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Research Article

Insecticide Seed Treatments Reduced Crop Injury from Flumioxazin, Chlorsulfuron, Saflufenacil, Pyroxasulfone, and Flumioxazin + Pyroxasulfone + Chlorimuron in Soybean

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With increased instances of weed resistance to applications of postemergence herbicides, the use of soil-applied herbicides that offer residual activity is becoming popular. Unfortunately, under some conditions, the use of residual herbicides can result in unintentional injury to crops. However, there are a number of ways to reduce these risks, including the use of in-crop herbicide safeners. Based on previous research conducted on rice, the potential may exist for crop injury from certain soil-applied herbicides to be reduced (safened) in seeds treated with insecticides. Field trials were conducted in Marianna, Arkansas, in 2015 and 2016, and near Colt, Arkansas, in 2016, to explore this possibility in soybean. Soybean seeds were treated with the insecticide thiamethoxam and subsequently the herbicides metribuzin, saflufenacil, pyroxasulfone, sulfentrazone, chlorimuron, flumioxazin, flumioxazin + pyroxasulfone + chlorimuron, mesotrione, and chlorsulfuron were applied immediately after planting. Of the nine herbicides evaluated, the insecticide reduced crop injury for flumioxazin, chlorsulfuron, saflufenacil, pyroxasulfone, and flumioxazin + pyroxasulfone + chlorimuron. The highest degree of injury reduction was seen 1 week after emergence (WAE) at Marianna, where injury from flumioxazin + pyroxasulfone + chlorimuron was reduced from 15% to 5%. Based on the results from this study, the insecticide seed treatment thiamethoxam may have the potential to safen soybean to applications of some soil-applied herbicides.

1. Introduction

Herbicide use in the US is a vital component of agriculture production. Gianessi and Reigner [1] estimate that herbicide use provides a labor equivalent of 70 million hand laborers and increases crop yields as much as 20%. The introduction of herbicide-resistant (HR) crops has also significantly improved the efficiency of crop production, both in the US and globally [2]. Beginning with the introduction of glyphosate-resistant soybean in 1996, the widespread adoption of HR crops provided growers with the ability to effectively control a broad spectrum of weeds by utilizing just one or two postemergence (POST) applications of a herbicide with a single mode of action [3]. Unfortunately, this approach resulted in weeds that were resistant to those control strategies [4]. For example, overreliance upon glyphosate has resulted in glyphosate-resistance in 37 individual weed species since 2000 [5]. In order to effectively combat herbicide resistance, the use of herbicides with residual activity is recommended [6, 7].

Residual herbicides are applied to the soil surface, and depending on climatic, chemical, and soil properties, they can control a broad spectrum of weeds for varying lengths of time [8, 9]. The use of a residual herbicide, as a part of a sequential herbicide program, can increase crop yields as a result of increased weed control compared to programs that do not include a residual component [10, 11]. The residual activity provided by these herbicides typically allows for later applications of POST-applied herbicides and, thus, improved flexibility for crop producers [12]. Apart from being applied by themselves, residual herbicides can be tank mixed with a number of POST-applied herbicides. In these instances, the

POST herbicide controls weeds that have already emerged, whereas the residual herbicide provides lasting control of weeds that have not yet germinated at the time of application. This approach results in high levels of weed control, which can consequently improve crop yield [10].

In addition to providing the obvious benefit of successfully controlling weeds, residual herbicides are also important herbicide-resistance management tools. Because residual herbicides greatly decrease the number of weeds present early in the season, there is decreased resistance selection on POST herbicides in subsequent applications. Reduced selection results in less likelihood for herbicide resistance, which in turn increases the potential lifespan of a given herbicide [6, 13]. Including residual herbicides as part of a tank mixture with POST herbicides results in an increased number of herbicide modes of action (MOA) applied to weeds. Application of multiple effective herbicide MOA is one of the most important methods for delaying the onset of herbicide resistance [6, 14].

