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Flumioxazin effects on soybean canopy formation and soil-borne pathogen presence

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Research Article

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Abstract

Rapid crop canopy formation is important to reduce weed emergence and selection for herbicide resistance. Field experiments were conducted in 2017 and 2018 in Fayetteville, AR, to evaluate the impacts of PRE applications of flumioxazin on soybean injury, soybean density, canopy formation, and incidence of soil-borne pathogens. Flumioxazin was applied at 0, 70, and 105 g ai ha^{-1} to predetermined flumioxazin-tolerant and -sensitive soybean varieties. Flumioxazin at 70 g ha^{-1} injured the tolerant and sensitive varieties from 0% to 4% and 14% to 15%, respectively. When averaged over flumioxazin rates, density of the sensitive variety was only reduced in 2017 when activation of flumioxazin was delayed 7 d. Compared to the tolerant soybean variety, flumioxazin at 70 g ha^{-1} delayed the sensitive variety from reaching 20%, 40%, 60%, and 80% groundcover by 15, 16, 11, and 5 d, respectively. No delay in canopy closure (95% groundcover) was observed with either variety. Consequently, no yield loss occurred for either variety following a flumioxazin application. Flumioxazin did not impact root colonization of *Didymella, Fusarium, Macrophomina*, or *Rhizoctonia*. *Pythium* colonization of the soybean stem was increased by flumioxazin in 2017, but not in 2018. Increased injury, delays in percent groundcover, and an increase in *Pythium* colonization of soybean following a flumioxazin application may warrant the need for other soil-applied herbicides at soybean planting. Alternatively, soybean injury and delays in percent groundcover following flumioxazin applications can be mitigated through appropriate variety selection; however, comprehensive screening is needed to determine which varieties are most tolerant to flumioxazin.

Introduction

Flumioxazin is a protoporphyrinogen oxidase-(PPO) inhibiting herbicide Group 14 that is used in soybean production for preplant or PRE control of small-seeded broadleaves and annual grass weeds (Taylor-Lovell et al. 2001, 2002; Yoshida et al. 1991). PPO-inhibiting herbicides were used extensively to control *Amaranthus* ssp. before the release of glyphosate-resistant crops (Norsworthy et al. 2012). Following the evolution of glyphosate-resistant *Amaranthus* ssp. the use of PPO-inhibiting herbicides increased (Norsworthy et al. 2012). PPO-resistant Palmer amaranth [*Amaranthus palmeri* (S.) Wats.] was first confirmed in Arkansas in 2015 (Salas et al. 2016). Since then, PPO resistance has been confirmed in seven states (Heap 2019; Varanasi et al. 2018). The evolution and spread of PPO-resistant Palmer amaranth has called into question the utility and importance of these herbicides for weed control in soybean.

Historically, flumioxazin has been used in the mid-south over sulfentrazone, another PPO-inhibiting herbicide, because of lower risk for injury to soybean (Taylor-Lovell et al. 2001). The gene controlling soybean tolerance to sulfentrazone has been determined and was once screened for in most commercialized soybean varieties (Swantek et al. 1998). It has been suggested that flumioxazin and sulfentrazone tolerance in soybean are closely linked but not synonymous; nonetheless, more research is needed to determine the mechanism of soybean tolerance to flumioxazin (Taylor-Lovell et al. 2002). Current commercialized soybean varieties are not screened for flumioxazin tolerance, resulting in uncertainty as to the risk for injury from the herbicide. Two factors that contribute to flumioxazin injury to soybean are varietal sensitivity and the splashing of herbicide onto emerged seedlings. The latter may be more severe when a suspected sensitive variety is grown (Yoshida et al. 1991). Although herbicide injury at high levels can reduce yields (Kapusta et al. 1986), herbicide-induced injury may have alternative effects on soybean production such as delaying canopy formation (Nelson and Renner 2001) and increasing incidence of soil-borne pathogens infecting the seedling plants (Dann et al. 1999).

