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Rice tolerance to acetochlor with a fenclorim seed treatment

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Research Article

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Abstract

Rice producers in the United States need effective herbicides to control problematic weeds. Previous research has demonstrated that acetochlor can provide in-season weed control in rice; however, undesirable injury is common. Thus, trials were initiated in 2020 and 2021 to evaluate 1) rice cultivar tolerance to microencapsulated (ME) acetochlor with the use of a fenclorim seed treatment at 2.5 g ai kg^{-1} of seed; 2) a dose-response of a fenclorim seed treatment with ME acetochlor; and 3) rice tolerance to fenclorim and ME acetochlor under cool, wet conditions. For all trials, acetochlor was applied delayed-preemergence (4 to 7 d after planting). In the dose-response trials and in the presence of acetochlor, the fenclorim seed treatment rate of 2.5 g ai kg^{-1} reduced rice injury and increased rice plant heights and shoot numbers relative to acetochlor without fenclorim, and plant heights and shoot numbers were comparable to those of the nontreated control in all evaluations. In the cultivar screening, 14 of 16 cultivars exhibited <20% injury with acetochlor at 1,260 g ai ha^{-1} and fenclorim at 2.5 g ai kg^{-1} 2 wk after emergence (WAE) at the Pine Tree Research Station (PTRS). At the Rice Research and Extension Center (RREC) 2 and 4 WAE and at PTRS 4 WAE, all cultivars exhibited <20% injury with acetochlor and fenclorim. The fenclorim seed treatment in the presence of acetochlor provided comparable rice plant height, shoot numbers, groundcover, and rough rice yield to that of the nontreated control. Under cool, wet conditions, rice injury without fenclorim ranged from 15% to 60% with acetochlor at 1,050 g ai ha^{-1}, whereas injury from acetochlor with the fenclorim seed treatment ranged from 0% to 20%. Based on the results of these experiments, the fenclorim seed treatment appears to safen an assortment of rice cultivars from injury caused by ME acetochlor.

Introduction

Weed control remains one of the primary factors limiting rice production, especially when problematic weeds have developed resistance to available rice herbicides (Barber et al. 2020; Butts et al. 2022; Heap 2022). Three of the five most problematic weeds in Arkansas rice fields include barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv], Amazon sprangletop [*Diplachne panicoides* (J. Presl) A.S. Hitchc], and weedy rice (*Oryza sativa* L.). These grasses can cause potential yield losses up to 82% if left uncontrolled throughout the season (Smith 1988).

Within the mid-southern United States, barnyardgrass has developed resistance to propanil [categorized by the Weed Science Society of America (WSSA) as a Group 5 photosystem II inhibitor), quinclorac (WSSA Group 4, synthetic auxin), clomazone (WSSA Group 13, 1-deoxy-D-xyulose-5-phosphate synthase inhibitor), fenoxaprop/cyhalofop (WSSA Group 1, acetyl-coenzyme A carboxylase inhibitors), and WSSA Group 2 acetolactate synthase (ALS) inhibitors (Heap 2022). Additionally, barnyardgrass resistance to thiobencarb (WSSA Group 8, lipid synthesis inhibitors) has been reported in California rice production. In comparison to barnyardgrass, Amazon sprangletop and weedy rice have been reported to be resistant to only a few herbicides. Amazon sprangletop has developed resistance to cyhalofop and fenoxaprop, while weedy rice has developed resistance to imidazolinone herbicides (ALS inhibitors) through backcrossing with Clearfield® rice (Dauer et al. 2018; Gealy et al. 2015). A survey conducted in 2012 with Mississippi and Arkansas rice consultants rated the "control of herbicide-resistant weeds" as one of the top five concerns, with the need for new effective sites of action for rice producers as the third highest concern (Norsworthy et al. 2013).

Current rice recommendations suggest the use of overlapping residual herbicides to limit the dependence on postemergence herbicides to control weeds prior to flooding (Barber et al. 2020). However, the lack of preemergence residual herbicide options to control weedy rice pressures producers to plant nontransgenic, herbicide-resistant cultivars to allow for control of emerged weedy rice. Even with optimum applications, escapes are inevitable and threaten the durability of current options (Bagavathiannan and Norsworthy 2012). Therefore, mid-southern rice producers need a nontraited residual control option for weedy rice and an alternative site of action for residual barnyardgrass control.

Herbicides that inhibit very long-chained fatty acid elongase (VLCFA) are currently unavailable for use in U.S. rice production but are labeled for use rice production systems in Asia. Recent research has evaluated VLCFA herbicides for use in current rice herbicide programs (Avent et al. 2020; Bertucci et al. 2019; Fogleman 2018; Godwin 2017; Norsworthy et al. 2019). An experiment evaluated acetochlor application timings in rice and determined that undesirable injury occurred at delayed preemergence (DPRE) and spiking timings (Fogleman et al. 2019; Godwin et al. 2018). However, good rice tolerance was observed when applications occurred at the 1- to 2-leaf stage or later. Additionally, microencapsulated (ME) formulations provided better rice tolerance than emulsifiable concentrate formulations of acetochlor (Fogleman et al. 2019).

Acetochlor absorption occurs through root and shoot uptake by germinated seedlings emerging through the soil and provides little to no control of emerged weeds (Babczinski et al. 2012). Consequently, control of emerged weeds with early postemergence applications of acetochlor alone is not possible (Anonymous 2018; Babczinski et al. 2012). A DPRE application timing is ideal for weed control since most rice producers plant to a seedbed with no weeds present. However, Fogleman (2018) and Godwin (2017) have demonstrated the variability in rice tolerance to ME acetochlor at this application timing. Therefore, a herbicide safener seed treatment has been pursued to provide adequate rice tolerance to acetochlor (Avent et al. 2020).

Fenclorim was developed by the Ciba-Geigy company in the 1980s and released as a mixture with pretilachlor, another chloroacetamide herbicide similar to acetochlor (Quadranti and Ebner 1983). Pretilachlor has been widely used to control weeds in rice production systems in Asia when fenclorim is used in conjunction with the herbicide to mitigate injury (Chauhan et al. 2014; Chen et al. 2013). Fenclorim mitigates rice injury from pretilachlor by reduced total uptake and improved degradation of the herbicide (Scarponi et al. 2003). Additionally, fenclorim causes an upregulation of several metabolic responses in rice (Chen et al. 2013; Deng and Hatzios 2002; Hu et al. 2020; Scarponi et al. 2005; Shahzad et al. 2017; Usui et al. 2001; Wu et al. 1999).

Upregulation of glutathione-*S*-transferase (GST) genes is considered the primary metabolic pathway in which pretilachlor detoxification is improved by fenclorim. Moreover, a recent study (Hu et al. 2020) has illustrated the wide variety of metabolic processes upregulated by fenclorim and identified that fenclorim reduces lipid peroxidation and production of reactive oxygen species induced by pretilachlor. Pretilachlor detoxification is well linked to GST activity, and GST enzymes have been well described to react with foreign compounds with similar molecular shape and structure (Deng and Hatzios 2002; Shahzad et al. 2017; Wu et al. 1999). Therefore, the metabolic processes induced by fenclorim for pretilachlor will likely be reciprocated to acetochlor. Furthermore, both herbicides belong to the same chemical family, and acetochlor lacks only two hydrogen-saturated carbons compared to pretilachlor.

