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Oxyfluorfen-resistant rice tolerance and weed control when using oxyfluorfen

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Research Article

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Abstract

Herbicide-resistant barnyardgrass and weedy rice control, without crop injury, is a challenge for rice producers in the United States. Herbicides not initially labeled for rice, such as oxyfluorfen, are now being evaluated as new tools for weed control. The ROXY® trait allows for the use of oxyfluorfen in rice for weed control preemergence and postemergence. Experiments were initiated in 2021 and 2022 to evaluate (1) the effectiveness of preemergence- and postemergence-applied oxyfluorfen on barnyardgrass and weedy rice, (2) the sensitivity of oxyfluorfen-resistant rice to oxyfluorfen as a function of application timing, and (3) the influence of soil moisture on oxyfluorfen-resistant rice sensitivity to oxyfluorfen. In the field, a rate response was observed for oxyfluorfen applied to weedy rice when averaged over application timings of 1-leaf, 2-leaf, 3-leaf, and tillering, with oxyfluorfen at 1,680 g ai ha^{-1} resulting in 81% and 72% control 7 d after application (DAA) in 2021 and 2022, respectively. Under greenhouse conditions, barnyardgrass and weedy rice control averaged by the rate of oxyfluorfen was ≥85 and ≥70%, respectfully, 7 DAA for the 1-, 2-, and 3-leaf rice growth stage timings. Preemergence applications of oxyfluorfen under 100% soil saturation resulted in 75% injury to oxyfluorfen-resistant rice, greater than all other soil moisture at 7 DAA. All postemergence applications of oxyfluorfen resulted in 63% to 70% injury to oxyfluorfen-resistant rice at 7 DAA, regardless of soil moisture. Barnyardgrass and weedy rice control with oxyfluorfen is achieved with timely applications; however, injury to oxyfluorfen-resistant rice is likely.

Introduction

United States producers planted 1,000,000 ha of rice in 2021, with Arkansas accounting for approximately 48% of total domestic production (USDA-NASS 2022). Rice producers worldwide face an increasing demand for rice on a diminishing number of hectares (Nguyen and Ferrero 2006; Rosa et al. 2020). Abiotic and biotic stresses, such as water limitations, are significant challenges facing rice producers (Labrada 2006; Norsworthy et al. 2013). Standard weed control methods for rice production consist of crop rotation, permanent flooding, and chemical control (Al-Khatib et al. 2019; Butts et al. 2022). When using chemical control tactics, timely applications are required to reduce weed competition with the crop and to reduce the likelihood of selecting herbicide-resistant individuals within a given weed population (Metzger et al. 2019).

Herbicide applications in rice production fields may not always be timely and can lead to the selection of herbicide-resistant weeds (Norsworthy et al. 2012). Additionally, spraying weeds that are larger than what is recommended on the label can lead to herbicide failure. Weeds escaping an herbicide application may result in plants reaching the reproductive stage and producing seeds. The surviving seeds from plants exposed to an herbicide application will be deposited into the soil seedbank, germinate in subsequent growing seasons, and produce plants that are likely to be less sensitive to the herbicide (Belz 2020).

Barnyardgrass is currently resistant to seven herbicide modes of action (MOAs) in the United States, whereas weedy rice is resistant to two MOAs (Heap 2023; J. K. Norsworthy, personal communicati, January 2023). Weedy rice is tolerant to all conventional rice herbicides due to genetic similarity with cultivated rice (Burgos et al. 2021). In a survey of Arkansas crop consultants and producers, barnyardgrass and weedy rice were the first and third most problematic weeds, respectively, in flooded rice, whereas barnyardgrass was the most problematic weed in furrow-irrigated rice (Butts et al. 2022). Rice producers have reported spending \$266 ha^{-1} on chemical weed control, with 81% of the costs focused on controlling barnyardgrass. Reduced rice yield, dockage on grain quality due to weed seed contamination, and extra cost for weed control through cultural or mechanical practices are additional economic detriments associated with uncontrolled herbicide-resistant barnyardgrass and weedy rice (Norsworthy et al. 2012).

