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Impact of fluopyram fungicide and preemergence herbicides on soybean injury, population, sudden death syndrome, and yield

Yuba R. Kandel[a], Daren S. Mueller[a], Travis Legleiter[b], William G. Johnson[b], Bryan G. Young[b], Kiersten A. Wise[b,*]

[a] Department of Plant Pathology and Microbiology, Iowa State University, Ames 50011, United States
[b] Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907-2054, United States

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ABSTRACT

Field experiments were conducted in Indiana and Iowa in 2014 and 2015 to examine the effect of preemergence herbicides and fluopyram seed treatment on soybean (*Glycine max* (L.) Merr.) injury, plant population, sudden death syndrome (SDS; *Fusarium virguliforme* O'Donnell & T. Aoki), and yield. Sulfentrazone + cloransulam-methyl + S-metolachlor and flumioxazin + chlorimuron ethyl + S-metolachlor herbicides resulted in higher phytotoxicity at growth stage VC-V1 compared to a non-treated control. Phytotoxicity due to preemergence herbicide was rarely observed at V4. Seed treated with fluopyram resulted in higher phytotoxicity at VC-V1 than seed without fluopyram, regardless of preemergence herbicide treatment. The combination of preemergence herbicide and fluopyram did not increase the severity of soybean injury in any year or location compared to either applied alone. Preemergence herbicide treatment reduced plant population in Indiana in 2014 and Iowa in 2015 compared to the non-treated control, but did not affect yield. Fluopyram seed treatment reduced foliar symptoms of SDS by over 70% and increased yield up to 12% in Indiana, but had no effect on SDS or yield in Iowa. These results indicate that while injury can occur with both preemergence herbicides and fluopyram-treated seed, phytotoxicity is not more severe when both pesticides are used together, and yield is not reduced by their use. Farmers should continue to use preemergence herbicide programs if they treat their seed with fluopyram to manage SDS, and use production practices that minimize the risk of preemergence herbicide injury in soybean.

1. Introduction

Sudden death syndrome (SDS) of soybean (*Glycine max* (L.) Merr.) caused by the fungus *Fusarium virguliforme* O'Donnell & T. Aoki, is an annual threat in the Midwestern United States, and can cause yield loss of up to 80% in susceptible varieties (Roy et al., 1997). Symptoms of SDS include interveinal chlorosis and necrosis on the upper trifoliates, which usually appears during the reproductive growth stages of the soybean plant. Although symptoms are often first observed in the foliage, the fungus is soil-borne and infects shortly after seedlings germinate (Gongora-Canul and Leandro, 2011), producing a fungal toxin that is translocated to the foliar tissue, resulting in the characteristic symptoms of SDS (Pudake et al., 2013).

Sudden death syndrome is best managed using an integrated approach, since no single management tactic is 100% effective in years where environmental conditions favor disease. High soil moisture and low temperatures (15 °C) favor root rot, while high soil moisture (rainfall 12–15 cm/month) with moderate temperatures (approximately 25 °C) during reproductive stages favors the foliar symptoms of SDS (Kandel et al., 2016b; Scherm and Yang, 1996) Therefore, farmers are encouraged to manage SDS by using cultivars with genetic resistance, managing soybean cyst nematode (SCN), which has been shown to increase SDS severity (Westphal et al., 2014; Xing and Westphal, 2006), and through cultural practices such as crop rotation (Rupe et al., 1997) and tillage (Wrather et al., 1995). In addition to these cultural practices, the fungicide fluopyram (ILeVO®, Bayer CropScience, Research Triangle Park, NC) was registered in December 2014 on soybean, and has reduced SDS in several research trials across the Midwest and Ontario, Canada (Kandel et al., 2016a, b). Fluopyram is a succinate dehydrogenase inhibiting fungicide (SDHI; Fungicide Resistance Action Committee (FRAC) group 7) and moves systemically from the seed into the cotyledon and first true leaves of the soybean plant (J. Riggs, personal communication). This "pooling" of the fungicide can cause a phytotoxic response in the outer tissue of the cotyledon

* Corresponding author. Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907-2054, United States.
E-mail address: kawise@purdue.edu (K.A. Wise).

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Fig. 1. Phytotoxicity of fluopyram seed treatment in soybean seedlings.

resulting in a brown to black discoloration on affected tissues. The phytotoxicity is sometimes referred to as the "halo effect," and is typically uniform across fluopyram-treated seed (Fig. 1). Shortly after the release of fluopyram, anecdotal observations by farmers and those in the agribusiness industry suggested that the phytotoxicity associated with fluopyram on soybean seedlings was more severe in fields where certain preemergence herbicides were applied to soybeans, and in some instances the combined injury was reported to have reduced plant population. This apparent synergism between the two chemicals is concerning to farmers, particularly due to the increase in preemergence herbicides for broadleaf weed control.