There is not a good understanding of the adverse effects that flumioxazin-induced herbicide injury has on soybean canopy formation. Soybean canopy formation or light interception by the crop can be measured using digital imagery (Purcell 2000). Light interception of 95% or greater

is considered full canopy closure (Board et al. 1992; Gardner et al. 1985; Harder et al. 2007; Purcell 2000). An increase in soybean population or spatial distribution of soybean increases light interception, promoting early canopy formation (Bertram and Pederson 2004; Harder et al. 2007). Crop canopy development in turn affects weed emergence (Burnside and Moomaw 1977; Chandler et al. 2001; Dalley et al. 2004; Harder et al. 2007; Légère and Schreiber 1989; Nelson and Renner 1997; Nice et al. 2001; Young et al. 2001). An increase in canopy closure decreases weed seed germination by decreasing soil temperature and light quantity and quality that reaches the soil surface (Harder et al. 2007; Jha and Norsworthy 2009; Yelverton and Coble 1991). Changes in crop canopy formation have the potential to impact weed emergence and disease presence by altering environmental conditions surrounding the crop (Jha and Norsworthy 2009; Levene et al. 1998).

PPO-inhibiting herbicides have been shown in the past to affect pathogen presence and disease severity (Dann et al. 1999; Levene et al. 1998; Sanogo et al. 2001). Lactofen, a PPO-inhibiting herbicide, has been found to reduce soybean stem rot [*Sclerotinia sclerotiorum* (Lib.) de Bary] severity by 40% to 60% (Dann et al. 1999). A high level of glyceollin was found in soybean leaves treated with lactofen. It is believed that an increase in glyceollin production caused by lactofen injury to soybean is responsible for the control of soybean stem rot (Dann et al. 1999).

Another PPO-inhibiting herbicide, acifluorfen, increases glyceollin in soybean, resulting in a decline in soybean cyst nematode egg production by 50% to 60% (Levene et al. 1998). This type of interaction between herbicide and pest is considered an indirect response (Duke et al. 2007). An indirect response is when the herbicide causes a physiological change within the plant that increases tolerance or changes the environment to the point that it is unsuitable for the disease.

Flumioxazin and sulfentrazone have the ability to reduce root colonization of root rot (*Pythium arrhenomanes*). Flumioxazin has been shown to reduce in vitro mycelium growth of *P. arrhenomanes* and *P. aphanidermatum* (Daugrois et al. 2005). This type of interaction is described as a direct response of a herbicide on pathogens (Duke et al. 2007). A direct relationship between herbicide and disease is the ability of the herbicide to inhibit growth and reproduction by the compound itself (Duke et al. 2007). Herbicide and disease interactions are complex, requiring the need for additional studies to understand the possible underlying benefits or negative impacts on a cropping system. An area of study that is not thoroughly researched is how early season flumioxazin-induced injury to soybean affects the crop, including incidence of soil-borne pathogens. The objective of this research was to determine whether flumioxazin resulted in delays in soybean canopy development and affected the incidence of soil-borne pathogens.

Materials and Methods

Field experiments were conducted in 2017 and 2018 at the University of Arkansas-Agricultural Research and Extension Center, in Fayetteville, AR. The experiments were planted on June 15, 2017, and May 11, 2018. The soil at the site of the experiment was a Leaf silt loam (Fine, mixed, active, thermic Typic, Albaquults) with 31% sand, 50% silt, 18% clay, 1.4% organic matter, and pH 6.5 and 6.0 on those dates, respectively. In both years, the fields were prepared prior to planting by disking and hipping beds that were 91 cm wide. The plot size was 7.6 m long and 3.6 m wide. The experiment was conducted in adjacent field sites each year. Trials were planted in fields where soybean was grown the previous year to increase the likelihood that soil-borne pathogens were present.

The experiment was designed as a two-factor factorial randomized complete block design with four replications. The factors were soybean variety [Credenz 4818LL and Credenz 4748LL (Bayer CropScience, Triangle Park, NC 27709)] and flumioxazin (Valor 51WG, Valent USA, Walnut Creek, CA 94596) at three rates (0, 70, 105 g ai ha^{-1}). A greenhouse screening was conducted prior to field experimentation to categorize the two indeterminate soybean varieties. The Credenz 4818LL was flumioxazin-tolerant and Credenz 4748LL was flumioxazin-sensitive. Seed treatments were applied to simulate practices commonly used in soybean production. Both varieties of soybean were treated with commercial seed treatments PONCHO®/VOTiVO®, which contains 40.3% clothianidin, 8.1% *Bacillus firmus* I-1582; ILeVO®, which contain 48.4% of fluopyram; and REDIGO® 480, which contains 41% prothioconazole and 28.35% metalaxyl, also commonly known as ALLEGIANCE®-FL. Soybean is commonly categorized into medium, medium bushy, and bushy to correctly describe growing characteristics of varieties. CDZ 4748LL is considered a medium bushy and CDZ 4818LL would be considered bushy. Soybean varieties were seeded at 346,000 seed ha^{-1} at a 2.2-cm depth. The experiments were kept weed free with glufosinate, *S*-metolachlor, and hand-weeding. Visual estimates of soybean injury to flumioxazin were rated 21 d after planting (DAP) on a scale of 0% to 100%, with 0% being no crop injury and 100% being crop death (Frans and Talbert 1977).

