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Research Article

Author for correspondence:
Steven M. Martin, Department of Crop, Soil, and Environmental Sciences, University of Arkansas, Fayetteville, AR 72704.
(Email: steven_martin2010@yahoo.com)

Insecticide Seed Treatments Partially Safen Rice to Low Rates of Glyphosate and Imazethapyr

Steven M. Martin[1], Jason K. Norsworthy[2], Robert C. Scott[3], Jarrod Hardke[4], Gus M. Lorenz[5] and Ed Gbur[6]

[1]Graduate Research Assistant, Department of Crop, Soil, and Environmental Sciences, University of Arkansas, Fayetteville, AR, USA, [2]Professor and Elms Farming Chair of Weed Science, Department of Crop, Soil, and Environmental Sciences, University of Arkansas, Fayetteville, AR, USA, [3]Professor, Department of Crop, Soil, and Environmental Sciences, University of Arkansas, Fayetteville, AR, USA, [4]Rice Extension Agronomist, Department of Crop, Soil, and Environmental Sciences, University of Arkansas, Fayetteville, AR, USA, [5]Distinguished Professor and Extension Entomologist, Department of Entomology, University of Arkansas, Fayetteville, AR, USA and [6]Professor, Agricultural Statistics, University of Arkansas, 1366 W Altheimer Drive, Fayetteville, AR

Abstract

Each year there are multiple reports of drift occurrences, and the majority of drift complaints in rice are from imazethapyr or glyphosate. In 2014 and 2015, multiple field experiments were conducted near Stuttgart, AR, and near Lonoke, AR, to evaluate whether insecticide seed treatments would reduce injury from glyphosate or imazethapyr drift or decrease the recovery time following exposure to a low rate of these herbicides. Study I was referred to as the "seed treatment study," and Study II was the "drift timing study." In the seed treatment study the conventional rice cultivar 'Roy J' was planted, and herbicide treatments included imazethapyr at 10.5 g ai ha^{-1}, glyphosate at 126 g ae ha^{-1}, or no herbicide. Each plot had either a seed treatment of thiamethoxam, clothianidin, chlorantraniliprole, or no insecticide seed treatment. The herbicides were applied at the two- to three-leaf growth stage. Crop injury was assessed 1, 3, and 5 wk after application. Averaged over site-years, thiamethoxam-treated rice had less injury than rice with no insecticide seed treatment at each rating, along with an increased yield. Clothianidin-treated rice had an increased yield over no insecticide seed treatment, but the reduction in injury for both herbicides was less pronounced than in the thiamethoxam-treated plots. Overall, chlorantraniliprole was generally the least effective of the three insecticides in reducing injury from either herbicide and in protecting rice yield potential. A second experiment conducted at Stuttgart, AR, was meant to determine whether damage to rice from glyphosate and imazethapyr was influenced by the timing (15, 30, and 45 d after planting) of exposure to herbicides for thiamethoxam-treated and nontreated rice. There was an overall reduction in injury with the use of thiamethoxam, but the reduction in injury was not dependent on the timing of the drift event. Reduction in damage from physical drift of glyphosate and imazethapyr as well as increased yields over the absence of an insecticide seed treatment appear to be an added benefit.

Introduction

Conventional rice is often grown in close proximity to glyphosate-resistant soybean [*Glycine max* (L.) Merr.] and imidazolinone-resistant rice in Midsouth cropping systems. This policy—along with poor herbicide application techniques, especially of glyphosate and imazethapyr —can lead to off-target movement of herbicides onto conventional rice. Several factors determine the severity of the drift event and the concentration of herbicide drift, such as wind speed, distance from targeted area, droplet size, and application method (Smith et al. 2000). Glyphosate drift of 800 m can occur with a 3.46 m s^{-1} wind when applied with an airplane, as opposed to less than 100 m when properly sprayed with a ground sprayer during similar wind speeds (Yates et al. 1978). Depending on rice growth stage, concentration, and herbicide, injury can range from barely noticeable to complete necrosis and plant death (Ellis et al. 2003; Kurtz and Street 2003).