Unfortunately, one main drawback associated with the use of residual herbicides is crop injury following application. In some cases, herbicides that are labeled for use in crop can cause injury to young plants. Flumioxazin, sulfentrazone, chlorimuron, *S*-metolachlor, and pyroxasulfone are some examples in soybean production [15, 16]. Crop response to these preemergence (PRE) herbicides can be greatly variable depending upon both soil and environmental conditions, with cool, wet, and low pH conditions causing the most crop injury in soybean following applications of flumioxazin and sulfentrazone [16]. In addition to temperature, moisture, and pH, soil organic matter (SOM) and texture can impact the activity of herbicides to varying degrees, depending upon the herbicide [17, 18]. Aside from environmental effects, varietal selection can cause substantial variation in response to soil-applied herbicides [19]. Early-season injury from herbicides typically dissipates quickly with no adverse effects on crop yield, but in some cases, more severe injury symptoms and stand loss can cause reduced yields [15, 16].

Another concern with residual herbicides is injury to successive crops. Due to their relatively long half-lives, plant-back restrictions are needed for many soil-applied herbicides in order to protect crops in replant situations following crop failure, as well as crops grown the next season [20]. These plant-back restrictions can greatly limit rotational options and can drive growers' decisions on what to plant the following year. One notable example of how crop rotation is directly influenced by herbicide use in the state of Arkansas can be seen in imidazolinone-resistant (Clearfield®, BASF Corporation, Research Triangle Park, NC) rice (*Oryza sativa* L.). Imidazolinone-resistant rice is tolerant to applications of the herbicide imazethapyr, an acetolactate synthase- (ALS-) inhibiting imidazolinone. According to Renner et al. [21], imidazolinones can persist in the soil for as long as two years after their initial application. Grain sorghum, cotton, and conventional rice all have a rotational restriction of 18 months following imazethapyr applications, meaning that rice producers in Arkansas are limited to planting soybean, corn (*Zea mays* L.), or imidazolinone-resistant rice the following season [20].

A possible solution to preventing or reducing the effects of crop injury when using residual herbicides is the use of herbicide safeners. Safeners have been effectively used in crops for both PRE and POST herbicide applications and typically reduce crop injury from herbicides by increasing a plant's ability to metabolize certain herbicidal compounds [22, 23]. Through the use of safeners, crop injury can be reduced such that a herbicide can be used in crops where it would cause unacceptable levels of injury when applied without a safener [24]. One such example can be seen with the herbicide safener fluxofenin (Concep III, Syngenta Crop Protection, LLC, Greensboro, NC), which is already used extensively in grain sorghum production to prevent injury from PRE herbicides. Without a fluxofenin seed treatment, chloroacetamide herbicides such as *S*-metolachlor and alachlor cannot be applied in sorghum production due to high levels of injury to the crop from these herbicides [25]. While the use of safeners has generally been more successful in monocotyledonous crops [26, 27], some examples of safeners do exist in dicots [28]. As such, the potential may exist for novel herbicide safeners to be found in soybean.

Safeners can be applied to the soil, to foliage, or as a seed coating to maximize their efficacy [29]. The benefits of applying herbicide safeners as seed treatments are twofold: injury from herbicides is greatly decreased, and the safener is selectively applied to the crop [24]. Applying the safener only to the crop ensures that safening effects are not conferred to the weeds present in a field, maintaining herbicidal efficacy. This property is highly desirable, and thus, seed-applied safeners have great value. Recently, Miller et al. [30] reported that the insecticide seed treatment thiamethoxam (Cruiser 5S, Syngenta Crop Protection, LLC, Greensboro, NC), in addition to protecting seedling rice from early-season insect damage, also provided a reduction in crop injury following application of some POST herbicides. Although in-plant concentrations of insecticides decrease substantially 3 to 4 weeks after planting [31], enough insecticidal material was still present in the rice at this time to produce a safening effect. Since safening effects were seen even in the case of low thiamethoxam presence, it was hypothesized that similar effects may be seen at crop emergence, when thiamethoxam concentration is much higher in the plant. Thus, research was conducted to determine whether thiamethoxam could be used to reduce crop injury from select soil-residual herbicides in soybean.