To determine soybean canopy formation over time, photos were taken weekly after planting until soybean reached canopy closure with an unmanned aerial vehicle (DJI Phantom 4 Pro equipped with a 1080p gimbal-mounted camera; Shenzhen, China, 518057). Photographs were taken of the whole trial and were divided into plots using the software program Field Analyzer (https://www.turfanalyzer.com/). Field analyzer produced a proportion of green pixels for the center two rows within the four-row treated plot; thus, an accurate representation of percent groundcover could be calculated (Purcell 2000). Canopy height and width of five soybean plants in the center two rows of each plot were also recorded on a weekly basis. The measurements were then averaged by plot, and soybean volume was calculated using the following equation $(\pi \times plant\ hieght)(plant\ width \div 2)^2$ (Norsworthy 2004). Soybean grain yield was determined following physiological maturity by harvesting the center two rows in each plot using a small-plot combine and then adjusting moisture to 13%.

When soybean reached V1 in the nontreated plots, 10 plants were sampled from the outside two rows of the plots that received flumioxazin at 107 g ha^{-1} and from nontreated plots. These plants were dug from the plots, with roots remaining intact, and placed in sterile plastic bags. All samples were placed in a cooler and immediately transported to the laboratory. Individual plants were cut 1.5 cm below and above the soil line, keeping the portion of the soybean plant that contained the soil line. The samples remained grouped by plot and were washed with running water for 20 min. Samples were then soaked in a 6% 87.5 ml L^{-1} bleach dilution for 30 s. Soybean stems were then placed in 100-mm-diameter petri dishes containing agar (part number 97064-336, VWR International, Arlington Heights, IL 60004) for 3 to 4 d. One sample of hyphal growth that differed in morphological characteristics

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within a petri dish was selected and transferred by removal using a flame-sterilized scalpel to petri dishes containing an amended potato dextrose agar medium PDArad (18 g Difco potato dextrose agar, 10 mg and 250 mg of the antibiotics rifampicin and ampicillin, respectively) and the miticide fenpropathrin (0.14 mg ai L^{-1}; Danitol 2.4 EC, Valent Chemical Co. Mahomet, IL 59639). Isolates of similar morphological characteristics were grouped 7 to 8 d after the transfers were made. The number of isolates per group was recorded. Isolates of the same group were randomly selected and sent for DNA analysis at the University of Arkansas Plant Pathology laboratory in Monticello, AR.

Pure cultures of fungi and oomycete isolates were obtained and transferred to the Monticello laboratory using the method described previously. Representative pure cultures of fungi and oomycete were randomly selected from each group for DNA analysis. Deoxyribonucleic acid (DNA) was collected from pure cultures by scraping 0.25 ml to 0.5 ml of mycelia and spores from the tops of colonies using a sterile scalpel blade. Mycelia and spores were placed into a microfuge tube, where 500 µl of 0.9% (w/v) NaCl prepared with sterile distilled water was added. Genomic DNA extractions were obtained by using a Norgen Biotek Genomic DNA Purification kit (Kit 27300, Norgen Biotek Corp., Thorold, ON, L2V 4Y6 Canada). Polymerase chain reaction was achieved by following the Go*Taq* Green Master Mix 2X (Promega Corp., Madison, WI 53711) using a 25-µl reaction and following the accompanying amplification guidelines. Primers used in reactions were internal transcribed spacer-4 (ITS-4; reverse) and ITS-5 (forward; ThermoFisher Scientific, Waltham, MA 02454). Confirmation of amplification was determined by gel electrophoresis, followed by soaking in GelRed (Biotium, Freemont, CA 94538) nucleic acid stain for 20 min, and viewing the gel on an ultraviolet light box. Digestion of excess nucleotides was achieved by using the ExoSAP-IT protocol (catalog number 78201, ThermoFisher Scientific). Quantification of DNA concentrations were achieved by using a microvolume spectrophotometer (SimpliNano, GE Health Care Life Sciences, Logan, UT 84321). Samples were sent premixed to Eurofins Genomics (Louisville, KY 40299) for sequencing following standard protocol. Sequences were trimmed, aligned using ClustalW in Bio-Edit (version 7.0.5, Ibis Therapeutics, Carlsbad, CA 92008), and identified using the nucleotide basic local alignment search tool in GenBank (BLASTn, NCBI, Bethesda, MD 20892).