Clearfield® (BASF, Research Triangle Park, NC, USA) rice was the first commercially grown herbicide-resistant rice technology, allowing for the use of acetolactate synthase (ALS)-inhibiting herbicides within the imidazolinone family to control grass weeds and weedy rice during the growing season (Linscombe 2015). Imazethapyr (Newpath®, BASF) and imazamox (Beyond®, BASF) are both Herbicide Resistance Action Committee/Weed Science Society of America (HRAC/WSSA) Group 2 herbicides that inhibit ALS. Weedy rice populations in some areas have confirmed resistance to these two herbicides commonly used in imidazoline-resistant rice (Heap 2023). Provisia® (BASF) rice was released in 2018 to allow for in-crop use of the acetyl coenzyme A carboxylase–inhibiting herbicide quizalofop-p-ethyl. Quizalofop-p-ethyl is one of the few options for weedy rice control in areas where weedy rice is resistant to ALS-inhibiting herbicides (Barber et al. 2022; Heap 2023). To prevent weedy rice from developing target-site resistance to quizalofop-p-ethyl, producers should rotate herbicide MOA and use cultural control techniques, among other strategies (Barber et al. 2022; Norsworthy et al. 2012).

Rice resistant to oxyfluorfen (ROXY®, Albaugh, St. Joseph, MO, USA) is currently under development (McKenzie 2017). An HRAC/WSSA Group 14 herbicide, oxyfluorfen inhibits the protoporphyrinogen IX oxidase (PPO) enzyme. Oxyfluorfen is currently labeled for control of barnyardgrass and other weeds around conifers and deciduous trees, almonds [*Prunus dulcis* (Mill.) D.A. Webb], pistachios (*Pistacia vera* L.), walnuts (*Juglans regia* L.), and windbreaks (Anonymous 2014). Incorporating the oxyfluorfen-resistant trait into rice production systems could lead to the herbicide being used as an additional option to control barnyardgrass and weedy rice in the crop (Boyd 2021).

Acifluorfen, carfentrazone, and saflufenacil are PPO-inhibiting herbicides used in rice weed control programs; however, most PPO herbicides are ineffective against grasses, partly because of limited translocation throughout the plant, especially to the belowground growing point (EPA 2002; VanGessel 2014). These three herbicides are used primarily to control broadleaf weeds and sedges (Barber et al. 2022). Weedy rice and barnyardgrass have no documented resistance to oxyfluorfen or other PPO herbicides (Heap 2023). Although oxyfluorfen-resistant rice is a new technology, producers must follow good stewardship practices, such as crop rotation, effectively controlling all weeds before planting, using multiple MOAs to help prevent resistance, and preventing surviving plants from producing seed (Boyd 2021; Norsworthy et al. 2012).

This research aimed to understand the efficacy of oxyfluorfen on barnyardgrass and weedy rice over a range of application timings and weed sizes in a paddy rice system. Additionally, experiments were conducted to evaluate the tolerance of oxyfluorfen-resistant rice to preemergence- and postemergence-applied oxyfluorfen over a range of soil moisture regimes.

Materials and Methods

Barnyardgrass, Weedy Rice, and Oxyfluorfen-Resistant Rice Response to Oxyfluorfen in the Greenhouse

Greenhouse studies were conducted in 2021 and 2022 at the Milo J. Shult Agriculture Research and Extension Center in Fayetteville, AR. A Captina silt loam soil (fine-silty, siliceous, active, mesic Typic Fragiudults) (USDA-NRCS 2022) with 20% sand, 66% silt, 14% clay, and 2.3% organic matter was collected and sieved to remove large pieces of residue and reduce the size of large soil aggregates. Oxyfluorfen-resistant rice, barnyardgrass, and weedy rice were hand planted in separate 11-cm-diameter by 15-cm-depth pots at a depth of 1.3 cm. The trials were initiated on November 5, 2021, and March 3, 2022. After emergence, the plants in each pot were thinned to four. The soil was saturated for both experiments until the plants began to tiller to simulate a situation in which the herbicide should be readily available for uptake. When the nontreated plants began to tiller, the pots were placed in 1.2×0.51 m trays and flooded to a depth of 2.5 cm. Urea (46-0-0) was applied to all species after flooding at a rate of 317 kg ha^{-1}.

The experiment was designed as a randomized complete block with two factors (application timing and herbicide rate) and four replications. Oxyfluorfen was applied at 560, 1,120, and 1,680 g ai ha^{-1} to plants at three different development stages: 1-, 2-, and 3-leaf. A nontreated control was included for comparison. Each herbicide application was mixed with 1% v/v methylated seed oil (MSO). All applications were made in a spray chamber with two flat-fan 1100067 nozzles (TeeJet® Technologies, Springfield, IL, USA) calibrated to deliver 187 L ha^{-1} at 1.61 km h^{-1}. Visible control and injury ratings were recorded at 7, 14, 21, and 28 DAA d after application (DAA). In addition, aboveground biomass was collected 28 DAA, dried at 66 C for 3 d to constant mass, weighed, and compared to the nontreated control.