In the summer of 2019 in Fayetteville, AR, preliminary studies were conducted with 'Diamond' rice and a technical-grade fenclorim seed treatment at 0.25 and 2.5 g ai kg^{-1} of seed. The fenclorim seed treatment improved rice tolerance to DPRE applications of emulsifiable concentrate (EC) and ME acetochlor. However, <20% injury was not observed with the EC formulation (Avent et al. 2020). Additionally, the fenclorim seed treatment at 0.25 g ai kg^{-1} provided adequate tolerance to the lower rates of ME acetochlor, but not at 1,260 g ai ha^{-1}. The fenclorim treatment at 2.5 g ai kg^{-1} of seed reduced rice injury from acetochlor to <10% injury 21 d after treatment with acetochlor at 1,260 g ai ha^{-1}. However, these trials were split-plots initiated in the middle of the summer; therefore, yield data could not be collected, and injury was likely not representative of what would occur in typical rice-growing conditions. Thus, trials were initiated to 1) determine an optimum fenclorim seed treatment rate to provide adequate rice tolerance to acetochlor; 2) evaluate common Arkansas rice cultivars tolerance to acetochlor and fenclorim; and 3) determine whether fenclorim can provide adequate rice tolerance to acetochlor under cool, wet growing conditions.

Materials and Methods

Fenclorim Dose Response

Two experiments were initiated at the Rice Research and Extension Center (RREC) near Stuttgart, AR, on May 3, 2020, and April 23, 2021, to evaluate the safening effects of varying fenclorim rates on rice. These trials were established on a Dewitt silt loam soil composed of 27% sand, 54% silt, and 19% clay with a soil pH of 6.2 and 1.7% organic matter. Each trial was managed culturally and for pest management based on University of Arkansas Cooperative Extension Services recommendations for direct-seeded, delayed-flooded rice production. Both trials were amended preplant based on University of Arkansas System Division of Agriculture Marianna Soil Test Laboratory fertility recommendations with no preplant nitrogen. The site was cultivated at trial establishment to remove any weeds present, and the entire site was oversprayed with clomazone at 336 g ai ha^{-1} at the time of planting.

'RT 7521 FP' and ''RT 7321 FP' (Table 1) rice cultivars were planted at 36 seeds m^{-1} of row at a 1-cm depth with a 10-row Almaco (Nevada, IA) cone drill on 19-cm-wide rows. All rice seed was base-treated with clothianidin, carboxin, thiram, metalaxyl, fludioxonil, and gibberellins at 0.75, 0.38, 0.33, 0.16, 0.03, and 0.04 g ai kg^{-1} of seed, respectively. Plots were 1.5 m by 5.2 m with 1.5 m between plots within a block, and a 0.9-m alley between blocks. Last, all herbicides, including treatments and oversprays, were applied using a CO_2-pressurized backpack sprayer calibrated to deliver 140 L ha^{-1} at 276 kPA with AIXR 110015 (TeeJet Technologies, Glendale Heights, IL) nozzles at 4.8 km h^{-1}. Urea (46-0-0) was applied at 316 kg ha^{-1} less than 6 h before flooding.

The experiments were conducted as a three-factor factorial within a randomized complete block design with four replications. Factor A was the two different cultivars (Table 1). Factor B (herbicide) consisted of no acetochlor or a DPRE application of ME acetochlor at 1,260 g ai ha^{-1}. Last, factor C was fenclorim rates of 0, 0.625, 1.25, 2.5, and 5 g ai kg^{-1} of seed, allowing for a total of 20 treatments. Since these experiments were focused on

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Table 1. Cultivars, designation, producer, and seeding rate.[a]

Cultivar	Designation	Producer	Experiment	Seeding rate
				seeds m^{-1} of row
RT 7521 FP	Long-grain hybrid	RiceTec, Inc., Alvin, TX	FDR CS	36
RT 7321 FP	Long-grain hybrid	RiceTec, Inc., Alvin, TX	FDR CS	36
XP 753	Long-grain hybrid	RiceTec, Inc., Alvin, TX	CS	36
RTV 7231 MA	Long-grain pureline	RiceTec, Inc., Alvin, TX	CS	52
PVL02	Long-grain pureline	Horizon Ag, LLC., Memphis, TN	CS	72
PVL03	Long-grain pureline	Horizon Ag, LLC., Memphis, TN	CS	72
CLL15	Long-grain pureline	Horizon Ag, LLC., Memphis, TN	CS	72
CLL16	Long-grain pureline	Horizon Ag, LLC., Memphis, TN	CS	72
CLL17	Long-grain pureline	Horizon Ag, LLC., Memphis, TN	CS	72
CLJ01	Long-grain pureline	Horizon Ag, LLC., Memphis, TN	CS	72
Diamond	Long-grain pureline	UADA, Stuttgart, AR	CS GC	72
Jewel	Long-grain pureline	UADA, Stuttgart, AR	CS	72
Jupiter	Medium-grain pureline	UADA, Stuttgart, AR	CS	72
Lynx	Medium-grain pureline	UADA, Stuttgart, AR	CS	72
Titan	Medium-grain pureline	UADA, Stuttgart, AR	CS	72
DG263L	Long-grain pureline	Nutrien Ag Solutions, Inc. Saskatoon, SK, Canada	CS	72

[a]Abbreviations: FDR, fenclorim dose response; CS, cultivar screening; GC growth chamber; UADA, University of Arkansas System Division of Agriculture.

tolerance, all plots were kept weed-free using conventional rice herbicides (clomazone, thiobencarb, propanil, halosulfuron-methyl, florpyrauxifen-benzyl) and hand-removal. Evaluations included an average of five rice plant heights, an average of two 0.5-m^{-1} of row shoot counts, and visual rice injury 2 and 4 wk after emergence (WAE). Rice injury was evaluated on a 0% to 100% scale, with 0% being no injury and 100% representing crop death (Frans and Talbert 1977). Rough rice grain yield was collected following crop maturity using an Almaco (ALMACO, Nevada, IA) small-plot combine harvesting the entire plot, and grain was adjusted to 12% moisture.

Initially, regression analysis with a nonlinear three-parameter model was considered; however, due to poor R^2 (<0.50), an ANOVA was deemed more appropriate. All data distributions were analyzed using the distribution platform of JMP Pro software (version 16.1; SAS Institute Inc, Cary, NC), and heights, shoots, and yield were normally distributed, whereas injury was gamma-distributed. Data distributions were selected based on best fit using least log-likelihood and Akaike information criterion. All data were subjected to ANOVA, and means were separated using Fisher's protected LSD at $\alpha = 0.05$. Dunnett's procedure ($\alpha = 0.05$) was conducted to evaluate whether the relative shoots, heights, and yields were comparable to those of the nontreated (no fenclorim, no acetochlor). Normally distributed data were analyzed within JMP Pro software using the fit-model platform, while injury was analyzed using the generalized linear mixed model add-in with a gamma distribution (Gbur et al. 2012).

All quantitative data are reported relative to the nontreated control for each cultivar and each year. Because the objective of this experiment was to determine the optimum fenclorim seed treatment rate to use with ME acetochlor, all data were analyzed separately by year and herbicide effect, considering there were differences in rainfall activation both years.

Cultivar Screening

Two experiments were initiated in spring 2021 to evaluate differential cultivar response to applications of acetochlor with and without fenclorim seed treatment. On April 19, 2021, the first trial was initiated at the Pine Tree Research Station (PTRS) near Colt, AR, on a Calloway silt loam (11% sand, 70% silt, 19% clay, 7.8 pH, and 1.69% organic matter). The second trial was established on April 20, 2021, at the RREC on a Dewitt silt loam soil composed of 27% sand, 54% silt, and 19% clay, soil pH 5.5, and 1.8% organic matter. Each trial was managed culturally and for pest management based on University of Arkansas Cooperative Extension Services recommendations for direct-seeded, delayed-flooded rice production. Both trials were amended preplant based on Marianna Soil Test Laboratory fertility recommendations with no preplant nitrogen. Both RREC and PTRS sites were cultivated before trial establishment to remove any weeds that were present, and both sites were oversprayed with clomazone at 336 g ai ha^{-1} at the time of planting. Sixteen different cultivars were planted at a 1-cm depth with a 10-row Almaco cone drill on 19-cm-wide rows at 36, 52, and 72 seeds m^{-1} of row for hybrid, RTV 7231 MA, and inbred cultivars, respectively (Table 1). All rice seed was base-treated, and all herbicide treatments and overspray applications were applied the same as the dose response experiment. Urea (46-0-0) was applied at 316 kg ha^{-1} less than 6 h before flooding.