Statistical Analysis

Data collected for soybean volume and percent groundcover were analyzed similarly. Data were regressed in the Fit Curve platform of JMP 14.1 (SAS Institute Inc., SAS Campus Drive, Cary, NC 27513). A mechanistic curve {$y = a\ [1 - b * \text{EXP}\ (-c*\text{days})]$} where a = asymptote, b = scale, and c = growth was fit to the soybean volume and percent groundcover data by days after planting in a similar manner to that used in other research (SAS Institute 2014). Parameters to fit the mechanistic growth curves are found in Table 1. From the mechanistic curves, inverse predictions of the days until soybean achieved 20%, 40%, 60%, 80%, 95%, and 1,000, 3,000, and 5,000 cm^3 were predicted for percent groundcover and soybean volume, respectively. The 95% confidence interval for the mean of the inverse prediction was used to differentiate herbicide treatment and variety effects.

The percent injury data, collected 21 DAP, were not normally distributed; therefore, injury data were subjected to log transformation, determined by the lambda value of a box cox test (Box and Cox 1964) and back-transformed for data interpretation. Soybean density, pathogen isolates, and yield data relative to the nontreated plants of the same variety passed all assumptions of ANOVA. Site years were analyzed separately due to differences in soybean emergence prior to a rainfall event that activated the herbicide. In 2017, the experiment went 7 d without rainfall, and in that time period, soybean emerged prior to herbicide activation (data not shown), thus impacting the amount of herbicide injury observed. In 2018, flumioxazin was activated by rainfall prior to soybean emergence (data not shown). Means were separated using a Fisher's protected LSD test with an α value of 0.05. P-values for each ANOVA are displayed in Table 2.

Results and Discussion

Soybean Injury

In general, injury levels in 2017 were higher than in 2018 likely because of the 7-d delay in herbicide activation versus the 4-d delay in the second year. In both site years, a significant interaction between variety and flumioxazin rate was observed ($P < 0.0001$ and $P = 0.0002$ in 2017 and 2018, respectively; Table 2). Similarly, the sensitive variety CDZ 4748LL suffered greater injury from flumioxazin than the tolerant CDZ 4818LL (Table 3), further validating that soybean has differing levels of flumioxazin tolerance as hypothesized by Taylor-Lovell et al. (2001). The sensitive and tolerant soybean varieties displayed 14% to 15% and 0% to 4% visible injury, respectively, due to an application of flumioxazin at 70 g ha^{-1}. Injury increased as flumioxazin rate increased. Flumioxazin applied at 105 g ha^{-1} to the sensitive and tolerant varieties caused 21% to 30% and 4% to 8% visible injury, respectively, at 21 DAP. The difference in variety tolerance affected the injury level observed. The delay in activation in 2017 increased the chance for herbicide injury to soybean to occur. Yoshida et al. (1991) concluded that a delay in activation allows for soybean to emerge prior to the herbicide infiltrating the soil surface, resulting in a splashing of herbicide onto emerged soybean when subsequent rainfall occurs. A delay in herbicide activation may be key in determining variety tolerance of soybean to flumioxazin at labeled field use rates. Through knowledge of variety sensitivity to flumioxazin, injury to soybean may be mitigated when activation of the herbicide is delayed.