The prevalence of weed species resistant to postemergence soybean herbicide products such as glyphosate, acetolactate synthase inhibitors (Group 2 - ALS-inhibitors), and protoporphyrinogen oxidase inhibitors (Group 14 – PPO inhibitors) has increased across the primary soybean production areas of the United States (Heap, 2016). Currently, 16 weed species have evolved resistance to glyphosate in the United States (Heap, 2016). Control of herbicide resistant broadleaf weeds has become a major challenge in soybean production. In order to control herbicide resistant weeds, especially problematic species such as Palmer amaranth (*Amaranthus palmeri* S. Wats), waterhemp (*Amaranthus tuberculatus* (Moq.) J.D. Sauer), and marestail (*Conyza canadensis* (L.) Cronquist), farmers rely on preemergence herbicides. These herbicides reduce weed seedling populations and the need for multiple postemergence herbicide applications (Ellis and Griffin, 2002; Legleiter et al., 2009; Whitaker et al., 2010). For example, preemergence followed by postemergence herbicide applications resulted in greater control of glyphosate-resistant waterhemp through a growing season compared to postemergence applications alone (Sarangi et al., 2017).

Although preemergence herbicides are needed for weed control on soybeans, there are several application factors that increase the risk for these products to cause herbicide injury to soybean. Certain preemergence herbicide active ingredients can cause injury when they are applied in conditions when the soybean seedling is unable to rapidly metabolize the herbicide, such as in wet conditions (Taylor-Lovell et al., 2001). Injury can also occur if the application is delayed after planting and preemergence herbicides are applied close to soybean emergence. Other factors that can increase risk of injury include shallow planting, or inadequate soil to seed contact (row closure) as these factors increase the risk of contact between the herbicide and the germinating seed.

The soil conditions that favor risk of preemergence herbicide injury, such as cool, wet soil after planting and at emergence, are also the conditions that favor infection by *F. virguliforme*, meaning that farmers with fields at high-risk for SDS may choose to use fluopyram seed treatment, and need to understand the potential risk for soybean injury or loss from using preemergence herbicide applications along with fluopyram seed treatment. The objectives of this study were to examine the effect of common preemergence herbicide programs on soybean injury, stand, and final yield for seed treated with and without fluopyram, and determine if fluopyram + preemergence herbicides results in a synergistic phytotoxic effect on soybean seedlings.

2. Materials and methods

2.1. Field experiments in Indiana

Field experiments were established at the Pinney Purdue Ag Center (PPAC) in LaPorte County, Indiana (41.4431028, −86.9294834) in 2014 and 2015. Experiments were arranged as a randomized complete block design with four replications each year. Treatments consisted of a factorial arrangement of seed treatment by preemergence herbicide program. Each year, a cultivar moderately susceptible to SDS was selected for planting (Pioneer 92Y60 (SDS rating 4, 1 = worst, 9 = best) in 2014 and Beck's 278R4 (SDS rating 7, 1 = worst 9 = best) in 2015) and treated with either a commercial base seed treatment (CB) containing a combination of prothioconazole + penflufen + metalaxyl (EverGol® Energy, Bayer CropScience, 0.019 mg a.i./seed), metalaxyl (Allegiance®, Bayer CropScience, 0.02 mg a.i/seed), and clothianidin + *Bacillus firmus* (Poncho®/Votivo™, Bayer CropScience, 0.13 mg a.i/seed) or fluopyram standard rate (ILeVO®, Bayer CropScience, 0.15 mg a.i./seed) in addition to the CB. Preemergence herbicide treatments were applied to all experimental plots treated with both CB and fluopyram + CB seed each year. Preemergence herbicide treatments are listed in Table 1. The treatment of flumioxazin + chlorimuron ethyl + S-metolachlor is not a labeled application of these products, because of the known crop injury risk. This herbicide combination was intentionally included to attempt to injure soybeans and assess the effect of injury on the interaction between fluopyram and preemergence herbicide treatments.

Table 1
Preemergence herbicide active ingredients, common names, rate, and herbicide groups for treatments applied to experimental plots in 2014 and 2015 in Indiana and Iowa.

Active ingredient	Trade name	Rate (g/ha)	Herbicide group (s)	Test location and year
Flumioxazin + chlorimuron ethyl + S-metolachlor	Valor® XLT + Dual II Magnum	128 + 188	2, 14, 15	Indiana – 2014, 2015
Flumioxazin + pyroxasulfone	Fierce®	188 (Indiana)	14, 15	Indiana – 2014, 2015
		224 (Iowa)		Iowa – 2014, 2015
Metribuzin	Sencor®	425	5	Iowa – 2014
Metribuzin + chlorimuron ethyl + metribuzin	Canopy + metribuzin	450 + 425	2, 5	Indiana – 2014, 2015
Saflufenacil + dimethenamid-P	Verdict®	256 (Indiana)	14, 15	Indiana – 2014, 2015
		622 (Iowa)		Iowa – 2015
Sulfentrazone + chlorimuron- ethyl	Authority® XL	561	2, 14	Iowa – 2014
Sulfentrazone + cloransulam-methyl	Authority® First	196	2, 14	Iowa – 2015
Sulfentrazone + cloransulam-methyl + S-metolachlor	Authority® First + Dual II Magnum	159 + 188	2, 14, 15	Indiana – 2014, 2015