2. Materials and Methods

An experiment was conducted at the Lon Mann Cotton Research Station (LMCRS) in Marianna, Arkansas, United States (34 43.4368N, 90 44.0390W), in 2015 to assess the potential for insecticide seed treatments to reduce crop injury following applications of residual herbicides in soybean. In 2016, experiments were repeated at LMCRS, in addition to those conducted at the Pine Tree Research Station

Table 1: General description of experimental sites[a].

Location	Year	Planting date	Application date	Sand	Silt	Clay	pH
					%		
LMCRS	2015	5/14/2015	5/14//2015	0.8	90.5	8.7	7.5
LMCRS	2016	5/5/2016	5/5/2016	0.8	90.5	8.7	7.5
PTRS	2016	5/19/2016	5/19/2016	0.4	78.1	21.5	7.8

[a]LMCRS: Lon Mann Cotton Research Station in Marianna, AR; PTRS: Pine Tree Research Station near Colt, AR.

(PTRS) near Colt, Arkansas, United States (35 06.3584N, 90 56.2437W). DG5067LL (Delta Grow Seed Company Inc., England, AR), a glufosinate-resistant, non-STS, maturity group 5.2 soybean, was planted at a seeding rate of 340,000 seeds ha^{-1} to an approximate 2.5-cm depth. Four-row plots were established utilizing a randomized complete block design with four replications. Row spacings were 96 cm at LMCRS and 76 cm at PTRS, with plot length at all locations of 7.2 m. Plots were managed using agronomic recommendations provided in the University of Arkansas Soybean Production Handbook [32]. The soils at LMCRS and PTRS were a Convent silt loam (fine-silty, mixed, active thermic Typic Glossaqualf) and Calhoun silt loam (coarse-silty, mixed, superactive, nonacid, thermic, Fluvaquentic Endoaquepts), respectively [33]. Prior to planting, all seeds received a fungicide seed treatment of mefenoxam + fludioxonil + sedaxane (Cruiser plus Vibrance, Syngenta Crop Protection, LLC, Greensboro, NC) at a rate of 0.075 + 0.025 + 0.025 g ai kg^{-1} seed. In addition to fungicides, seeds were treated with either no insecticide or thiamethoxam (Cruiser 5S, Syngenta Crop Protection, LLC, Greensboro, NC) at 0.5 g ai kg^{-1} seed. Both fungicide and insecticide seed treatments were made using a water-based slurry. Herbicide applications were made at planting, using a CO_2-pressurized backpack sprayer calibrated to deliver 143 L ha^{-1} at 276 kPa (Table 1). Seven herbicides that are labeled for use in soybean were applied at, or slightly above, their recommended PRE rates to encourage injurious symptomology. These herbicides included metribuzin (841 g ha^{-1}), saflufenacil (75 g ha^{-1}), pyroxasulfone (268 g ha^{-1}), sulfentrazone (533 g ha^{-1}), chlorimuron (79 g ha^{-1}), flumioxazin (107 g ha^{-1}), and chlorimuron + flumioxazin + pyroxasulfone (29 + 108 + 136 g ha^{-1}). In addition, two herbicides that commonly cause injury to soybean via carryover—mesotrione (42 g ha^{-1}) and chlorsulfuron (1.8 g ha^{-1})—were applied at reduced rates to simulate amounts that may be present following applications in the previous growing season.

Following application, visual injury ratings were collected weekly on a 0 to 100% scale, where 0% is no injury and 100% is soybean death. In addition, crop density and height measurements were made three weeks after application to allow for adequate germination across the test. Yield data were collected by harvesting the center two rows of each plot and correcting seed moisture to 13%. Data were subjected to analysis of variance, and significant means were separated using Fisher's protected LSD ($\alpha = 0.05$). Site years were analyzed separately due to considerable variation in environmental conditions at each location (Figures 1–3) and differing responses at each of the sites. For responses that did not produce a significant herbicide by insecticide seed treatment interaction, seed treatment main effects were evaluated. At evaluation timings where no measurable injury was observed for one or more herbicide treatments, the assumptions for ANOVA were not met. When either no interaction was identified, or the response did not meet the assumptions for ANOVA, individual t-tests were conducted to compare treatments with no insecticide to each insecticide seed treatment, within a herbicide.