Soybean Density

Soybean density was not affected by the interaction of flumioxazin rate by variety in 2017 or 2018 ($P = 0.8223$ and $P = 0.4529$, respectively; Table 2). However, the only significant main effect was variety in 2017 ($P = 0.0046$). In 2017, there was a 19% reduction in density of the sensitive soybean variety compared with the nontreated, averaged over flumioxazin rates (Table 4). The tolerant variety showed no reduction in density caused by applications of flumioxazin in either site year (Table 4). In 2018, soybean was planted and then went 4 d without an activating rainfall. Soybean seedlings had not yet emerged at the time of herbicide activation, eliminating the effect of herbicide splash onto soybean as a possible mechanism of stand reduction. Yoshida et al. (1991) observed that a delay in flumioxazin activation until after soybean emergence increased the splashing of herbicide onto cotyledons, resulting in an increase in crop injury. The lack of emerged plants in 2018 compared with the already emerged plants in 2017 at the

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Table 1. Parameters of the mechanistic growth curve {y = *a* [1 – *b* * EXP (-*c**days)]} where *a* = asymptote, *b* = scale, and *c* = growth rate, fit to groundcover and soybean volume data from 2017 and 2018.

			Parameters			
Response variable	Variety	Flumioxazin rate	Asymptote	Scale	Growth rate	R^2
		g ai ha^{-1}				
Soybean groundcover	CDZ 4818LL	0	0.0072	−7.2207	−0.0430	0.9725
		70	−0.0249	2.4583	−0.0380	0.8605
		105	−0.6617	0.9366	−0.0124	0.8698
	CDZ 4748LL	0	−0.0022	19.8330	−0.0441	0.9361
		70	−0.3201	0.9177	−0.0220	0.9456
		105	−0.3210	0.8254	−0.0238	0.9476
Soybean volume	CDZ 4818LL	0	202.7928	−0.1897	−0.1090	0.9245
		70	−280.0561	0.4339	−0.0851	0.9357
		105	−236.4815	0.3662	−0.0908	0.9257
	CDZ 4748LL	0	−146.0814	0.6990	−0.0863	0.9088
		70	−175.2448	0.3166	−0.0971	0.9804
		105	−397.6464	0.2604	−0.0836	0.9214

Table 2. Results of the ANOVA conducted on soybean injury, soybean density, and relative yield are displayed by P-values of all factors initially tested in the analysis.

	Soybean injury		Soybean density		Soybean yield	
Factors	2017	2018	2017	2018	2017	2018
	P-values					
Variety	<0.0001	<0.0001	0.0046	0.2980	0.3388	0.3284
Flumioxazin rate	<0.0001	<0.0001	0.8223	0.8048	0.9052	0.9452
Variety × flumioxazin rate	0.0001	0.0002	0.8223	0.4529	0.3293	0.3856

Table 3. Percent visual estimates of injury to soybean 21 d after planting as influenced by the interaction of flumioxazin rate by varietal tolerance to flumioxazin.

		Soybean injury	
Variety	Flumioxazin rate	2017	2018
	g ai ha^{-1}	%–	
CDZ 4818LL	0	0 d[a]	0 d
	70	4 cd	0 d
	105	8 c	4 c
CDZ 4748LL	0	0 d	0 d
	70	15 b	14 b
	105	30 a	21 a

[a]Within column, means followed by different letters are different according to Fisher's protected LSD test at α = 0.05.

Table 4. Relative soybean density as affected by variety in 2017 and 2018 at Fayetteville, Arkansas.

	Soybean density	
Variety	2017	2018
	% of nontreated[a]	
CDZ 4818LL	106 a[b]	100 a
CDZ 4748LL	81 b	89 a

[a]The nontreated plots of CDZ 4818LL and CDZ 4748LL in 2017 had soybean densities of 276,640 and 298,870 plants ha^{-1}, respectively and in 2018 had soybean densities of 266,760 and 251,000 plants ha^{-1}, respectively.
[b]Within column, means followed by different letters are different according to Fisher's protected LSD test at α = 0.05.

time of flumioxazin activation likely resulted in some plant death by the time of stand count assessments, explaining why the treated sensitive soybean variety had reduced density in 2017 but not in 2018.

Soybean Volume

There was no statistical delay in the number of days soybean required to reach the selected soybean volumes in both the sensitive and tolerant varieties (Table 5). Soybean has the ability to increase branching when soybean population per area is reduced (Shibles and Weber 1965), which may explain the lack of effect on soybean volume in 2017 for the sensitive variety. Also, measuring only five plants per plot may have made it difficult to detect subtle differences in canopy volume between treatments.