Prior to planting, fields were tilled with a field cultivator on May 30, 2014 and May 1, 2015. In both years, the previous crop was corn. Soybean seeds were planted at a rate of 340,00 seeds/ha with a 76-cm row spacing on June 3 in 2014 and 2015. Experimental plots were four rows wide, and 9 m long. The two center rows were used for evaluation. Each plot was inoculated at the time of planting with sorghum seed colonized by a local isolate of *F. virguliforme*. Inoculum was prepared using a previously established method (de Farias Neto et al., 2006) and applied to the soil at a rate of 4.1 g/m. Preemergence herbicide treatments were applied on June 5, 2014 using a CO_2 pressurized backpack sprayer and a hand-held boom fitted with four TJ-8001VS nozzles spaced 45 cm apart, which delivered 140 L/ha at 275 kPa. Control plots that received no preemergence herbicide treatment were included in each experiment. In 2015, preemergence herbicides were applied on June 4 using a self-propelled sprayer equipped with a compressed air system fitted with six TJ-VS 8002 nozzles spaced 45 cm apart, which delivered 140 L/ha at 275 kPa. Post-emergence weed control was achieved by applying lactofen (Cobra, Valent U.S.A. LLC, Walnut Creek, CA) at a rate of 175 g/ha with NIS at a rate of 0.25% v/v on July 22 at the R1 growth stage in 2014 and lactofen at a rate of 105 g/ha plus quizalofop-p-ethyl (Assure II, Dupont, Wilmington, DE) at 15.6 g/ha on July 15 in 2015.

Phytotoxicity was rated in all treatments at the VC growth stage (Fehr et al., 1971) on June 19, 2014, and June 24, 2015. Phytotoxicity was rated using a 1 to 5 scale, where 1 referred to no injury, and 5 referred to plant death. Additional phytotoxicity ratings were performed at V4 on July 11, 2014, and July 21, 2015. Plant population (stand) data was assessed at V3 on July 3, 2014 and July 15, 2015. Soybean population was calculated by counting the total live plants in 3.2 m of each of the two center rows of each experimental plot. Data were converted to plants/hectare prior to analysis.

Foliar SDS levels were rated at growth stage R5-R6 each year, which corresponded to September 5, 2014 and September 22, 2015. Disease ratings consisted of rating foliar disease incidence and severity. Disease incidence was recorded as the percentage of symptomatic plants in the center two rows of each plot, and severity was rated using a previously developed 0–9 scale where 0 = no disease and 9 = premature plant death. The foliar disease index (FDX) was calculated using the following equation: FDX = disease incidence x disease severity/9.

The center two rows of each plot were harvested using a Kincaid 8-XP (Kincaid Equipment Manufacturing, Haven, KS) small-plot combine on November 3, 2014 and October 19, 2015. Yield data were calculated with corrections for moisture content and yields were adjusted to 13% moisture.

2.2. Field experiments in Iowa

In Iowa, field experiments were performed at Iowa State University Research Farms in Boone (42.0092284, −93.7821937) and Story County (42.0586289, −93.6163745) in 2014 and 2015, respectively. Experiments were laid out in a factorial randomized complete block design with four replicates. Treatments consisted of seed treated with a commercial base (CB; described above), or CB + fluopyram standard rate (0.15 mg a.i./seed) and three preemergence herbicides. Preemergence herbicides used each year are listed in Table 1. The same cultivars were used in Iowa as Indiana in each year (Pioneer 92Y60 in 2014 and Beck's 278R4 in 2015). Rating scales were the same as described previously.

Fields were tilled with a cultivator approximately a week before planting. The previous crop was corn in both years. Soybean seeds were planted at the rate approximately 309,000 seeds/ha on June 10 in 2014 and April 29 in 2015. Planting was delayed in 2014 due to rain. (http://mesonet.agron.iastate.edu/request/coop/fe.phtml). Experimental plots were four rows wide with 76-cm row spacing and 5.3 m long. The two center rows were used for evaluation. Each plot was inoculated at the time of planting with sorghum seed colonized by a local isolate of *F. virguliforme* NE 305. Inoculum was prepared using a previously established method (de Farias Neto et al., 2006) and applied to the soil with soybean seed at planting at a rate of 8.3 g of infested sorghum per meter of row.

Preemergence herbicide treatments were applied on June 3, and May 1 in 2014 and 2015, respectively using a self-propelled small research plot sprayer equipped with six flat-fan nozzles (Teejet XR 11015) spaced 51 cm apart, which delivered 140 L/ha at a pressure of 241 kPa. Control plots that received no preemergence herbicide treatment were included in each experiment. Postemergence herbicides glyphosate (Tomahawk® United Suppliers, Eldora IA) with fluthiacet-methyl (Cadet®, FMC Corporation, Philadelphia PA), were applied on July 14 in 2014, when soybeans were at V3-V4 growth stage at a rate of 2.6 L a.i./ha. No postemergence herbicides were applied in 2015. Weeds were removed manually as needed.

Phytotoxicity was rated in all treatments at VE-VC on May 18, 2015. Phytotoxicity was rated using 1-to-5 scale where 1 = no injury and 5 = severe necrosis on new growth. In 2014, soybean population data was recorded at V2-V3 on July 14 by counting the total live plants per 3.2 m on two center rows of each plot. In 2015, plant population data were recorded twice at VE -VC on May 18 in 1 m and on June 1 at V1-V2 in 3.2 m in two center rows of each plot. Data were converted to plants/ha prior to analysis.

Foliar SDS levels were rated at growth stage R5-R6 each year, which corresponded to September 30, 2014 and August 27, 2015. Disease ratings, which included incidence and severity data, were collected for two center rows of each plot as described above and FDX was calculated using the incidence and severity using the equation FDX = disease incidence x disease severity/9.

The center two rows of each plot were harvested using an Almaco small-plot combine (Almaco, Nevada, IA) on October 28, 2014 and September 28, 2015. Yield data were adjusted to 13% moisture.