Glyphosate use has increased significantly since the release of glyphosate-resistant crops (Benbrook 2016). Glyphosate is a nonselective systemic herbicide that causes chlorosis followed by necrosis that eventually leads to plant death. Glyphosate inhibits 5-enolpyruvyl-shikimate-3-phosphate synthase, preventing the production of amino acids that are necessary for plant growth (Senseman 2007). Since the introduction of glyphosate-resistant crops in 1996, glyphosate has been primarily used as a POST-applied herbicide to control a wide

range of both broadleaf and grass weeds. The widespread adoption of glyphosate-resistant crops in the Midsouth includes soybean, corn (*Zea mays* L.), and cotton (*Gossypium hirsutum* L.). Adoption of genetically modified rice was never accepted globally, causing other herbicide options to be utilized in rice production.

In rice production, an imidazolinone-resistant line, developed through conventional breeding techniques, has been widely adopted since introduction in 2002 (Croughan 1994; Hardke 2015). The most widespread herbicide used in the imidazolinone-resistant rice is imazethapyr. Imazethapyr is an acetolactate synthase inhibitor that primarily ceases plant production of isoleucine, leucine, and valine (Shaner 1991). Symptomology caused by imazethapyr usually consists of chlorosis in the meristematic region followed by chlorosis and necrosis throughout the plant within 7 to 14 d after exposure (Shaner 1991).

In the southern United States, rice is an important agronomic crop in Arkansas, Louisiana, Mississippi, Missouri, and Texas. These states account for a majority of the rice hectares produced in the United States. Arkansas is the largest producer of rice in the United States with more than 50% of the rice hectares often planted to imidazolinone-resistant varieties (Norsworthy et al. 2013; NASS 2016). Arkansas also ranks 11th in US soybean production, with nearly 1.3 million ha planted in 2015. Nearly 98% of these planted hectares were herbicide-resistant, with most being glyphosate-resistant (NASS 2016).

Glyphosate and imazethapyr drift onto a conventional rice crop can cause adverse effects (Ellis et al. 2003; Kurtz and Street 2003; Hensley et al. 2012). Rice injury up to 94% has been reported from glyphosate at 140 g ae ha^{-1} when applied at the two- to three-leaf growth stage, subsequently leading to a 56% yield reduction (Ellis et al. 2003). The same glyphosate rate applied at panicle differentiation caused no more than 35% visible injury and 31% yield reduction. In another study, a similar rate of glyphosate caused up to 35% injury when applied at panicle initiation and 45% injury when applied at the three- to four-leaf growth stage (Kurtz and Street 2003).

Similar studies have been conducted to determine the effects of imazethapyr drift onto conventional rice. In an experiment evaluating rice response to simulated imazethapyr drift at 1/8 and 1/16 of the 70 g ai ha^{-1} rate, injury was greatest early in the season when the drift event occurred on one-tiller rice, yet yield loss was greatest when simulated drift occurred at the boot stage (Hensley et al. 2012).

Studies have been conducted to determine the effects of low rates of imazethapyr and glyphosate onto rice, and some have determined that thiamethoxam can partially safen rice to glyphosate and imazethapyr drift (Miller et al. 2016); however, further research is needed to understand if safening occurs across insecticide seed treatments. The objective of this research was to determine if three commercially available insecticide seed treatments would lessen rice injury from low rates of glyphosate and imazethapyr exposure and whether possible injury reduction would be influenced by time after planting.

Materials and Methods

Two field studies were conducted in the summers of 2014 and 2015 to determine the effects of glyphosate and imazethapyr drift

Table 1. Planting dates, application dates of herbicides, and permanent flood dates for seed treatment experiment.

Location	Year	Planting date	Application date	Permanent flood
Stuttgart, AR	2014	April 23	May 9	June 6
	2015	May 5	June 2	June 17
Lonoke, AR	2014	May 20	June 5	July 2
	2015	June 8	June 22	July 14

onto conventional rice. The first experiment evaluated different insecticide seed treatments (referred to as the seed treatment study). The second experiment evaluated the timing of rice exposure to low rates of glyphosate and imazethapyr (referred to as the drift timing study).