The objective of this experiment was not to determine the differences of each cultivar, but rather to determine whether fenclorim provided a safening effect for each cultivar. The experiments were conducted over 16 different cultivars as a two-factor factorial within a randomized complete block design with four replications. The factors included 1) no acetochlor or DPRE application of ME acetochlor at 1,260 g ai ha^{-1} and 2) a fenclorim seed treatment of 0 or 2.5 g ai kg^{-1} of seed, allowing for a total of 64 treatments. Because these experiments focused on tolerance, all plots were kept weed-free using conventional rice herbicides (clomazone, thiobencarb, propanil, halosulfuron-methyl, florpyrauxifen-benzyl) and hand-removal. Evaluations included an average of five rice plant heights, an average of two 0.5-m^{-1} of row shoot counts, and visual rice injury 2 and 4 WAE. An unmanned aerial system (DJI Mavic 2; DJI Technology Co., Ltd., Nanshan, Shenzhen, China) captured groundcover images 5 WAE. Overhead images were then analyzed using Field Analyzer (Green Research Services, LLC, Fayetteville, AR). Green pixel counts were measured to determine percentage groundcover. Rough rice grain yield was collected at harvest with a small-plot combine, and grain was adjusted to 12% moisture and kilograms per hectare for yield estimates. The center four rows of each plot at PTRS were harvested, and seven rows were harvested at RREC.

Table 2. Effect of fenclorim seed treatment doses on rice injury with and without acetochlor and averaged over cultivars 'RT 7321 FP' and 'RT 7521 FP'.[a–d]

	Injury							
	Acetochlor at 0 g ai ha^{-1}				Acetochlor at 1,260 g ai ha^{-1}			
	2 WAE		4 WAE		2 WAE		4 WAE	
Fenclorim rate	2020	2021	2020	2021	2020	2021	2020	2021
g ai kg^{-1} of seed	%							
0	0	0	0	0	30 A	66 A	22 A	70 A
0.625	9	3	1	3	27 A	41 B	17 AB	36 B
1.25	9	4	1	4	23 AB	34 B	13 BC	24 C
2.5	10	2	1	5	18 B	15 C	5 D	13 D
5	10	3	3	2	19 B	19 C	8 CD	14 D
P-value	NA	NA	NA	NA	0.0104	<0.0001	0.0004	<0.0001

[a]Abbreviation: WAE, weeks after emergence.
[b]Means within a column not containing the same letter are significantly different according to Fisher's protected LSD ($\alpha = 0.05$).
[c]P-values were generated using the generalized linear mixed model add in with a gamma distribution within JMP Pro 16.1 software (SAS Institute, Cary, NC).
[d]P-values for rice injury without acetochlor were not displayed because injury was relative to the nontreated check, which is always 0%. The presence of the fenclorim seed treatment did cause an effect but injury was ≤10% and no differences ($P < 0.05$) were observed for the fenclorim doses ≥0.625 g ai kg^{-1} of seed.

All data distributions were analyzed using the distribution platform of JMP Pro 16.1 software. Data distribution selections were based on best fit using least log-likelihood and Akaike information criterion. Heights, shoots, groundcover, and yield were normally distributed, and injury was gamma-distributed. All data were subjected to ANOVA, and means were separated using Fisher's protected LSD at $\alpha = 0.05$. Normally distributed data were analyzed using the fit model platform in JMP Pro software, while injury was analyzed using the GLIMMIX procedure in SAS software (version 9.4; SAS Institute Inc.; Gbur et al. 2012). Site was analyzed separately due to differences in rainfall activation, which caused varying injury between the two locations.

Growth Chamber Experiment

Two growth chamber experiments were initiated at the Milo J. Shult Research and Extension Center, Fayetteville, AR, in 2021, to evaluate rice injury potential from applications of acetochlor with and without fenclorim under cool, wet conditions. Both growth chambers were set to provide a 12-h photoperiod with day and nighttime temperatures of 23.8 C and 12.8 C, respectively. Light intensity was set to 1,000 μmol m^{-2} s^{-1}. Before trial initiation, a silt loam soil was collected and sieved. Soil analysis was conducted at the University of Arkansas Diagnostic Laboratory in Fayetteville, AR, using loss on ignition for organic matter and the hydrometer method for texture, resulting in a 6.4 of pH and 2.3%, 20%, 66%, and 14% organic matter, sand, silt, and clay, respectively. The soil was then dried at 33 C for 2 wk, and 8 kg was added to 11.4-L pots. Soil bulk density and volumetric field capacity were calculated using Soil Plant Air Water (SPAW) software (U.S. Department of Agriculture–Agricultural Research Service, Washington DC) with inputs of soil texture and organic matter to determine how much water was required to maintain 100% field capacity of the soil:

$$MW = \frac{VFC}{BD} \times \%M \times MS \qquad [1]$$

where MW represents the estimated mass of water required, VFC is the volumetric field capacity provided by SPAW, BD is the bulk density calculated by SPAW using texture and organic matter, $\%M$ is the percent soil moisture established as the testing parameter, and MS is the mass of the dried soil (31.5% ÷ 1.42 × %100 × 8,000g = 1,775 g of water).

Temperatures and soil moisture were set to provide a "worst-case" scenario for rice tolerance to a chloroacetamide herbicide. Under these conditions, rice should accumulate only 15 growing degree units each day, prolonging elongating root and shoot exposure to acetochlor. At 100% field capacity, maximum acetochlor efficacy should be expected (Dhareesank et al. 2006). The experiment was designed as a three-factor factorial completely randomized design with four replications. Factors consisted of with and without a fenclorim seed treatment at 2.5 g ai kg^{-1} of seed; with and without ME acetochlor at 1,050 g ai ha^{-1}; and planting depths of 0.6 and 2.5 cm. 'Diamond' rice was planted at 40 seeds pot^{-1} (30.5 cm diameter) at 80% field capacity. Acetochlor was applied 5 d after planting (DPRE) using a spray chamber calibrated at 187 L ha^{-1} with two flat-fan 1100067 nozzles (TeeJet, Glendale Heights, IL).

After application, pots were watered to 100% field capacity and maintained every 3 d. Evaluations included visual injury estimates (0% to 100%, with 0% being no injury and 100% being rice death), an average of five rice plant heights per pot, and shoot counts in the pot at 1 and 4 WAE. Rice aboveground biomass was collected at the final evaluation timing and weighed after drying at 60 C for 5 d until no moisture was present in samples. Soil in pots was flooded at approximately 3 WAE (4-leaf to tillering growth stage), and urea at 316 kg ha^{-1} was applied immediately after flooding to simulate field conditions. The experiment was analyzed within JMP Pro 16.1 software, and all data were subjected to ANOVA. Distributions were checked using the distribution platform, and all distributions were normal except injury, which was gamma-distributed. Data distribution selections were based on best fit using least log-likelihood and Akaike information criterion. All data were pooled over the two different experimental runs, which was considered random, and means were separated using Fisher's protected LSD ($\alpha = 0.05$).