2.3. Data analysis

Analysis of variance was performed using PROC GLIMMIX in SAS version 9.4 (SAS Institute Inc. Cary, NC) to determine the effect of seed treatment, herbicide application and their interaction on plant population, FDX, and yield. Seed treatment, herbicide application, and their interaction were treated as fixed effects and replication was considered a random effect. Mean separation was performed using Fisher's protected LSD at $\alpha = 0.05$. Because phytotoxicity was scored on ordinal scales, these data were analyzed using non-parametric statistical analysis (Shah and Madden, 2004). Analysis of variance (ANOVA)-type statistics (ATS) (Brunner and Puri, 2001) was used to test the hypothesis. First, data were ranked using PROC RANK and PROC MIXED and macros (Shah and Madden, 2004) were used to get treatment effects and corresponding statistics. Data were analyzed and presented by year and state since herbicide treatments differed in 2014 and 2015, and treatments in Iowa were different than those established in Indiana. Growing conditions and weather varied in 2014 and 2015 in both locations. In Indiana, year × herbicide treatment interaction was significant for plant population ($P = 0.02$) and year × seed treatment interaction was also significant for yield ($P = 0.03$), further justifying the need to analyze data by year.

Although interaction terms were included in the analysis to test the effects of preemergence herbicides on fluopyram-treated seedlings, the interaction term itself does not quantify an observed response as synergistic, antagonistic or additive. Therefore, Colby's analysis used to quantify herbicide interactions was performed to determine if synergistic or antagonistic responses occurred in the experiments. Colby's analysis (1967) was calculated for phytotoxicity and plant population in each state and year. This formula is calculated as: $E = 100-[((100-x) \times (100-y))/100]$, where E is the expected injury or plant population reduction expressed as a percentage of the control, and x represents the injury or plant population reduction as a percentage of the control from

Table 2
Probability of rejecting null hypothesis ($P > F$) observed based on analysis of variance (ANOVA) tests for the effects of fungicide seed treatment (ST), preemergence herbicides (Herbicide) and their interaction on soybean population, Fusarium damage index (FDX) and soybean yield recorded in Indiana and Iowa in 2014 and 2015.

		Phyto VC-V1[a]	PhytoV4	Plant Population	FDX	Yield
Indiana 2014	ST	< 0.01	.	0.74	< 0.01	< 0.01
	Herbicide	< 0.01	.	< 0.01	0.44	0.24
	ST × herbicide	0.95	.	0.98	0.34	0.98
Indiana 2015	ST	< 0.01	0. 41	0.51	< 0.01	0.17
	Herbicide	< 0.01	0.046	0.33	0.85	0.71
	ST × herbicide	0.45	0.55	0.99	0.54	0.07
Iowa 2014	ST	.	.	0.51	0.25	0.92
	Herbicide	.	.	0.64	0.41	0.31
	ST × herbicide	.	.	0.55	0.61	0.53
Iowa 2015	ST	< 0.01	.	0.08	0.18	0.88
	Herbicide	0.28	.	< 0.01	0.12	0.09
	ST × herbicide	0.24	.	0.04	0.91	0.89

[a] Phyto = Phytotoxicity data were analyzing non parametric ANOVA. "." denotes data were not recorded.

fluopyram alone, and y represents the injury or plant population reduction as a percentage of control from the preemergence herbicide treatment alone. Observed vs. expected values for each treatment were compared using *t*-tests in SAS. If the observed response was significantly greater than the expected value at the $P = 0.05$ level, the treatment combining fluopyram and a preemergence herbicide was considered synergistic. If the observed response was significantly less than the expected value, the treatment was determined to be antagonistic. If *t*-tests did not detect a significant difference between observed and expected responses, the treatment combination was considered additive.

3. Results

3.1. Field experiment results in Indiana

Preemergence herbicide treatment affected phytotoxicity at VC-V1 both years of the study (Table 2). No interaction between fungicide seed treatment and preemergence herbicide treatment on phytotoxicity was observed in either year of the study (Table 2). In 2014, flumioxazin + chlorimuron ethyl + S-metolachlor and sulfentrazone + cloransulam-methyl + S-metolachlor resulted in greater phytotoxicity compared to other preemergence herbicide treatments and the non-treated control with 0.75 and 0.74 relative treatment effect, respectively (Table 3). In 2015, flumioxazin + chlorimuron ethyl + S-metolachlor, resulted in the highest levels of phytotoxicity at this growth stage. Metribuzin + chlorimuron ethyl and saflufenacil + dimethenamid-P did not increase phytotoxicity compared to the non-treated control at VC-V1 in either year. None of the preemergence herbicide treatments increased phytotoxicity compared to the non-treated control at V4 in 2014, while in 2015 only flumioxazin + chlorimuron ethyl + S-metolachlor and sulfentrazone + cloransulam-methyl + S-metolachlor had higher phytotoxicity at V4 compared to the non-treated control (Table 3).

Fungicide seed treatment affected phytotoxicity at VC-V1 in both years ($P < 0.01$). At this growth stage, the CB + fluopyram seed treatment resulted in greater phytotoxicity compared to the CB alone (Table 3).

Colby's analysis demonstrated that observed values for phytotoxicity and plant population were not different than expected values in 2014 and 2015, indicating that preemergence herbicide treatments and fungicide seed treatments did not have synergistic or antagonistic relationships in these trials (Table 4).