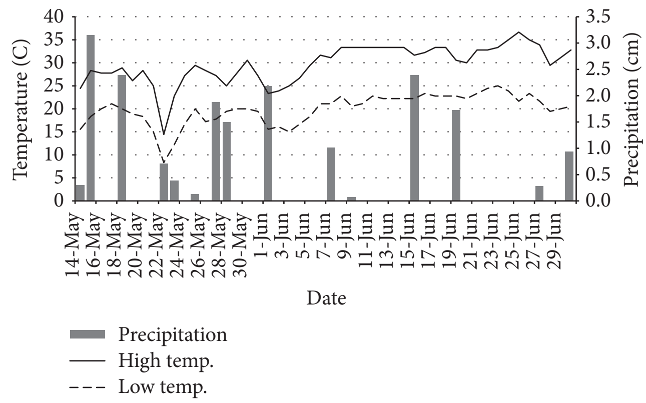

Figure 1: Environmental conditions at the Lon Mann Cotton Research Station in Marianna, AR, in 2015 beginning at planting date May 14.

3. Results and Discussion

Of the nine herbicides evaluated, five showed reductions in injury (safening) in at least one site year. Injury reduction was seen at two site years for flumioxazin and at one site year for chlorsulfuron, saflufenacil, pyroxasulfone, and flumioxazin + pyroxasulfone + chlorimuron. Injury from flumioxazin was reduced at LMCRS (2016) at 1 and 2 weeks after emergence (WAE), where thiamethoxam reduced injury from 13% at both evaluation timings to 8% and 5% at 1 and 2 WAE, respectively (Table 2). Additionally, at PTRS, injury caused by flumioxazin at 2 WAE was reduced from 15% to 8% (Table 3). The highest level of injury reduction occurred at LMCRS (2016), where injury was reduced 1 WAE from 15% to 5% when treated with thiamethoxam (Table 2). Chlorsulfuron

Table 2: Visible soybean injury, density, height, and yield at the Lon Mann Cotton Research Station in Marianna, AR in 2016[a,b].

Herbicide	Seed treatment	1 WAE	Injury 2 WAE %	4 WAE	Density Plants m^{-1} row	Height cm	Yield kg ha^{-1}
None	None	0	0	0	27	8	2520
	Thiamethoxam	0	0	0	27	9	2550
Metribuzin	None	1	2	1	25	8	2540
	Thiamethoxam	1	0	0	26	9	2460
Saflufenacil	None	3	14	6	24	8	2400
	Thiamethoxam	1	14	1	26	8	2730
Pyroxasulfone	None	4	2	0	27	9	2630
	Thiamethoxam	1	3	0	26	8	2610
Sulfentrazone	None	1	14	4	25	8	2650
	Thiamethoxam	1	14	3	25	9	2460
Chlorimuron	None	4	5	6	27	8	2380
	Thiamethoxam	3	4	1	25	9	2540
Flumioxazin	None	13	13	5	24	8	2600
	Thiamethoxam	5*	8*	1	24	8	2480
Chl + Flu + Pyr	None	15	17	6	27	8	2620
	Thiamethoxam	5*	12*	1	25	8	2710
Mesotrione	None	10	4	3	25	8	2740
	Thiamethoxam	7	4	0	26	8	2470
Chlorsulfuron	None	5	7	8	27	8	2690
	Thiamethoxam	2	3*	4	27	8	2480
Main effect[c]	None			4	NS	NS	NS
	Thiamethoxam			1†	NS	NS	NS

[a]WAE: weeks after emergence; NS: nonsignificant; Chl + Flu + Pyr: chlorimuron + flumioxazin + pyroxasulfone; [b]means followed by an asterisk indicate a significant herbicide by insecticide interaction ($\alpha = 0.05$) or a significant injury reduction via insecticide seed treatment, within the same herbicide, compared to no insecticide. [c]Where no significant interaction is present, insecticide seed treatment's main effect is given below and marked with a cross.