Results and Discussion

Fenclorim Dose Response

Injury between the two cultivars of RT 7321 FP and RT 7521 FP did not differ ($P > 0.05$); therefore, the data are presented averaged over the two cultivars. Injury to rice from acetochlor at 1,260 g ai ha^{-1} was 30% in 2020 and 66% in 2021 at 2 WAE in the absence of fenclorim (Table 2). The variation in injury from one year to the next is likely caused by an activating rainfall occurring 7 d after

Table 3. Effect of fenclorim seed treatment doses on rice shoots with and without acetochlor and averaged over cultivar.[a–e]

	Relative shoots							
	Acetochlor at 0 g ai ha^{-1}				Acetochlor at 1,260 g ai ha^{-1}			
	2 WAE		4 WAE		2 WAE		4 WAE	
Fenclorim rate	2020	2021	2020	2021	2020	2021	2020	2021
g ai kg^{-1} of seed	———	———	———	———	%	———	———	———
0	100 AB	100	100	100	57 C*	44 C*	67 C*	54 B*
0.625	80 B	113	95	110	69 C*	61 BC*	66 C*	60 B*
1.25	109 A	118	79	108	97 A	78 AB	81 BC	70 B*
2.5	90 AB	102	93	105	81 B	93 AB	99 A	97 A
5	81 B	111	86	108	83 B	81 AB	96 AB	105 A
P-value	0.0343	0.2740	0.2780	0.2744	<0.0001	0.0003	0.0025	<0.0001

[a]Abbreviation: WAE, weeks after emergence.
[b]Average shoots in nontreated at 2 WAE were 24 and 20 m^{-1} of row and at 4 WAE were 42 and 30 m^{-1} of row for 2020 and 2021, respectively.
[c]Means within a column not containing the same letter are significantly different according to Fisher's protected LSD (α = 0.05).
[d]An asterisk indicates a mean significantly different from the nontreated (no fenclorim and no acetochlor) according to Dunnett's test (α = 0.05).
[e]P-values were generated using the fit model platform with JMP Pro 16.1 software (SAS Institute, Cary, NC).

acetochlor application in 2020 when the rice was spiking as compared with 3 d after application in 2021 when the rice had yet to emerge. The increase in injury for 2021 is expected since chloroacetamide herbicides are highly dependent upon water activation, and the emerging rice was at a more susceptible growth stage than the emerged rice in 2020 (Babczinski et al. 2012). In similar studies evaluating DPRE applications of acetochlor with rates ranging from 630 to 1,570 g ai ha^{-1}, injury varied from 18% to 89% (Fogleman et al 2019; Godwin et al. 2018; Norsworthy et al. 2019).

Rice injury from the fenclorim seed treatment in the absence of acetochlor was ≤10% for all rates of the safener at both evaluations (Table 2). Slight rice injury from seed treatments has been observed, where carbendazim reduced root length of the crop relative to no seed treatment (Sandhya et al. 2018). The injury associated with the fenclorim treatments 2 WAE was due to a delay in emergence of the crop. Across several field studies, fenclorim generally delayed emergence by 1 to 2 d, which caused the rice to appear stunted. By 4 WAE, rice in plots with fenclorim-treated seed generally recovered or even surpassed growth of rice not treated with fenclorim. In recent greenhouse trials, fenclorim-applied to rice at 2.5 g ai kg^{-1} of seed caused an increase in root and shoot biomass compared to nontreated plants by 4 WAE (JKN, nonpublished data).

The fenclorim seed treatment increased rice tolerance to acetochlor, and lowered the year-to-year variability in injury (Table 2). In both years and evaluation timings, injury trended downward as the fenclorim seed treatment rate increased. By 4 WAE, the fenclorim seed treatment rate of 2.5 g ai kg^{-1} of seed reduced injury compared to fenclorim rates ranging from 0 to 1.25 g ai kg^{-1} of seed. Increasing the fenclorim rate beyond 2.5 g ai kg^{-1} of seed did not further reduce injury to rice. Since fenclorim at 5 g ai kg^{-1} of seed provided no extra benefit compared with 2.5 g ai kg^{-1}, the recommended seed treatment rate should remain at 2.5 g ai kg^{-1} of seed to reduce the potential cost for producers.

A significant effect of the fenclorim seed treatment rate on rice shoot counts in the absence of acetochlor occurred 2 WAE in 2020 (Table 3); however, at all other timings and years, this effect was not significant and appears to be due to field variability. When comparing fenclorim seed treatment rates for plots treated with acetochlor, rice shoots were greatest for the two highest rates of fenclorim at 4 WAE in both years. Additionally, according to a Dunnett's test, the number of shoots in the presence of acetochlor and fenclorim at 0 or 0.625 g ai kg^{-1} were less than those from the nontreated control (no fenclorim and no acetochlor) at 2 WAE (31% to 66% reduction) and 4 WAE (33% to 46% reduction). Conversely, fenclorim rates of 2.5 and 5 g ai kg^{-1} of seed were comparable to those of the nontreated control in both evaluation timings and years. Godwin et al. (2018) observed a 44% reduction in shoots following a DPRE application of acetochlor at 1,050 g ai ha^{-1}.

Acetochlor reduced the average height of rice by 35% at 2 WAE in 2021, when seed did not receive the fenclorim treatment (Table 4). Meanwhile, rice heights with acetochlor and fenclorim at 2.5 g ai kg^{-1} were greater than rice treated with acetochlor and no fenclorim at each evaluation. Previous research reported an 18% reduction in rice height approximately 4 wk after treatment with ME acetochlor applied DPRE averaged over acetochlor at 1,050 and 2,100 g ai ha^{-1} (Fogleman et al. 2019). In 2020 and 2021, higher injury levels were observed without fenclorim, which likely caused the height reductions. Similar to shoots and injury, rice height was less impacted by acetochlor if fenclorim of 2.5 or 5.0 g ai kg^{-1} of seed was employed. Additionally, fenclorim rates of 0.625 and 1.25 g ai kg^{-1} of seed resulted in shorter rice than the nontreated control in 2021 at 2 WAE.

The rates of fenclorim did not influence rough rice grain yield in the presence or absence of acetochlor (Table 5). The lack of a response is likely due to the ability of hybrid rice to tiller and compensate for stand loss. Across other field studies by previous researchers, acetochlor affected yield in some trials but not others. Fogleman and others (2019) reported 14% to 22% reductions in yield from acetochlor applied DPRE at 1,050 g ai ha^{-1}. In another experiment with the same acetochlor application timings, yield was reduced numerically by 1% in 2015 and significantly by 42% in the following year, indicating the variable influence acetochlor has on rice yield (Godwin et al. 2018).

The fenclorim seed treatment rate of 2.5 g ai kg^{-1} of seed was derived from the maximum use rate of pretilachlor and fenclorim of 450 and 225 g ai ha^{-1}, respectively (Chauhan et al. 2014; Quadranti and Ebner 1983). With an inbred rice cultivar planting rate of 90 kg of seed ha^{-1}, the amount of fenclorim per hectare is equivalent to the foliar use rate. Conversely, when planting a hybrid cultivar at 30 kg ha^{-1}, as opposed to 90 kg ha^{-1}, the amount of fenclorim per hectare is reduced by one-third. However, the amount of fenclorim per seed is equivalent across cultivars regardless of planting rate, because the application rate is based on weight of seed, not area treated. Therefore, based on the results from this experiment, the optimum seed treatment rate appears to be

Table 4. Effect of fenclorim seed treatment doses on rice heights with and without acetochlor and averaged over cultivar.[a–e]

	Relative height							
	Acetochlor at 0 g ai ha^{-1}				Acetochlor at 1,260 g ai ha^{-1}			
	2 WAE		4 WAE		2 WAE		4 WAE	
Fenclorim rate	2020	2021	2020	2021	2020	2021	2020	2021
g ai kg^{-1} of seed	%							
0	100	100	100	100	73 B	65 B*	79 B*	90 BC
0.625	97	104	99	103	86 AB	73 B*	95 A	101 AB
1.25	105	104	103	91	87 A	74 B*	100 A	88 C
2.5	101	109	101	104	92 A	100 A	101 A	108 A
5	100	113	98	109	83 AB	88 A	100 A	110 A
P-value	0.8169	0.7260	0.9060	0.1039	0.0363	0.0004	0.0001	0.0039

[a]Abbreviations WAE, weeks after emergence/
[b]Average heights in nontreated at 2 WAE were 17 and 11 cm and at 4 WAE were 35 and 15 cm for 2020 and 2021, respectively.
[c]Means within a column not containing the same letter are significantly different according to Fisher's protected LSD (α = 0.05).
[d]An asterisk indicates a mean significantly different from the nontreated (no fenclorim and no acetochlor) according to Dunnett's test (α = 0.05).
[e]P-values were generated using the fit model platform with JMP Pro 16.1 software (SAS Institute, Cary, NC).