The preemergence herbicide flumioxazin + chlorimuron ethyl + S-metolachlor reduced plant population by 16% compared to the non-treated control in 2014, but herbicide treatment did not significantly

Table 3
Mean rank (MR) and relative treatment effect (RE) with 95% confidence intervals (CI) for phytotoxicity (Phyto) at VC-V1 and phytotoxicity at soybean growth stage V4 due to seed treatment and preemergence herbicides recorded in field experiments performed in Iowa and Indiana in 2014 and 2015.

Site, year	Effect		PhytoVC-V1[a]			PhytoV4		
			MR	RE	95% CI for RE	MR	RE	95% CI for RE
Indiana, 2014	ST[b]	CB	19.4	0.39	0.33, 0.47	.	.	.
		CB + Fluopyram	29.6	0.61	0.53, 0.67		.	.
	PH[c]	Flumioxazin + chlorimuron ethyl + S-metolachlor	36.4	0.75	0.63, 0.82	.	.	.
		Flumioxazin + pyroxasulfone	30.8	0.63	0.53, 0.72	.	.	.
		Metribuzin + chlorimuron ethyl + metribuzin	16.1	0.32	0.23, 0.45	.	.	.
		Saflufenacil + dimethenamid-P	13.9	0.28	0.19, 0.40	.	.	.
		Sulfentrazone + cloransulam-methyl + S-metolachlor	36.1	0.74	0.55, 0.85	.	.	.
		None	13.9	0.28	0.19, 0.40	.	.	.
Indiana, 2015		CB	18.4	0.38	0.32, 0.47	25.4	0.53	0.46, 0.60
		CB + Fluopyram	30.0	0.62	0.54, 0.69	22.8	0.47	0.40, 0.54
		Flumioxazin + chlorimuron ethyl + S-metolachlor	41.0	0.86	0.76, 0.90	35.4	0.74	0.53, 0.85
		Flumioxazin + pyroxasulfone	28.4	0.59	0.42, 0.74	24.1	0.5	0.35, 0.65
		Metribuzin + chlorimuron ethyl + metribuzin	17.8	0.37	0.25, 0.52	24.1	0.5	0.35, 0.65
		Saflufenacil + dimethenamid-P	16.4	0.34	0.22, 0.49	20.6	0.43	0.31, 0.56
		Sulfentrazone + cloransulam-methyl + S-metolachlor	25.8	0.52	0.37, 0.66	24.9	0.51	0.35, 0.66
		None	15.7	0.32	0.21, 0.48	15.5	0.32	0.26, 0.39
Iowa, 2015		CB	8.5	0.25	.	.	.	.
		CB + Fluopyram	24.5	0.75	.		.	.
		Flumioxazin + pyroxasulfone	18.6	0.57	0.38, 0.72	.	.	.
		Saflufenacil + dimethenamid-P	16.1	0.49	0.31, 0.66	.	.	.
		Sulfentrazone + cloransulam-methyl	16.6	0.50	0.32, 0.68	.	.	.
		None	14.8	0.45	0.31, 0.60	.	.	.

[a] Phytotoxicity was rated using a 1-to-5 scale where 1 referred to no injury, and 5 referred to plant death. "." denotes no data were recorded.
[b] Seed treatment (ST): Commercial base (CB) seed treatment by Bayer CropScience with a combination of prothioconazole + penflufen + metalaxyl (EverGol Energy, 0.019 mg a.i./seed; Bayer CropScience), metalaxyl (Allegiance, 0.02 mg a.i./seed; Bayer CropScience), and clothianidin + *Bacillus firmus* (Poncho/VOTiVO, 0.13 mg a.i./seed; Bayer CropScience); and CB + fluopyram (ILeVO, 0.15 mg a.i/seed; Bayer CropScience).
[c] Details of the preemergence herbicides (PH) with active ingredient and application rate have been provided in Table 1.

Table 4
Effect of fluopyram and preemergence herbicides on soybean phytotoxicity and plant population based on Colby's analysis in field experiments performed in Indiana and Iowa during 2014 and 2015.