Percent Groundcover

In the two site years within this study, early-season groundcover of the sensitive soybean was delayed by a flumioxazin application (Table 6). An application of flumioxazin at 70 g ai ha^{-1} increased the number of days required for sensitive soybean to reach 20%, 40%, 60%, and 80% groundcover by 15, 16, 11, and 5 d, respectively. No delay in canopy formation was observed in the tolerant variety following a flumioxazin application at 70 or 105 g ha^{-1}. Additionally, flumioxazin did not affect the time (days) to 95% groundcover in either the sensitive and tolerant variety. Thus, flumioxazin applied to a sensitive or tolerant variety will not delay canopy closure; canopy formation could be delayed when

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Table 5. The number of days predicted for soybean to reach a volume of 1,000, 3,000, and 5,000 cm^3. Differences between treatments occur when the 95% confidence intervals of the mean do not overlap.

Variety	Flumioxazin	Soybean volume	Predicted	Confidence limits
	g ai ha^{-1}	cm^3	days[a]	lower, upper[b]
CDZ 4818LL	0	1,000	28	25, 31
	70		28	25, 30
	105		29	27, 32
	0	3,000	39	38, 40
	70		39	38, 40
	105		40	39, 41
	0	5,000	44	43, 45
	70		44	43, 45
	105		45	44, 46
CDZ 4748LL	0	1,000	28	26, 30
	70		31	29, 33
	105		31	29, 33
	0	3,000	40	39, 41
	70		42	41, 43
	105		42	41, 43
	0	5,000	45	44, 47
	70		47	44, 48
	105		47	45, 49

[a]The number of days for the soybean to reach the predicted soybean volume (cm^3)
[b]The 95% confidence interval of the true (population mean) number of days for soybean to reach each predicted soybean volume

Table 6. The number of days predicted for soybean treated with flumioxazin at 0, 70, and 105 g ai ha^{-1} to reach 20%, 40%, 60%, 80%, and 95% groundcover.

Variety	Flumioxazin	Groundcover	Predicted[a]	CI of mean[b]
	g ai ha^{-1}	%	days	lower, upper
CDZ 4818LL	0	20	21	18, 23
	70		22	19, 25**
	105		24	21, 27**
	0	40	34	31, 37
	70		35	32, 38**
	105		38	35, 41**
	0	60	47	43, 50
	70		48	44, 51**
	105		50	47, 54**
	0	80	59	56, 63
	70		60	56, 63**
	105		61	58, 65**
	0	95	69	64, 73
	70		68	64, 72
	105		69	65, 73
CDZ 4748LL	0	20	25	22, 28
	70		40	34, 46* **
	105		41	36, 46* **
	0	40	40	37, 44
	70		56	52, 60* **
	105		55	51, 59* **
	0	60	53	50, 56
	70		64	62, 67* **
	105		64	61, 66* **
	0	80	65	62, 67
	70		70	68, 72* **
	105		69	68, 71* **
	0	95	72	68, 76
	70		73	72, 75
	105		73	71, 75

[a]The number of days for soybean to reach the predicted percent groundcover (%).
[b]The 95% confidence limits of the number of days required for soybean to reach the predicted percent groundcover.
*Designates the confidence limits of a treatment not overlapping with the nontreated of the same variety and groundcover (%).
**Shows significant differences due to nonoverlapping confidence intervals of same treatment and same percent groundcover between varieties.

flumioxazin is applied to a sensitive variety, which could increase weed emergence. Similarly, Nelson and Renner (2001) observed soybean injury following a POST herbicide application delayed leaf area index, soybean growth, and development. A delay in soybean canopy formation is undesirable, as it may lead to an increase in weed emergence (Jha and Norsworthy 2009). In turn, this rise in weed emergence increases selection for herbicide resistance (Norsworthy et al. 2012).

Soybean Yield

The interaction of variety by flumioxazin rate was not significant in 2017 or 2018 ($P = 0.3293$ and $P = 0.3856$, respectively; Table 2). Likewise, the main effects in 2017 and 2018 of soybean variety ($P = 0.3388$ and $P = 0.3284$, respectively) and flumioxazin rate ($P = 0.9052$ and $P = 0.9452$, respectively) did not affect soybean yield (Table 2). Similarly, Taylor-Lovell et al. (2001) did not observe yield loss in 15 soybean varieties treated with flumioxazin at 105 g ha^{-1}, even when 59% injury was observed soon after emergence.