Table 5. Effect of fenclorim seed treatment doses on rough rice yields with and without acetochlor and averaged over cultivar.[a,b]

	Relative yield			
	Acetochlor at 0 g ai ha^{-1}		Acetochlor at 1,260 g ai ha^{-1}	
Fenclorim rate	2020	2021	2020	2021
g ai kg^{-1} of seed	%			
0	100	100	123	84
0.625	112	97	118	83
1.25	100	94	115	97
2.5	103	102	108	97
5	104	103	115	97
P-value	0.6602	0.4303	0.2147	0.1039

[a]Average rough rice yield in nontreated were 10,200; 7,600; 10,500; and 8,400 kg ha^{-1} for cultivars '7521' and '7321' and 2020 and 2021, respectively.
[b]P-values were generated using the fit model platform with JMP Pro 16.1 software (SAS Institute, Cary, NC).

2.5 g ai kg^{-1} of seed. Injury, average height, and number of shoots obtained with acetochlor plus fenclorim seed treatment at 2.5 g ai kg^{-1} of seed were comparable to that of the nontreated control. Additionally, rice treated with acetochlor in the presence of fenclorim at 2.5 g ai kg^{-1} of seed showed greater tolerance to the herbicide compared to acetochlor-treated rice without fenclorim.

Cultivar Screening

Rice injury at PTRS was >40% without fenclorim when acetochlor was applied across all cultivars (Table 6). In comparison, rice injury following acetochlor was <20% when seeds were treated with fenclorim across all cultivars, and a reduction in injury relative to non-fenclorim treatments within a cultivar was observed for all evaluations at this location. Similarly, at the RREC location, reductions in injury with the addition of fenclorim were observed for all cultivars and evaluations except 'Titan' at 4 WAE; however, injury overall for this cultivar was ≤13%. Among three previous studies evaluating rice tolerance to DPRE-applied acetochlor, injury ranged from 18% to 89% with four separate cultivars: 'CL151', 'CL111', CL172', and 'PVL01' (Fogleman et al. 2019; Godwin et al. 2018; Norsworthy et al. 2019).

All cultivars exhibited <20% injury with acetochlor and fenclorim, except for 'DG263L' and 'XP753' 2 WAE at PTRS where 23% and 24% injury was observed (Table 6). However, a reduction in yield was not observed for DG263L or XP753 with acetochlor and fenclorim. Previous research has reported up to 35% bleaching of rice with clomazone applied at 1,120 g ai ha^{-1}, but rice yield was not negatively impacted (Zhang et al. 2005). At PTRS, 7 out of 16 cultivars suffered reductions in rough rice grain yield when acetochlor was applied without fenclorim. However, grain yield was never reduced at either site for any cultivar when acetochlor was used in conjunction with the fenclorim seed treatment.

Frans and Talbert (1977) historically classified crop injury at 20% to 30% as a slight effect that is unlikely to persist; however, rice producers may not accept >20% injury. Therefore, if ME acetochlor were to become labeled for use in rice with a fenclorim seed treatment, the rate of acetochlor should likely be reduced from 1,260 g ai ha^{-1}. Future studies should consider reducing the acetochlor rate and continue to screen XP753 and DG263L cultivars, which appear to be more susceptible to acetochlor injury. Regardless, the safening potential of a fenclorim seed treatment to provide <20% injury to acetochlor at 1,260 g ai ha^{-1} across 14 of 16 cultivars is quite promising.

A safening response is defined in this study as a significant improvement relative to rice treated with acetochlor without fenclorim. For rice height, shoots, and groundcover, there were 160 total observations (Table 7). Out of these observations, fenclorim improved rice tolerance to acetochlor for 70 assessments, and there were no rice evaluations where fenclorim increased rice sensitivity to the herbicide. Of those 70 observations, rice treated with fenclorim and acetochlor was comparable to the nontreated control 60 times.

The lack of a safening effect for the other 90 of 160 observations is due to heights being a poor predictor for rice tolerance to acetochlor (Table 7). Of the 64 observations for heights, 46 were insignificant and 25 provided no trend numerically, indicating that acetochlor does not always affect rice heights. Furthermore, only 16 of 64 observations for height resulted in a significant interaction, where fenclorim provided a safening effect to acetochlor. For shoots and groundcover data, 35 of 64 and 21 of 32 observations had significant differences, respectively. For all significant P-values of shoots and groundcover, fenclorim elicited a safening response to acetochlor, and the acetochlor plus fenclorim treatment achieved numerically greater shoots or groundcover than acetochlor alone for the remaining, insignificant observations. Significant differences were likely not detected due to overall variation in quantitative data from field variability or the lack of

Table 6. Rice cultivar injury and yield as influenced by acetochlor and fenclorim.[a–e]