State	Year	Treatment[a]	Phytotoxicity[b] (VC-V1) % of control observed	Phytotoxicity (VC-V1) % of control expected	P value	Stand (plants/ha) % of control observed	Stand (plants/ha) % of control expected	P value
Indiana	2014	CB + no preemergence herbicide	100	.	.	100	.	.
		CB + fluopyram	87.5	.	.	100.7	.	.
		CB + flumioxazin + chlorimuron ethyl + S-metolachlor	68.8	.	.	117.7	.	.
		CB + flumioxazin + pyroxasulfone	75.0	.	.	104.6	.	.
		CB + metribuzin + chlorimuron ethyl + metribuzin	100	.	.	105.2	.	.
		CB + saflufenacil + dimethenamid-P	100	.	.	106.9	.	.
		CB + sulfentrazone + cloransulam-methyl + S-metolachlor	68.8	.	.	106.5	.	.
		CB + fluopyram + flumioxazin + chlorimuron ethyl + S-metolachlor	50.0	59.4	.18	121.4	118.6	.62
		CB + fluopyram + flumioxazin + pyroxasulfone	62.5	65.6	.60	103.2	105.8	.76
		CB + fluopyram + metribuzin + chlorimuron ethyl + metribuzin	81.3	87.5	.45	107.9	106.4	.89
		CB + fluopyram + saflufenacil + dimethenamid-P	87.5	87.5	1.00	104.3	107.9	.63
		CB + fluopyram + sulfentrazone + cloransulam-methyl + S-metolachlor	50.0	60.9	.48	108.5	107.8	.93
	2015	CB + no preemergence herbicide	100	.	.	100	.	.
		CB + fluopyram	81.3	.	.	97.3	.	.
		CB + flumioxazin + chlorimuron ethyl + S-metolachlor	62.5	.	.	117.7	.	.
		CB + flumioxazin + pyroxasulfone	78.1	.	.	104.6	.	.
		CB + metribuzin + chlorimuron ethyl + metribuzin	92.2	.	.	105.2	.	.
		CB + saflufenacil + dimethenamid-P	92.2	.	.	106.9	.	.
		CB + sulfentrazone + cloransulam-methyl + S-metolachlor		.	.	106.5	.	.
		CB + fluopyram + flumioxazin + chlorimuron ethyl + S-metolachlor	62.5	50.4	.08	106.2	100.9	.35
		CB + fluopyram + flumioxazin + pyroxasulfone	71.9	64.1	.42	108.3	109.8	.91
		CB + fluopyram + metribuzin + chlorimuron ethyl + metribuzin	76.6	74.9	.58	99.3	100.1	.89
		CB + fluopyram + saflufenacil + dimethenamid-P	78.1	74.8	.39	98.3	101.0	.47
		CB + fluopyram + sulfentrazone + cloransulam-methyl + S-metolachlor	72.9	69.7	.66	99.9	99.2	.86
Iowa	2014	CB + no preemergence herbicide	.	.	.	100	.	.
		CB + fluopyram	.	.	.	99.9	.	.
		CB + flumioxazin + pyroxasulfone	.	.	.	98.8	.	.
		CB + metribuzin	.	.	.	93.6	.	.
		CB + sulfentrazone + Chlorimuron- ethyl	.	.	.	92.6	.	.
		CB + fluopyram + flumioxazin + pyroxasulfone	.	.	.	99.2	85.0	.44
		CB + fluopyram + metribuzin	.	.	.	97.9	94.2	.84
		CB + fluopyram + sulfentrazone + chlorimuron- ethyl	.	.	.	99.5	94.1	.77
	2015	CB + no preemergence herbicide	100	.	.	100	.	.
		CB + fluopyram	75.0	.	.	97.7	.	.
		CB + flumioxazin + pyroxasulfone	99.1	.	.	131.5	.	.
		CB + saflufenacil + dimethenamid-P	100	.	.	105.1	.	.
		CB + sulfentrazone + cloransulam-methyl	100	.	.	96.6	.	.
		CB + fluopyram + flumioxazin + pyroxasulfone	63.3	74.3	< .01[c]	109.6	129.3	.27
		CB + fluopyram + saflufenacil + dimethenamid-P	63.1	74.3	< .01[c]	120	101.4	.09
		CB + fluopyram + sulfentrazone + cloransulam-methyl	66.9	75	.02[c]	84.8	94.8	.34

[a] Seed planted for all treatments received a commercial base seed treatment (CB) containing a combination of prothioconazole + penflufen + metalaxyl (EverGol® Energy, Bayer CropScience, 0.019 mg a.i./seed), metalaxyl (Allegiance®, Bayer CropScience, 0.02 mg a.i/seed), and clothianidin + *Bacillus firmus* (Poncho®/Votivo™, Bayer CropScience, 0.13 mg a.i/seed). Fluopyram was applied at the standard rate (ILeVO®, Bayer CropScience, 0.15 mg a.i./seed) in addition to the CB. Details of preemergence herbicides with active ingredient and application rate have been provided in Table 1.

[b] Phytotoxicity was rated using a 1-to-5 scale where 1 referred to no injury, and 5 referred to plant death. Phytotoxicity was converted to percent of control for Colby's analysis using equation 100- (phytotoxicity rating-1) x 25.

[c] Indicates that the observed value is significantly different than the expected value, according to t-tests.

affect plant population in 2015 (Table 5). Yield and FDX were not affected by herbicide treatment in any year of the study (Table 2). No interaction between fungicide seed treatment and preemergence herbicide treatment was observed in either year of the study for FDX or yield (Table 2).

Fungicide seed treatment did not affect plant population, but did affect FDX in both years ($P < 0.01$), with the CB + fluopyram treatment reducing FDX by 71 and 72% compared to the CB alone in 2014 and 2015, respectively (Fig. 2). Yield was increased by 12% in the CB + fluopyram treatment in 2014 compared to CB alone (Fig. 2).

3.2. Field experiment results in Iowa

In 2014, preemergence herbicide treatment and fungicide seed treatment did not have an effect on stand, FDX, or yield (Table 2). Phytotoxicity data was not recorded for Iowa in 2014.

In 2015, preemergence herbicide treatment had no effect on phytotoxicity ($P = 0.25$), but did affect plant population ($P < 0.001$) (Table 2). Flumioxazin + pyroxasulfone and saflufenacil + dimethenamid-P reduced plant population by 17 and 10%, respectively, compared to the non-treated control (Table 6). The interaction between fungicide seed treatment and preemmergence herbicide treatment for plant population was significant in 2015 ($P = 0.04$). The preemergence herbicide effect was significant for both seed treatments ($P < 0.01$). Flumioxazin + pyroxasulfone and saflufenacil + dimethenamid-P had significantly lower plant population than no herbicide treatment in CB and CB + fluopyram, respectively (data not shown).