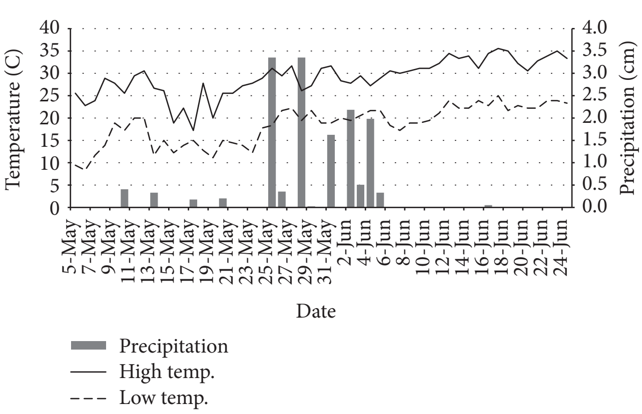

Figure 2: Environmental conditions at the Lon Mann Cotton Research Station in Marianna, AR, in 2016 beginning at planting date May 5.

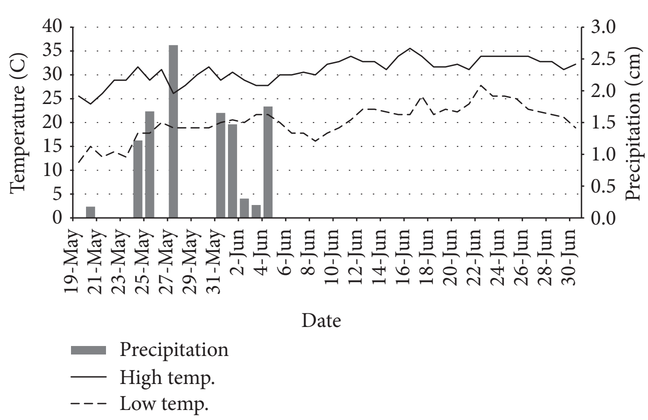

Figure 3: Environmental conditions at the Pine Tree Research Station near Colt, AR, in 2016 beginning at planting date May 19.

injury was reduced 1 WAE at LMCRS (2016) from 7% to 3% (Table 2). Soybean was also safened to saflufenacil at PTRS 2 WAE, where injury was reduced from 22% to 15% (Table 3). Injury from pyroxasulfone was also reduced via a thiamethoxam seed treatment, with injury being reduced at PTRS 1 and 2 WAE, from 13% to 4% and from 14% to 5%, respectively.

Table 3: Visible soybean injury, density, and yield at the Pine Tree Research Station near Colt, AR in 2016[a].

Herbicide	Seed treatment	Injury[b] 1 WAE	2 WAE	4 WAE	Density	Yield
			%		Plants m^{-1} row	kg ha^{-1}
None	None	0	0	0	16	2770
	Thiamethoxam	0	0	0	20	2930
Metribuzin	None	6	9	7	19	2700
	Thiamethoxam	0	6	0	18	3130
Saflufenacil	None	12	22	5	17	2950
	Thiamethoxam	9	15‡	6	18	2780
Pyroxasulfone	None	13	14	6	18	3000
	Thiamethoxam	4‡	5‡	5	17	3210
Sulfentrazone	None	8	13	0	18	3180
	Thiamethoxam	2	8	3	19	3040
Chlorimuron	None	8	10	1	17	2300
	Thiamethoxam	8	7	3	15	2810
Flumioxazin	None	9	15	10	19	3090
	Thiamethoxam	5	8‡	5	19	3170
Chl + Flu + Pyr	None	18	19	6	19	2850
	Thiamethoxam	15	15	5	19	2930
Mesotrione	None	9	9	5	20	2970
	Thiamethoxam	8	5	6	19	3050
Chlorsulfuron	None	3	10	8	20	2860
	Thiamethoxam	6	5	5	19	2730
Main effect	None	9	13	NS	NS	NS
	Thiamethoxam	6†	8†	NS	NS	NS

[a]WAE: weeks after emergence; NS: nonsignificant; Chl + Flu + Pyr: chlorimuron + flumioxazin + pyroxasulfone; [b]where no significant interaction ($\alpha = 0.05$) is present, insecticide seed treatment main effect is given below and marked with a cross. For responses that did not produce a herbicide by insecticide seed treatment interaction, a t-test was conducted to compare treatments with no insecticide to each insecticide seed treatment within an herbicide. Where use of an insecticide seed treatment reduced injury or increased height or yield compared to no insecticide, means are marked with a double dagger (‡).