Seed Treatment Study

The seed treatment study was conducted at the Rice Research and Extension Center located near Stuttgart, AR (34°28′01.39″ N, 91°24′11.74″ W) (hereafter referred to as Stuttgart) and the University of Arkansas Pine Bluff farm located near Lonoke, AR (34°50′54.60″ N, 91°52′56.21″ W) (hereafter referred to as Lonoke). Studies at Stuttgart were conducted on a Dewitt silt loam soil (Fine, smectitic, thermic Typic Albaqualfs), whereas the studies at Lonoke were conducted on a Calhoun silt loam soil (Fine-silty, mixed, active, thermic Typic Glossaqualfs). Plot sizes at Stuttgart and Lonoke were 1.9 by 5.2 m and 1.9 by 7.6 m, respectively. Each plot contained 10 drill rows spaced 19 cm apart and was planted to 'Roy J' rice at 375 seed m^{-2}. Planting dates, herbicide application dates, and permanent flood establishment dates are provided in Table 1. Plots were fertilized according to the University of Arkansas System Division of Agriculture recommendations for both locations (Hardke 2012). Plots were kept weed free throughout the growing season using conventional POST herbicides to avoid any additional injury (Table 2).

In each year at each location, the experimental design was a randomized complete block with a two-factor factorial treatment arrangement with four replications. The two factors were

Table 2. Herbicides used to maintain weed-free plots.

Herbicide trade name	Herbicide common name	Rate	Application Timing[a]	Manufacturer
		g ai ha^{-1}		
Command 3 ME®	Clomazone	340	PRE	FMC Corp., Philadelphia, PA
Facet L®	Quinclorac	280	PRE	BASF Corp., Research Triangle Park, NC
Ricestar HT®	Fenoxaprop	123	MPOST	Bayer CropScience, Research Triangle Park, NC
Clincher®	Cyhalofop	314	LPOST	Dow AgroSciences LLC, Indianapolis, IN
Permit®[b]	Halosulfuron	40	MPOST	Gowan Co., Yuma, AZ

[a]Abbreviations: LPOST, application applied after establishment of permanent flood; MPOST application applied prior to establishing permanent flood; PRE, application applied at planting.
[b]Applied only at the Stuttgart location.

Table 3. Insecticide seed treatments and rates evaluated in seed treatment experiment.

Seed treatment trade name	Insecticide common name	Rate	Manufacturer
		mg g^{-1} seed	
CruiserMaxx Rice®	Thiamethoxam	1.405	Syngenta Crop Protection, Greensboro, NC
NipIt INSIDE®	Clothianidin	0.75	Valent U.S.A. Corp., Walnut Creek, CA
Dermacor X-100®	Chlorantraniliprole	1.0175	du Pont de Nemours and Co., Wilmington, Delaware

herbicide and insecticide seed treatments. The three levels of herbicide treatments were (1) glyphosate (Roundup PowerMax®, Monsanto Co., St. Louis, MO) at 126 g ae ha^{-1} (1/10× rate labeled for glyphosate-resistant soybean), (2) imazethapyr (Newpath®, BASF Corp., Research Triangle Park, NC) at 10.5 g ai ha^{-1} (1/10× rates for labeled for imidazolinone-resistant rice), and (3) a nontreated control (no herbicide). It is difficult to know the exact drift rate with off-target movement of these herbicides under field conditions; however, the drift rates in this research are similar to those evaluated by Bond et al. (2006) for imazethapyr on rice and Hensley et al. (2013) for glyphosate on rice. Other researchers have evaluated even higher rates of glyphosate on rice (Davis et al. 2011; Koger et al. 2005). Herbicide applications were made at the two- to three-leaf (V2 to V3) growth stage (Counce et al. 2000). The four levels of insecticide seed treatments included thiamethoxam, clothianidin, and chlorantraniliprole at labeled rates listed in Table 3, along with no insecticide seed treatment. All treatments (including the no insecticide seed treatment) received a fungicide seed treatment of azoxystrobin at 0.071 mg g^{-1} of seed, mefenoxam at 0.088 mg g^{-1} of seed, and fludioxonil at 0.015 mg g^{-1} of seed.

Rice water weevil (*Lissorhoptrus oryzophilus* Kuschel) counts were taken in each plot 3 wk after the permanent flood was established at both locations for 2015 only. Three 10-cm-diam soil cores were taken from each plot and washed to count the number of rice water weevil larvae in each core.