Fungicide seed treatment affected phytotoxicity at VC-V1, with the CB + fluopyram treatment resulting in greater phytotoxicity levels than the CB alone in 2015 (Table 2). Preemergence herbicide treatment and

Table 5
Effect of preemergence herbicide treatments on soybean plant population, foliar disease index (FDX) of sudden death syndrome (SDS), and yield in 2014 and 2015 in Indiana.

Herbicide[a]	Least square means					
	Plant population[b] (plants/ha)		FDX		Yield (kg/ha)	
	2014	2015	2014	2015	2014	2015
Flumioxazin + chlorimuron ethyl + S-metolachlor	259,727 b	282,595	7.7	10.0	3025	3447
Flumioxazin + pyroxasulfone	298,386 a	268,438	10.7	12.7	2968	3608
Metribuzin + chlorimuron ethyl + metribuzin	294,302 a	289,946	9.7	13.6	3035	3561
Saflufenacil + dimethenamid-P	293,486 a	289,130	14.2	12.4	2905	3566
Sulfentrazone + cloransulam-methyl + S-metolachlor	288,585 a	289,451	8.7	11.3	3111	3510
None	308,459 a	285,318	9.8	12.5	2788	3628

[a] Details of the preemergence herbicides with commercial trade name and application rate have been provided in Table 1.
[b] Plant population was recorded around soybean growth stage V2 by counting the number of live plants per 3.2 m in two central rows of each plot. FDX of SDS was calculated as follows: FDX = disease incidence × disease severity/9. Disease incidence was estimated as percentage of plants with SDS symptoms per plot and disease severity was scored on a 0-to-9 scale (0 = no disease and 9 = premature death) based on percentage of the chlorotic and necrotic leaf area and defoliation. Yield was adjusted to 13% moisture.

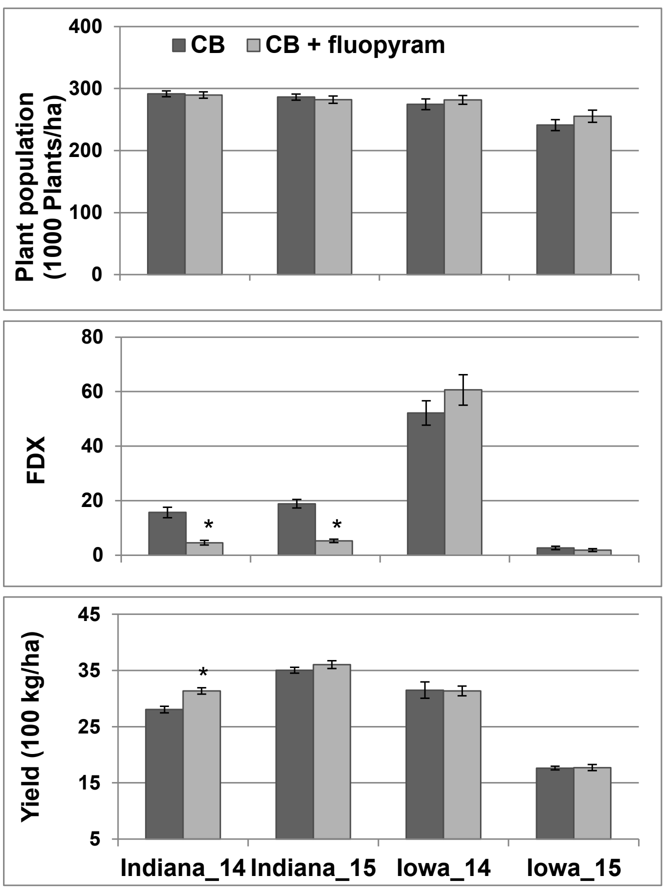

Fig. 2. Effect of fungicide seed treatment on plant population, foliar disease index (FDX) of sudden death syndrome, and yield of soybean recorded in field experiments carried out in Indiana and Iowa during 2014 and 2015. Vertical bar represents the mean for each parameter and lines extending from each bar represent the standard error of mean. Fungicide treatments were commercial base (CB) seed treatment by Bayer CropScience with combination of prothioconazole + penflufen + metalaxyl (EverGol Energy, 0.019 mg a.i./seed; Bayer CropScience), metalaxyl (Allegiance, 0.02 mg a.i./seed; Bayer CropScience), and clothianidin + *Bacillus firmus* (Poncho/VOTiVO, 0.13 mg a.i./seed; Bayer CropScience) and CB + fluopyram (ILeVO, 0.15 mg a.i/seed; Bayer CropScience). Plant population was recorded at soybean growth stage V2 by counting number of live plants per 3.2 m in two central rows of each plot. FDX of SDS was calculated as follows: FDX = disease incidence × disease severity/9. Disease incidence was estimated as percentage of plants with SDS symptoms per plot and disease severity was scored on a 0-to-9 scale (0 = no disease and 9 = premature death) based on percentage of the chlorotic and necrotic leaf area and defoliation. Yield was adjusted to 13% moisture.
*indicates CB + fluopyram treatment was significantly different from CB at α 0.05 level.