Injury from metribuzin, sulfentrazone, chlorimuron, and mesotrione was not reduced at any evaluation timing at each of the three locations (Tables 2–4). Similar to studies by McNaughton et al. [15], soybean injury from chlorimuron, flumioxazin, or pyroxasulfone alone was less than injury seen when the three were combined. Aside from a significant seed treatment main effect at LMCRS (2016), where crop height was increased from 47 cm to 50 cm when treated with thiamethoxam, plant height was not affected by seed treatment (Tables 2–4). Additionally, while injury reduction was seen in a number of herbicide-insecticide combinations, crop yield relative to a nontreated check was not increased in these situations (Tables 2–4).

All herbicides evaluated, except for chlorsulfuron and mesotrione, are labeled for use in soybean. As a result, overall soybean injury was low in many cases. Additionally, based on the low levels of injury following application of both metribuzin and sulfentrazone, it is likely that the variety chosen for these studies was tolerant to these herbicides. Choosing a susceptible variety would likely increase crop injury response to these herbicides, which may make the safening benefits associated with insecticide seed treatments more obvious than the ones in this study. In future research, variety selection should be heavily scrutinized in order to select crops that will exhibit high levels of injury.

4. Conclusions

In these experiments, insecticide seed treatments caused significant reductions in soybean injury following applications of flumioxazin, chlorsulfuron, saflufenacil, pyroxasulfone, and flumioxazin + pyroxasulfone + chlorimuron. Because thiamethoxam is a commonly used insecticide seed treatment in soybean production, and all of these herbicides except chlorsulfuron are frequently applied PRE in soybean, it is likely that some growers who use these insecticide/herbicide combinations will likely see reduced early-season injury from these herbicides. Although yield increases were not seen as a result of decreased crop injury in the trials in this study, reduced injury to seedling crops has been shown to result in increased yield in some cases. Future research examining injury reduction from other insecticide seed treatments (aside from thiamethoxam) and PRE herbicide combinations, under a variety of environmental conditions, may show that these yield increases are possible in soybean.

Table 4: Visible soybean injury, density, height, and yield at the Lon Mann Cotton Research Station in Marianna, AR in 2015[a].

Herbicide	Seed treatment	Injury[b] 1 WAE	Injury[b] 2 WAE	Injury[b] 4 WAE	Density	Height[c]	Yield
			%		Plants m^{-1} row	cm	kg ha^{-1}
None	None	0	0	0	21	57	3890
	Thiamethoxam	0	0	0	23	58	3740
Metribuzin	None	2	5	3	19	59	3900
	Thiamethoxam	0	6	3	22	62	3720
Saflufenacil	None	15	29	13	17	51	4040
	Thiamethoxam	14	24	15	18	57	3640
Pyroxasulfone	None	14	24	14	19	53	3650
	Thiamethoxam	10	25	11	22	53	3800
Sulfentrazone	None	24	43	24	15	47	3740
	Thiamethoxam	21	40	21	17	48	3500
Chlorimuron	None	3	6	4	20	38	3790
	Thiamethoxam	1	4	3	23	45	3820
Flumioxazin	None	2	1	3	21	57	3700
	Thiamethoxam	1	0	1	22	61	3770
Chl + Flu + Pyr	None	28	49	39	15	39	3250
	Thiamethoxam	26	48	41	13	41	3290
Mesotrione	None	1	13	3	20	57	3880
	Thiamethoxam	1	9	3	20	58	4050
Chlorsulfuron	None	18	53	83	21	12	1870
	Thiamethoxam	13	51	81	21	12	1350
Main effect	None	12	NS	NS	NS	47	NS
	Thiamethoxam	10†	NS	NS	NS	50†	NS

[a]WAE: weeks after emergence; NS: nonsignificant; Chl + Flu + Pyr: chlorimuron + flumioxazin + pyroxasulfone; [b]where no significant interaction ($\alpha = 0.05$) is present, insecticide seed treatment main effect is given below and a significant main effect is denoted with a cross (†).

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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