Drift Timing Study

The drift timing study was conducted in a manner similar to the seed treatment study. The drift timing study was conducted only at Stuttgart in 2014 and 2015 with soil texture, planting dates (Table 4), plot size, and application equipment and setup similar to the seed treatment study. This study was also kept weed free in a manner similar to the seed treatment study.

Table 4. Planting date and application dates of herbicides for drift timing experiment at the Rice Research and Extension Center near Stuttgart, AR.

		Application date		
Year	Planting date	15 DAP[a]	30 DAP	45 DAP
2014	April 24	May 9	May 20	June 3
2015	May 6	May 21	June 5	June 19

[a] Abbreviation: DAP, days after planting application.

Table 5. Main effect of insecticide seed treatment on observable injury, groundcover, and rough rice yield pooled over herbicides (glyphosate and imazethapyr) and the 2014 and 2015 growing seasons near Lonoke and Stuttgart, AR.

Insecticide seed treatment	Injury[a]			Groundcover	
	1 WAT[b]	3 WAT	5 WAT	5 WAT	Yield
	------------%----------			%	kg ha^{-1}
Thiamethoxam	18	27	16	50	9,600
Clothianidin	23	29	23	52	9,490
Chlorantraniliprole	26	37	28	47	9,040
No insecticide	30	39	31	42	8,790
LSD (0.05)[c]	4	9	9	7	510

[a] The "no herbicide" treatment was excluded from the injury evaluations, because no injury was observed; thus, the analysis for injury involved four levels of insecticide seed treatment by two levels of herbicide treatment.
[b] Abbreviation: WAT, weeks after treatment.
[c] Fisher's protected LSD is for comparing means within a column.

In each year, the experimental design was a randomized complete block with four replications. The three factors were seed treatment (two levels), herbicide (three levels), and timing of the herbicide application (three levels), with all combinations completely randomized within each block. All insecticide-treated seed contained thiamethoxam at 1.405 mg g^{-1} of seed (referred to as "treated seed"). All seeds, including the insecticide-treated seeds, were treated with the fungicides azoxystrobin at 0.071 mg g^{-1} of seed, mefenoxam at 0.088 mg g^{-1} of seed, and fludioxonil at 0.015 mg g^{-1} of seed. The seed receiving only the fungicide seed treatments will be referred to as "nontreated seed." Herbicide applications were 15, 30, and 45 d after rice planting (DAP).

Methods Common to Both Studies

All herbicides were applied using a CO$_2$-pressurized backpack sprayer calibrated to deliver 140 L ha^{-1} using a six-nozzle, 2.5-m spray boom, with AIXR 110015 nozzles. Rice injury was assessed visually 1, 3, and 5 wk after the herbicide treatment (WAT) on a scale of 0 to 100, with 0 being no injury and 100 being plant

Table 6. Main effect of herbicide on visible injury, groundcover, and rough rice yield for the seed treatment experiment, pooled over insecticide seed treatments and the 2014 and 2015 growing seasons near Lonoke and Stuttgart, AR.

Herbicide	Injury			Groundcover	
	1 WAT[a]	3 WAT	5 WAT	5 WAT	Yield
	-----------%----------			%	kg ha^{-1}
Glyphosate	27	42	28	45	8,790
Imazethapyr	22	24	21	51	8,940
No herbicide[b]	–	–	–	59	10,000
LSD (0.05)[c]	3	6	6	5	460

[a] Abbreviation: WAT, weeks after treatment.
[b] The "no herbicide" treatment was excluded from the injury evaluations, because no injury was observed; thus, the analysis for injury involved four levels of insecticide seed treatment by two levels of herbicide treatment.
[c] Fisher's protected LSD is for comparing means within a column.

Table 7. Average number of rice water weevil (RWW) larvae found per 10-cm-diam core in 2015 seed treatment studies averaged over experiments near Lonoke and Stuttgart, AR.