Pathogen Response

In 2018, soybean root colonization of pathogens was not affected by an application of flumioxazin application rate. *Macrophomina*, a possible causal agent of charcoal rot in soybean (Khan 2007), was found to be influenced by variety selection in both 2017 and 2018 ($P = 0.0132$ and $P = 0.0196$, respectively; Table 7). Thus, soybean varietal tolerance to *Macrophomina* colonization may be present as noted in a previous study (Pearson et al. 1984). In 2017, both variety and flumioxazin rate affected the degree of soybean stem colonization by *Pythium*. *Pythium* is the causal agent for root rot in soybean (Hendrix and Campbell 1973). The flumioxazin-tolerant soybean variety (CDZ 4818LL) had an average of 0.67 isolates of *Pythium* per 10 soybean plants, and the sensitive variety had 1.77 isolates per 10 plants (data not shown). Flumioxazin increased the likelihood of *Pythium* colonizing the stems of soybean. The nontreated averaged 0.46 isolates of *Pythium* per 10 plants, whereas isolates found in plots treated with flumioxazin increased to an average number of isolates of 1.94 per 10 plants (data not shown). The increase of *Pythium* is contrary to in vitro studies that showed that flumioxazin has a direct effect on reducing mycelium growth of *Pythium* (Daugrois et al. 2005).

It was hypothesized that the splashing of flumioxazin on to soybean stems near the soil line results in necrotic wounds, which allowed for an increase in *Pythium* colonization. It does not appear that flumioxazin increased glyceollin in soybean to compensate for the injury at levels similar to those caused by POST application of lactofen and acifluorfen (Dann et al. 1999; Levene et al. 1998). Soybean injury from flumioxazin also resulted in delays in growth, as observed in the percent groundcover data (Table 5). Delays in growth caused by environmental stresses can contribute to an increase in root rot severity (Kirkpatrick et al. 2006). Thus, the delay in soybean growth resulting from flumioxazin-induced injury could have contributed to an increase in *Pythium* colonization.

Conclusions and Practical Implications

Preemergence flumioxazin injury had season-long effects on the growth of a sensitive soybean variety, and full recovery was not achieved until late in the season when soybean approached canopy

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Table 7. The effects of variety, flumioxazin rate, and the interaction of variety × flumioxazin rate on the incidence of soybean root colonization of soil-borne pathogens.

Year	Factors	Pathogen groups						
		Fusarium	*Pythium*	*Macrophomina*	*Didymella*	*Rhizoctonia*	Outcast	Total
		P values						
2017	Variety	0.6393	0.0413	0.0132	na[a]	0.7796	0.0778	0.0897
	Flumioxazin rate	0.7011	0.0112	0.1134	na	0.6613	0.3305	0.4849
	Variety × flumioxazin rate	0.7809	0.0852	0.3593	na	0.6613	0.9897	0.4477
2018	Variety	0.2562	0.1671	0.0196	0.8665	0.5216	0.6279	0.8582
	Flumioxazin rate	0.5477	0.1139	0.3027	0.5199	0.5425	0.6312	0.9748
	Variety × flumioxazin rate	0.8774	0.8405	0.9344	0.1867	0.4495	0.8991	0.5254

[a]Abbreviation: na, not applicable.

closure. Although the herbicide injury to soybean did not impact yield, other monetary and cultural aspects may be directly affected. Delaying canopy formation of the sensitive variety by 15 d would expose the weed seedbank to environmental conditions that were conducive for emergence, thus potentially increasing the need for additional weed control measures. Increased weed emergence through reducing crop competitiveness via herbicide-induced injury to soybean may place added selection for herbicide resistance on POST herbicides.

Flumioxazin injury to soybean can be mitigated through tolerant varietal selection; however, large-scale flumioxazin variety screening is needed to make this practical. Soybean root colonization of *Didymella, Fusarium, Macrophomina*, and *Rhizoctonia* were not affected by an application of flumioxazin, but *Pythium* colonization of soybean roots was increased when flumioxazin was applied in one of two years. The necrotic wounding following a delayed activation of flumioxazin, as observed in 2017, may lead to an increase in *Pythium* colonization. For soybean varieties that are sensitive to flumioxazin, the risk for crop injury, delayed canopy formation, and increased disease incidence likely outweighs any weed control benefit from the herbicide, especially in areas infested with PPO-resistant *Amaranthus* spp.

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