			Injury				Yield	
			2 WAE		4 WAE			
Cultivar	Fenc	Acet	PTRS	RREC	PTRS	RREC	PTRS	RREC
			%				kg ha^{-1}	
RT7321FP	no	None	–	–	–	–	11,200	10,700
		Treated	76 A	75 A	74 A	65 A	9,700	10,200
	yes	None	4 C	4 C	0 C	5 B	11,000	10,700
		Treated	18 B	17 B	20 B	13 B	11,000	10,900
P-value			<0.0001	<0.0001	<0.0001	0.0052	0.1534	0.618
RT7521FP	no	None	–	–	–	–	10,500	11,000
		Treated	55 A	42 A	63 A	55 A	10,600	10,200
	yes	None	5 C	5 B	0 C	4 B	11,400	10,600
		Treated	14 B	9 B	15 B	20 B	10,600	10,700
P-value			0.0003	0.0006	<0.0001	0.0138	0.305	0.3101
CLJ01	no	None	–	–	–	–	6,400	9,000
		Treated	81 A	50 A	70 A	53 A	5,800	8,300
	yes	None	1 C	2 C	0 C	3 B	6,600	8,200
		Treated	19 B	10 B	19 B	11 B	6,400	8,600
P-value			<0.0001	<0.0001	<0.0001	0.0157	0.7771	0.1389
CLL 15	no	None	–	–	–	–	7,400	8,300
		Treated	39 A	24 A	55 A	28 A	6,100	8,300
	yes	None	3 C	6 B	0 C	3 B	6,700	8,100
		Treated	17 B	9 B	8 B	9 B	7,100	8,500
P-value			0.0006	0.0043	<0.0001	0.0015	0.1241	0.6812
CLL 16	no	None	–	–	–	–	8,300	8,400
		Treated	40 A	28 A	53 A	28 A	7,900	8,300
	yes	None	4 C	1 B	0 C	1 C	8,300	7,500
		Treated	10 B	10 B	6 B	14 B	8,200	8,200
P-value			<0.0001	0.0037	<0.0001	0.0016	0.7011	0.2328
CLL 17	no	None	–	–	–	–	8,400	8,000
		Treated	51 A	35 A	68 A	34 A	7,800	7,700
	yes	None	5 C	3 C	0 C	5 B	8,600	8,100
		Treated	14 B	7 B	11 B	18 B	8,100	8,600
P-value			<0.0001	<0.0001	<0.0001	0.037	0.8104	0.4641
DG263L	no	None	–	–	–	–	10,800	10,400
		Treated	70 A	41 A	68 A	45 A	9,300	9,600
	yes	None	4 C	3 C	0 C	1 C	11,500	10,000
		Treated	23 B	12 B	16 B	13 B	10,200	10,600
P-value			<0.0001	0.0016	<0.0001	0.0011	0.7631	0.1226
Diamond	no	None	–	–	–	–	8,200	9,200
		Treated	56 A	49 A	58 A	43 A	6,500	8,900
	yes	None	5 C	5 B	0 C	3 B	8,800	9,200
		Treated	14 B	7 B	9 B	10 B	7,900	9,800
P-value			<0.0001	0.0003	<0.0001	0.0313	0.3624	0.4431
Jewel	no	None	–	–	–	–	8,200 A	10,000 A
		Treated	65 A	31 A	66 A	38 A	7,400 B	9,000 B
	yes	None	4 C	1 C	0 C	3 B	8,300 A	9,100 B
		Treated	15 B	7 B	9 B	13 B	8,600 A	9,700 AB
P-value			<0.0001	<0.0001	<0.0001	0.0104	0.0221	0.0065
Jupiter	no	None	–	–	–	–	9,000 A	8,700
		Treated	40 A	16 A	51 A	17 A	6,400 B	7,900
	yes	None	4 C	3 B	0 C	0 B	8,400 A	7,800
		Treated	12 B	7 B	13 B	0 B	7,700 A	8,700
P-value			<0.0001	0.013	<0.0001	<0.0001	0.0472	0.2468
Lynx	no	None	–	–	–	–	9,100 A	8,300
		Treated	34 A	28 A	54 A	18 A	6,300 B	8,200
	yes	None	2 C	4 B	0 C	1 B	9,100 A	8,200
		Treated	10 B	7 B	6 B	5 B	8,500 A	8,600
P-value			<0.0001	0.0004	<0.0001	0.0276	0.0358	0.6163
RT7231MA	no	None	–	–	–	–	7500	8300
		Treated	76 A	23 A	68 A	15 A	7,200	7,400
	yes	None	5 C	4 C	0 C	0 C	7,800	7,900
		Treated	16 B	12 B	10 B	5 B	7,000	8,200
P-value			0.0002	<0.0010	<0.0001	0.0004	0.46	0.2923
PVL02	no	None	–	–	–	–	8,400 A	7,300
		Treated	44 A	35 A	65 A	34 A	6,300 B	6,700

(*Continued*)

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Table 6. (*Continued*)

Cultivar	Fenc	Acet	Injury				Yield	
			2 WAE		4 WAE			
			PTRS	RREC	PTRS	RREC	PTRS	RREC
	yes	None	2 C	1 C	0 C	1 B	7,900 A	7,300
		Treated	11 B	10 B	19 B	11 B	7,800 A	7,800
P-value			0.0002	<0.0001	<0.0001	0.0068	0.0035	0.4946
PVL03	no	None	–	–	–	–	7,900 A	8,700
		Treated	75 A	36 A	69 A	39 A	6,700 B	8,600
	yes	None	0 C	2 C	0 C	1 B	7,600 A	8,500
		Treated	15 B	8 B	11 B	10 B	7,700 A	9,100
P-value			<0.0001	<0.0001	<0.0001	0.0047	0.0477	0.4884
Titan	no	None	–	–	–	–	9,700 A	8,200
		Treated	38 A	16 A	44 A	13	6,300 B	8,100
	yes	None	3 C	1 B	0 C	8	8,900 A	7,500
		Treated	13 B	5 AB	6 B	8	8,800 A	8,700
P-value			0.0001	0.0117	<0.0001	0.1777	0.0354	0.2219
XP753	no	None	–	–	–	–	10,800 AB	10,400
		Treated	83 A	64 A	73 A	58 A	7,100 C	9,700
	yes	None	1 C	2 C	0 C	8 B	11,300 A	9,800
		Treated	24 B	20 B	19 B	13 B	9,900 B	10,000
P-value			<0.0001	0.0003	<0.0001	0.004	0.0216	0.4816

[a]Abbreviations: WAE, weeks after emergence; Fenc, fenclorim; Acet, acetochlor; PTRS, Pinetree Research Station; RREC, Rice Research and Extension Center.
[b]Fenclorim seed treatment rate of 0 and 2.5 g ai kg^{-1} of seed for no and yes, respectively.
[c]Acetochlor rate of 0 and 1,260 g ai ha^{-1}.
[d]Means within a column for each cultivar not containing the same letter are significantly different according to Fisher's protected LSD ($\alpha = 0.05$).
[e]P-values were determined using SAS software version 9.4 and the GLIMMIX procedure with a gamma distribution for injury and JMP Pro 16.1 software using the fit model platform for yield data (both softwares: SAS Institute, Cary, NC).

reduction in height or groundcover caused by acetochlor; hence, a significant improvement was not possible. To better detect differences, future studies should include more replications. and height may be of little value when evaluating rice tolerance to acetochlor with fenclorim.

A previous study evaluating pretilachlor and fenclorim with three different genetic lines of rice reported similar safening responses, with varying tolerance for each rice cultivar to pretilachlor alone (Deng and Hatzios 2002). Pretilachlor reduced root lengths by 67%, 54%, and 34% for rice lines 'Teqing', 'Koshihikari', and 'Lemont', respectively. Conversely, the addition of fenclorim to pretilachlor caused rice root growth to be similar or greater than the nontreated control of each cultivar. Similarly, the fenclorim seed treatment improved rice tolerance to acetochlor by reducing injury or improving height, shoots, or groundcover across 16 different rice cultivars, illustrating the feasibility of a ME chloroacetamide herbicide option with a fenclorim seed treatment across most cultivars currently grown in the mid-southern U.S. rice region.

Growth Chamber Experiment

Rice in the growth chamber experiment accumulated 15 growing degree units each day at the daily maximum and minimum temperatures of 23.8 and 12.7 C. Under this temperature regime and at 100% field capacity, conditions represent a worst-case scenario for rice injury after the DPRE application of acetochlor. At 1 WAE, rice height averaged over planting depth was reduced from 6.6 cm without acetochlor and fenclorim to 2.9 cm when acetochlor was applied in the absence of fenclorim (Table 8). Additionally, the delay in emergence, which causes the appearance of shorter rice, can be observed when comparing with and without fenclorim in the absence of acetochlor at 1 WAE. By 4 WAE, rice height and shoot counts were similar among treatments, except when treated with acetochlor at 1,050 g ai ha^{-1} in the absence of fenclorim.

Rice aboveground biomass was also improved by the fenclorim seed treatment, averaged over planting depth (Table 8). In the absence of the herbicide, fenclorim increased aboveground biomass by 3 g, and in the presence of acetochlor, fenclorim provided a safening effect by improving aboveground biomass from 16.4 g without fenclorim to 23.7 g with fenclorim. Furthermore, with acetochlor and the fenclorim seed treatment, rice aboveground biomass was comparable to that of the nontreated control. The increase in aboveground biomass in the absence of acetochlor is likely due to improved root growth caused by fenclorim. Recent greenhouse research evaluating rice with and without fenclorim seed treatments demonstrated an increase in root biomass from the addition of the fenclorim seed treatment (TA and JKN, nonpublished data). The increased root growth could result in improved nutrient uptake, which would allow greater accumulation in aboveground biomass.