Insecticide seed Treatment	Glyphosate	Imazethapyr	No herbicide
		RWW larvae per core	
Thiamethoxam	22	21	9
Clothianidin	16	11	11
Chlorantraniliprole	14	10	8
No insecticide	52	35	19
LSD (0.05)[a]		--------------12--------------	

[a]Fisher's protected LSD is for comparing any two means.

death. Rice groundcover was estimated using Sigma Scan Pro® (Systat Software, Inc., 501 Canal Blvd. Suite E, Point Richmond, CA 94804) by determining the percentage of green pixels in photographs of each plot. Photographs of each plot were taken 5 WAT using a 1.8-m monopod (Purcell 2000). Plots were harvested at maturity using a small-plot combine, and rough rice yields were recorded and adjusted to 12% moisture.

All data for both studies were analyzed in JMP Pro 12 (SAS Institute Inc., Cary, NC). Site-year and replication nested within site-year were included in the model as random effects. Means were separated using Fisher's protected LSD test at $\alpha = 0.05$.

Results and Discussion

Seed Treatment Study

Only rice water weevil numbers had a significant interaction between seed treatment and herbicide. For all other evaluations there was no significant interaction; however, the main effects of seed treatment and herbicide were significant.

Averaged over the glyphosate and imazethapyr herbicide treatments, plants in all insecticide seed treatment plots had at least 18% injury 1 WAT, but injury was less for all insecticide seed treatments than that observed in plots without an insecticide seed treatment (Table 5). At 1 WAT, thiamethoxam safened rice to a greater extent than did clothianidin or chlorantraniliprole. By 3 WAT, rice treated with thiamethoxam and clothianidin (27% and

29% injury, respectively) were both injured less than the rice not treated with insecticide (39% injury). Injury to chlorantraniliprole-treated rice 3 and 5 WAT was comparable to the rice not treated with insecticide. By 5 WAT, rice plants had begun to recover from injury caused by the herbicides, with ranking of insecticide seed treatments similar to earlier ratings. Evaluation of green pixels in photographs taken 5 WAT also revealed a reduction in damage to the crop, as indicated by greater groundcover for thiamethoxam- and clothianidin-treated rice than for plots without an insecticide seed treatment (Table 5). The reduction in early-season damage to rice when seeds were treated with thiamethoxam or clothianidin, pooled over herbicides, translated into a 700 to 810 kg ha^{-1} yield improvement over plots without an insecticide seed treatment that received a low rate of the herbicides (Table 5). In addition to protecting yield, it is likely that the quicker canopy formation caused by the seed treatments would aid weed control, because weed interference is largely a function of the rate of canopy formation (Miller et al. 2016).

The 1/10× rates of imazethapyr (10.5 g ai ha^{-1}) and glyphosate (126 g ae ha^{-1}) had different effects on the rice after application. Overall, glyphosate caused more injury than imazethapyr to the rice at all three ratings (Table 6). Damage to rice from glyphosate at 3 WAT averaged 42% over seed treatments, similar to the levels observed by Hensley et al. (2013) when applied to one-tiller rice. Rice injury was 24% following imazethapyr at 3 WAT averaged over insecticide seed treatments. Injury from glyphosate and imazethapyr seemed to have a direct effect on groundcover 5 WAT (Table 6). Glyphosate, which caused the most injury, resulted in rice having the least groundcover (45%) averaged over insecticide seed treatments, whereas the imazethapyr-treated plots had 51% groundcover. In comparison, the plots that were not treated with herbicide averaged 59% groundcover. Based on previous neonicotinoid research in Asian honey bees (*Apis cerana cerana*) (Ming et al. 2016), we speculate that a possible upregulation of stress genes from the neonicotinoids could explain the lower herbicide injury and an overall healthier rice plant.

Rice water weevil samples were taken for both locations in 2015. Pooled over locations, rice water weevil numbers were greatest when rice was treated with a low rate of imazethapyr or glyphosate in the absence of an insecticide seed treatment (Table 7). All three insecticides performed equally well in reducing rice water weevil numbers. Previous research has found thiamethoxam and clothianidin to provide comparable rice water weevil control (Everett et al. 2015), but these two insecticide seed

Table 8. Effects of application timing and herbicide on observable injury to rice pooled over 2014 and 2015 at Stuttgart, AR.