Table 6
Effect of preemergence herbicide treatments on soybean plant population, foliar disease index (FDX) of sudden death syndrome (SDS), and yield in 2014 and 2015 in Iowa.

	Herbicides[a]	Least square means Plant population[b] (plants/ha)	FDX	Yield (kg/ha)
2014	Flumioxazin + pyroxasulfone	268,554	49.1	3156
	Metribuzin	288,467	65.6	3179
	Sulfentrazone + Chlorimuron- ethyl	276,627	52.9	3326
	None	278,780	58.0	2914
2015	Flumioxazin + pyroxasulfone	215,812 c	1.1	1659
	Saflufenacil + dimethenamid-P	233,034 c	2.3	1746
	Sulfentrazone + cloransulam-methyl	284,699 a	2.7	1892
	None	259,405 b	3.0	1772

[a] Details of the preemergence herbicides with active ingredient and application rate have been provided in Table 1.
[b] Plant population was recorded around soybean growth stage V2 by counting number of live plants per 3.2 m in two central rows of each plot. FDX of SDS was calculated as follows: FDX = disease incidence × disease severity/9. Disease incidence was estimated as percentage of plants with SDS symptoms per plot and disease severity was scored on a 0-to-9 scale (0 = no disease and 9 = premature death) based on percentage of the chlorotic and necrotic leaf area and defoliation. Yield was adjusted to 13% moisture.

fungicide seed treatment did not affect FDX or yield. The interaction of seed treatment and preemergence herbicide from non-parametric ANOVA was not significant for phytotoxicity at VC-V1 ($P = 0.28$); however, Colby's analysis for all preemergence herbicide combinations with fungicide seed treatments resulted in expected phytotoxicity values that were greater than the observed values, indicating preemergence herbicide treatments had an antagonistic relationship with fungicide seed treatments (Table 4).

4. Discussion

In our study, preemergence herbicides and the seed treatment fluopyram each resulted in greater phytotoxicity at VC-V1 growth stage compared to the non-treated controls. Preemergence herbicide injury, in combination with the "halo effect" caused by fluopyram can result in plants that appear severely injured. However, although plants in our experiments experienced increased injury in certain years and locations, there were no statistical interactions between these two factors, and no synergistic interactions were observed for phytotoxicity through Colby's analysis in any year or location. Therefore, although fluopyram and certain preemergence herbicides may cause phytotoxicity, that phytotoxicity is not consistently worse when these two pesticides are used together, despite anecdotal reports.

Preemergence herbicide treatment did affect soybean population in Indiana in 2014 and Iowa in 2015 independent of seed treatment, with several treatments reducing plant population compared to the non-treated control. Phytotoxic response and reduction in plant population due to preemergence PPO-inhibiting herbicides like flumioxazin and sulfentrazone have been reported, with levels of response dependent on soybean cultivar sensitivity (Dayan et al., 1997; Li et al., 2000; Taylor-Lovell et al., 2001) and environmental conditions (Poston et al., 2008). Despite reducing plant population, these treatments did not reduce yield, and no herbicide treatment had a synergistic effect with fluopyram on yield. Soybean has the ability to compensate for reduced plant population and adjust to the available growing space, which may have resulted in similar yields among treatments, despite some reduction in plant population (Board, 2000; Carpenter and Board, 1997).

Limited research exists on the effect of preemergence herbicides on *Fusarium virguliforme* infection on soybean, although previous studies have found that preemergence herbicides can increase disease severity of other soil-borne diseases, such as Rhizoctonia root rot, caused by *Rhizoctonia solani,* in greenhouse conditions (Bradley et al., 2002). In our study root rot was not assessed to determine if preemergence herbicides increased root injury due to SDS, but results indicate that if root injury occurred, it was not to a level that impacted yield.

This study supports previous findings that FDX is reduced and yield increased by the fluopyram seed treatment in environments conducive to SDS (Kandel et al., 2016a, b). In Indiana, FDX was reduced by 70% and yield increased up to 12% with fluopyram seed treatment. Weather conditions were cool and wet at planting (https://iclimate.org/hourly-purdue-automated), which likely favored disease development However, in environments not conducive to SDS, such as Iowa in 2015, fluopyram had no effect on FDX or yield. In Iowa in 2014, the experiment was planted during the second week of June. Very late planting reduces early stage root infection (Kandel et al., 2016b), which may have affected efficacy of fluopyram seed treatment. Influence of planting date on fluopyram efficacy has been documented in previous reports as well (Kandel et al., 2016b; Vosberg et al., 2017). Experimental plots were flooded for several days in 2015, and recent research has shown that continuous flooding for 5–7 days reduces SDS severity by lowering the *F. virguliforme* population in soil (Abdelsamad et al., 2017).

To conclude, the combination of preemergence herbicide and fluopyram does not increase phytotoxicity or affect yield compared to either applied alone. Our results suggest that farmers do not need to change their preemergence herbicide programs if they choose to treat their seed with fluopyram to manage SDS. However, when planting soybean seed treated with fluopyram, we encourage farmers to use production practices that minimize the risk of preemergence herbicide injury, including planting soybeans at depths of at least 2.54 cm, applying preemergence herbicides 1–2 weeks prior to planting, and planting in soil conditions that maximize seed to soil contact and full furrow closure. In high-risk areas, such as coarse, low organic matter soils, it is recommended to plant soybean cultivars that have been tested for tolerance to preemergence herbicides.

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