Planting depth as the main effect or within any interaction did not significantly influence heights, shoots, or biomass ($P > 0.05$). However, planting depth influenced injury, and deeper planting depth reduced visual injury in treatments with acetochlor and no fenclorim. Injury from shallow to deeper planting depth decreased from 41% to 29% at 1 WAE and from 35% to 23% at 4 WAE, respectively (Table 9). In treatments with acetochlor plus fenclorim, little difference was observed in the injury rates across the planting depths (from 7% to 11%), indicating that planting depth would not provide a secondary improvement with the addition of the fenclorim seed treatment.

The injury reduction at the deeper planting depth in the absence of fenclorim was likely from positional selectivity of the seed. Previous research has demonstrated a reduction in grain sorghum [*Sorghum bicolor* (L.) Moench] phytotoxicity with *S*-metolachlor due to planting in deeper soil depths that were treated with the

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Table 7. Cultivar height, shoots, and coverage in response to acetochlor and fenclorim.[a–e]

			Average height				Average shoots				Coverage	
			2 WAE		4 WAE		2 WAE		4 WAE		5 WAE	
Cultivar	Fenc	Acet	PTRS	RREC	PTRS	RREC	PTRS	RREC	PTRS	RREC	PTRS	RREC
			cm				count m^{-1}				%	
RT7321FP	no	none	8 A	11 A	8 AB	22	24 A	36 A	24	38	30 A	48
		treated	4 B	6 B	8 B	21	8 B	16 B	16	16	8 B	16
	yes	none	8 A	10 A	8 AB	19	24 A	36 A	24	40	26 A	56
		treated	7 A	10 A	9 A	20	24 A	34 A	20	30	24 A	38
P-value			0.0164	0.0008	0.0214	0.5787	0.0237	0.0087	0.4903	0.1594	0.005	0.0821
RT7521FP	no	none	7 A	11 A	9	19	32 A	34 A	26	36	24	50
		treated	5 B	7 C	8	19	16 B	18 B	18	20	20	30
	yes	none	8 A	10 B	9	19	32 A	40 A	26	36	26	56
		treated	7 A	9 B	8	19	26 A	54 A	22	30	26	40
P-value			0.0151	0.003	0.7969	0.1522	0.0138	0.012	0.1147	0.0734	0.2361	0.7314
CLJ01	no	none	5 A	13 A	9	19	46 AB	54 A	44	50 A	28	46
		treated	3 B	7 B	8	19	10 C	28 B	24	16 B	8	26
	yes	none	6 A	12 A	9	18	56 A	52 A	44	44 A	36	48
		treated	5 A	11 A	9	19	40 B	52 A	38	44 A	26	40
P-value			0.0461	0.002	0.2435	0.5001	0.0123	0.0104	0.1519	0.0032	0.0832	0.0832
CLL 15	no	none	8	13	10	19	56 A	56 A	42	52	34 A	58
		treated	6	10	9	18	30 B	38 B	22	46	18 B	40
	yes	none	7	11	10	18	52 A	56 A	42	54	36 A	62
		treated	6	10	9	18	46 A	58 A	34	48	38 A	52
P-value			0.1325	0.0983	0.8074	0.4319	0.0096	0.0138	0.1678	0.7228	0.0151	0.0641
CLL 16	no	none	8	13	9	20	54 A	58	38	50 A	34 A	48
		treated	7	10	9	20	30 B	40	34	32 B	6 B	34
	yes	none	7	13	9	21	54 A	62	40	48 A	36 A	56
		treated	7	11	9	19	50 A	56	42	52 A	30 A	40
P-value			0.0528	0.0626	0.6709	0.2711	0.0042	0.1114	0.4009	0.0126	0.0001	0.71
CLL 17	no	none	8 A	11	9 A	20	50 A	48	38	40	26 A	50 B
		treated	5 C	9	7 B	20	20 B	36	22	28	10 B	28 C
	yes	none	7 AB	12	8 AB	19	50 A	52	38	44	30 A	56 A
		treated	7 B	10	8 AB	17	44 A	54	34	44	34 A	48 B
P-value			0.0003	0.622	0.0354	0.1983	0.0011	0.1016	0.0769	0.0747	0.0006	0.019
DG263L	no	none	8	11	9	18	60 A	62 A	46 A	50 A	28 A	58 A
		treated	4	7	7	18	20 C	32 C	22 C	26 B	18 B	30 B
	yes	none	7	10	9	17	54 A	60 A	38 AB	54 A	36 A	58 A
		treated	6	8	8	15	44 B	52 B	32 B	48 A	34 A	52 A
P-value			0.1313	0.1878	0.2146	0.0944	0.0001	0.0501	0.0307	0.0384	0.0261	0.0041
Diamond	no	none	9 A	13 A	10	18 A	50 A	56 A	50 A	48	28	44 A
		treated	6 C	9 B	7	16 B	24 B	34 B	24 C	34	14	26 B
	yes	none	8 AB	11 AB	9	16 AB	54 A	54 A	34 B	44	42	48 A
		treated	7 B	11 AB	8	18 A	52 A	54 A	34 B	42	34	48 A
P-value			0.0115	0.043	0.0766	0.0112	0.041	0.0261	0.0012	0.3007	0.2734	0.0394
Jewel	no	none	8 A	11 A	8	18	64 A	60 A	44	48	34 A	44 A
		treated	5 C	9 C	7	15	20 B	40 B	18	32	4 C	30 B
	yes	none	7 A	11 A	9	17	60 A	58 A	50	50	30 AB	48 A
		treated	6 B	9 B	8	17	52 A	54 A	36	48	20 B	46 A
P-value			0.0246	0.0371	0.5662	0.2258	0.0013	0.02	0.088	0.0976	0.0042	0.0463
Jupiter	no	none	6	11	9	17	60 A	64 A	50	60 A	28 A	44 A
		treated	4	9	8	17	28 B	46 B	28	42 B	6 B	30 B
	yes	none	6	11	9	18	58 A	58 A	50	54 A	30 A	50 A
		treated	5	10	8	18	54 A	58 A	34	54 A	26 A	52 A
P-value			0.0787	0.4831	0.4567	0.2316	0.0063	0.024	0.3799	0.0163	0.0023	0.05
Lynx	no	none	7	11	9	19	56 A	56 A	46 A	52	28	52
		treated	5	9	8	19	36 B	38 B	26 B	40	22	36
	yes	none	7	11	8	18	54 A	58 A	44 A	46	32	54
		treated	6	10	9	18	48 A	64 A	44 A	46	32	54
P-value			0.163	0.4278	0.2249	0.8195	0.0133	0.0229	0.0312	0.0648	0.4071	0.1241
RT7231MA	no	none	5 A	10	8	23	36 A	40	34 A	44	32 A	42 A
		treated	3 B	10	8	20	10 B	28	22 B	34	14 B	32 B
	yes	none	5 A	10	8	21	36 A	44	32 A	42	34 A	50 A
		treated	5 A	8	8	22	30 A	38	34 A	40	30 A	48 A
P-value			0.0035	0.5959	0.137	0.1033	0.0262	0.4957	0.0096	0.1652	0.0041	0.0142
PVL02	no	none	7	11	9	20	54 A	56 A	50	50	28 A	52 A
		treated	5	8	8	19	34 B	34 B	28	40	16 B	34 B
	yes	none	7	12	8	19	54 A	58 A	52	52	28 A	56 A
		treated	6	10	8	20	54 A	52 A	40	54	26 A	56 A
P-value			0.1535	0.1558	0.8904	0.5608	0.0146	0.0472	0.3154	0.2647	0.002	0.037
PVL03	no	none	6	13 A	8	19	48 AB	54 A	32	48 A	28 A	58 A
		treated	5	10 B	8	20	22 C	34 B	26	24 B	8 B	32 B