	Injury				
	Glyphosate	Imazethapyr	Glyphosate	Imazethapyr	
Application timing	-------1 WAT[a]-------		-------3 WAT--------		5 WAT[b]
	—————————————%—————————————				
15 DAP[a]	13	7	31	26	25
30 DAP	35	32	32	34	20
45 DAP	67	39	41	20	38
LSD (0.05)[c]	--------10---------		---------12---------		7

[a]Abbreviations: DAP, days after planting; WAT, weeks after treatment.
[b]Herbicide effect was not significant 5 WAT.
[c]Fisher's protected LSD is for comparing means within a column.

Table 9. The effects of seed treatment on observable injury to rice pooled over 2014 and 2015 at Stuttgart, AR.

Insecticide seed treatment	Injury		
	1 WAT[a]	3 WAT	5 WAT
	----------------%----------------		
Thiamethoxam	29	26	23
No insecticide	35	36	33
LSD (0.05)[b]	6	7	6

[a]Abbreviation: WAT, weeks after treatment.
[b]Fisher's protected LSD is for comparing means within a column.

treatments are sometimes less effective than chlorantraniliprole (Taillon et al. 2018). Research also showing that a decrease in groundcover can cause an increase in rice water weevil larvae (Stout et al. 2009) may explain the high counts in the plots exhibiting the greatest damage in the absence of the insecticide seed treatment.

Drift Timing Study

At 1 and 3 WAT, there was a significant interaction between herbicide and application timing (Table 8). For glyphosate 1 WAT, as application timing was delayed, injury to rice increased, probably because the safening effect of the insecticide seed treatment was most effective soon after planting. However, imazethapyr at 1 WAT caused 39% injury when applied 45 DAP compared to 67% for glyphosate. There was no difference in injury between glyphosate and imazethapyr within application timings of 15 and 30 DAP. At 3 WAT, there were no differences in the timing of glyphosate applications. Imazethapyr applied 45 DAP caused less injury than when applied 30 DAP but was not different from imazethapyr applied 15 DAP. For both herbicides, injury increased from 1 WAT to 3 WAT for the 15-DAP application but stayed nearly the same for the 30-DAP application and decreased for the 45 DAP. At 5 WAT, herbicide effect was no longer significant and only application timing was significant. Applications 45 DAP caused more injury than the 15 and 30 DAP applications.

Seed treatment also played a role in injury to the rice. At all three ratings, plots having the thiamethoxam-treated seed exhibited less injury than those without the insecticide seed treatment (Table 9)—a finding similar to those of other research (Miller et al. 2016).

Groundcover images were taken 5 wk after final treatment for all plots and later converted to percentage of green pixels using Sigma Scan. The main effects of timing and seed treatment had no

Table 10. The effects of reduced herbicide rates on groundcover and rice yield pooled over insecticide seed treatment, application timing, and the 2014 and 2015 growing season at Stuttgart, AR.

Herbicide	Groundcover	Yield
	%	kg ha^{-1}
Glyphosate	53	10,610
Imazethapyr	55	10,810
No herbicide	68	11,670
LSD (0.05)[a]	6	660

[a]Fisher's protected LSD is for comparing means within a column.

effect on groundcover; however, the herbicides applied did have an effect. There was no difference between the herbicides; however, the herbicides did reduce groundcover when compared to plots that did not receive any herbicide. There was a 13 to 15 percentage point decrease in groundcover when the drift rate of either imazethapyr or glyphosate was applied (Table 10).

Similar to groundcover, the only factor that affected yield was the application of imazethapyr or glyphosate. Plots without any herbicide treatment yielded 11,670 kg ha^{-1}, whereas the application of glyphosate and imazethapyr reduced yields to 10,610 and 10,810 kg ha^{-1}, respectively (Table 10).

Rice plants receiving a thiamethoxam seed treatment showed reduced damage from glyphosate and imazethapyr along with some rice water weevil protection. This reduction in injury protected some of the yield potential of rice when the glyphosate or imazethapyr exposure occurred soon after planting. Clothianidin-treated seed reduced injury and provided yield protection in the presence of glyphosate or imazethapyr as well as rice water weevil protection. Chlorantraniliprole provided rice water weevil protection but did not provide significant protection against glyphosate or imazethapyr. It is important to note that the insecticide seed treatments did not completely alleviate the risk for injury from imazethapyr or glyphosate but instead reduced the damage and subsequent yield loss caused by early-season exposure of rice to these herbicides.

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