a grower's field in Jackson, TN (N 35.660918° W −88.705494°). 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. sojina*. 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. 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 liquid flowable premix of azoxystrobin (2.87 kg ai L^{-1}) and difenoconazole (1.8 kg ai L^{-1}), 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 flat fan XR11002VS (XR) nozzle was selected to represent an industry recommended standard for fungicide applications, while the TTI11002-VP (TTI), a venturi, turbulence chamber nozzle, was selected to represent a drift-reduction nozzle type that is required to be used on the label of dicamba-tolerant and 2–4,D-tolerant soybean (BASF, 2018; Dow AgroSciences, 2018; Monsanto, 2018), although a smaller orifice size was utilized to accommodate the desired carrier volume and application speed. Four row by 9.14 m plots were arranged in a randomized complete block design with four replications in each location. Treatments were applied to the two center rows once soybean reached the R3 growth stage using a CO_2-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 approximately 1.8 m s^{-1}, resulting in a carrier volume of approximately 140 L ha^{-1}. Mean air temperature, relative humidity, and wind speed was collected using a Kestrel 3000 m (Kestrel Meters, Minneapolis, MN) 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