(*Continued*)

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Table 7. (*Continued*)

			Average height				Average shoots				Coverage	
			2 WAE		4 WAE		2 WAE		4 WAE		5 WAE	
Cultivar	Fenc	Acet	PTRS	RREC	PTRS	RREC	PTRS	RREC	PTRS	RREC	PTRS	RREC
	yes	none	6	12 A	8	23	56 A	58 A	40	50 A	28 A	66 A
		treated	6	12 A	8	22	42 B	54 A	38	44 A	26 A	56 A
P-value			0.2888	0.0135	0.5089	0.7508	0.0172	0.0366	0.6504	0.0273	0.0002	0.0361
Titan	no	none	7	11	9 A	18	54 A	56	46	54	40 A	58 A
		treated	5	10	8 B	20	28 B	48	30	46	8 B	36 C
	yes	none	6	11	9 A	19	56 A	54	48	58	38 A	54 AB
		treated	6	10	9 A	18	56 A	58	38	50	36 A	46 B
P-value			0.2061	0.0172	0.0002	0.164	0.0071	0.2386	0.6148	0.943	< 0.0001	0.0382
XP753	no	none	8 A	9	8	18	26	34	18	32	28 B	42
		treated	4 C	7	7	20	8	22	14	14	4 C	30
	yes	none	7 AB	10	8	19	28	34	22	32	40 A	48
		treated	6 B	8	8	19	20	28	16	24	40 A	44
P-value			0.0136	0.5415	0.6523	0.3037	0.1801	0.6524	0.786	0.2177	0.0008	0.493

[a]Abbreviations: WAE, weeks after emergence; Fenc, fenclorim; Acet, acetochlor; PTRS, Pinetree Research Station; RREC, Rice Research and Extension Center.
[b]Fenclorim seed treatment rate of 0 and 2.5 g ai kg^{-1} of seed.
[c]Acetochlor rate of 0 and 1,260 g ai ha^{-1}.
[d]Means within a column for each cultivar not containing the same letter are significantly different according to Fisher's protected LSD (α = 0.05).
[e]P-values were determined using JMP Pro 16.1 software (SAS Institute, Cary, NC) using the fit model platform for all data.

Table 8. Effect of acetochlor and the fenclorim seed treatment on rice heights, shoots, and aboveground biomass averaged over planting depth.[a–d]

		Heights		Shoots		Aboveground biomass
Acetochlor	Fenclorim	1 WAE	4 WAE	1 WAE	4 WAE	4 WAE
g ai ha^{-1}		——— cm ———		——— count pot^{-1} ———		g
0	No	6.6 A	31.7 A	31 A	43 A	23.1 B
	Yes	5 B	32.4 A	31 A	46 A	26.1 A
1,050	No	2.9 C	26.4 B	21 B	32 B	16.4 C
	Yes	4.7 B	29.2 A	30 A	44 A	23.7 AB
P-value		<0.0001	0.0178	0.0002	0.0001	0.0374

[a]Abbreviations: WAE, weeks after emergence.
[b]Fenclorim seed treatment rate of 0 and 2.5 g ai kg^{-1} of seed.
[c]Different letters within each column indicate a significant difference between treatments; means separated using Fisher's protected LSD (α = 0.05).
[d]P-values were determined using JMP Pro 16.1 software (SAS Institute, Cary, NC) with the fit model platform.

Table 9. Effect of planting depth, acetochlor, and the fenclorim seed treatment on rice injury.[a–d]

			Injury	
Planting depth	Acetochlor	Fenclorim	1 WAE	4 WAE
cm	g ai ha^{-1}		——— % ———	
0.6	0	No	–	–
		Yes	5 C	2 D
	1,050	No	41 A	35 A
		Yes	7 C	6 CD
2.5	0	No	–	–
		Yes	3 C	1 D
	1,050	No	29 B	23 B
		Yes	8 C	11 C
P-value			0.0052	0.0027

[a]Abbreviations: WAE, weeks after emergence.
[b]Fenclorim seed treatment rate of 0 and 2.5 g ai kg^{-1} of seed.
[c]Different letters within each column indicate a significant difference between treatments; means separated using Fisher's protected LSD (α = 0.05).
[d]P-values were determined using JMP Pro 16.1 software (SAS Institute, Cary, NC) with the fit model platform.

herbicide (Procópio et al. 2001). The recommended seeding depth of rice is 1.3 cm, and based on the results of this experiment, a deeper planting depth would not provide sufficient tolerance to rice with acetochlor at 1,050 g ai ha^{-1} without the addition of the fenclorim seed treatment.

It is important to note that rice injury from acetochlor without the fenclorim seed treatment averaged over planting depth and evaluation timing ranged from 15% to 60%. In comparison, acetochlor injury with the addition of fenclorim ranged from 0% to 20% (data not shown). Rice injury from acetochlor alone is still highly variable despite controlled conditions provided by a growth chamber. However, with the addition of fenclorim, rice injury was <20%, and the variability in tolerance was reduced from a difference of 45 percentage points to only 20 percentage points. Based on the results of this experiment, the cultivar Diamond under these less-than-ideal conditions (cool and wet) appears tolerant to acetochlor at 1,050 g ai ha^{-1} if treated with fenclorim at 2.5 g ai kg^{-1} of seed.

Practical Implications

Fenclorim has been well described as a safener when used in mixture with pretilachlor in Asian transplanted rice (Chen et al. 2013; Deng and Hatzios 2002; Hu et al. 2020; Quadranti and Ebner 1983; Scarponi et al. 2003 and 2005; Usui et al. 2001; Wu et al. 1999). To date, only one publication has described the safening potential of a fenclorim seed treatment to acetochlor (Avent et al. 2020). The experiments conducted in 2020 and 2021 demonstrate the safening capability of a fenclorim seed

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treatment under typical drill-seeded rice production systems, which encompasses ~85% of Arkansas rice production (Hardke 2021). With the fenclorim seed treatment of 2.5 g ai kg^{-1} of seed and ME acetochlor applied DPRE at 1,260 g ai ha^{-1}, rice exhibited ≤24% injury across all trials and cultivars.

Based on the results of the fenclorim dose-response experiment, the optimum rate of the fenclorim seed treatment appears to be 2.5 g ai kg^{-1} of seed. Comparable tolerance levels were observed with 5 g ai kg^{-1} of seed; however, the 2.5 g ai kg^{-1} rate was sufficient and would be a more affordable solution for producers than 5 g ai kg^{-1} of seed. Fenclorim seed treatment rates lower than 2.5 g ai kg^{-1} of seed provided less consistent safening. Future studies should also consider a rate response of acetochlor on a heavy clay soil texture since acetochlor activity is negatively correlated with increasing clay content (Reinhardt and Nel 1990), and these studies were conducted on a silt loam soil.

If fenclorim and acetochlor become labeled for use in U.S. rice production, some initial delay in emergence from the fenclorim seed treatment might be observed. However, without comparing with and without fenclorim, the effects of the fenclorim seed treatment may not be apparent. Across all trials, no adverse effects in the form of stand or yield were observed from the fenclorim seed treatment. Additionally, acetochlor would provide an alternative site of action to control herbicide-resistant barnyardgrass populations that are common throughout mid-southern U.S. rice fields. Acetochlor would also provide a nontraited option for controlling weedy rice if the tolerance from fenclorim provided to the cultivated rice was not reciprocated to weedy rice.

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