Oxyfluorfen-Resistant Rice Sensitivity and Weedy Rice Control with Oxyfluorfen in the Field

Field experiments evaluated the influence of plant size on weedy rice control and oxyfluorfen-resistant rice injury with oxyfluorfen. In 2021, a field trial was conducted on a Dewitt silt loam soil (fine, smectitic, thermic Typic Albaqualfs) (USDA-NRCS 2022) with 27% sand, 54% silt, 19% clay, 1.75% organic matter, and pH 5.6 at the Rice Research and Extension Center, near Stuttgart, AR. In 2022, the trial was conducted on a Calloway silt loam soil (fine-silty, mixed, active, thermic Aquic Fraglossudalfs) (USDA-NRCS 2022) with 12% sand, 70% silt, 18% clay, and 1.02% organic matter at the Pine Tree Research Station, near Colt, AR. The seedbed was prepared using conventional tillage before planting at both locations. A long-grain rice cultivar resistant to oxyfluorfen (ROXY®, Albaugh) and weedy rice were planted at 72 seeds m row^{-1}. Oxyfluorfen-resistant rice and weedy rice were drill-seeded in separate rows using a nine-row, small-plot drill. The four left rows of the drill were planted to oxyfluorfen-resistant rice, followed by a skip row and then four rows of weedy rice on the right side of each plot. Each plot was 5.2 m × 1.8m, with 19 cm between rows. Alleys 1 m in width were maintained between plots to prevent contamination among plots. The trials were planted on May 16, 2021, and May 17, 2022. In both years, clomazone (Command® 3ME, FMC, Philadelphia, PA, USA) was applied preemergence at 336 g ai ha^{-1}. In both years, the rice was flooded to a 6- to 10-cm depth 2 d after the tillering application. Soil fertility was managed based on soil testing and published recommendations (Roberts et al. 2021).

The experiment was designed as a randomized complete block with two factors (application timing and herbicide rate) and four replications. Oxyfluorfen (Albaugh) was applied at 560, 1,120, and 1,680 g ai ha^{-1} at the 1-, 2-, and 3-leaf stages and at tillering. Additionally, a nontreated control was included for comparison.

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Each application contained MSO at 1% v/v. All herbicide applications were made with a CO_2-pressurized backpack sprayer and a four-nozzle boom, using AIXR 110015 nozzles (TeeJet® Technologies) calibrated to deliver 140 L ha^{-1} at 4.8 km h^{-1}. Visible oxyfluorfen-resistant rice injury and weedy rice control were rated every 7 d until 28 DAA for each application on a scale of 0% to 100%, where 0% equals no weed control or rice crop injury and 100% equals complete control or rice crop death (Frans and Talbert 1977). Shoot counts (two 1-m sections of row) of weedy rice were taken 28 d after each application timing. Relative weedy rice shoot counts were determined by dividing the shoot counts in the treated plots by the shoot counts of the nontreated control.

Soil Moisture Influence on Oxyfluorfen-Resistant Rice Tolerance to Oxyfluorfen

Two greenhouse experiments were conducted at the Milo J. Shult Agricultural Research and Extension Center in Fayetteville, AR, to evaluate the effect of soil moisture on oxyfluorfen-resistant rice following an application of oxyfluorfen. A Captina silt loam soil (fine-silty, siliceous, active, mesic Typic Fragiudults) with 20% sand, 66% silt, 14% clay, and 2.3% organic matter was dried for 14 d at an air temperature of 33 C before initiation of the experiment and then placed in 11.4-L buckets. Oxyfluorfen-resistant rice was planted at 18 seeds bucket^{-1} (bucket diameter = 25.4 cm). Bulk density and field capacity were calculated using Soil Plant Air Water (SPAW) software (Agricultural Research Service, U.S. Department of Agriculture, Washington, DC, USA) to determine the amount of water needed to maintain 40%, 60%, 80%, and 100% saturation:

$$\text{Field capacity/Bulk density} \times \text{Targeted \% moisture} \times \text{Mass of soil} = \text{Water needed}\,(31.5\% \div 1.42 \times 100\% \times 8{,}000\text{g} = 1{,}775\text{g}) \quad [1]$$

In the first experiment, oxyfluorfen was applied PRE at 1,120 g ai ha^{-1}. In the second experiment, oxyfluorfen at 1,120 g ai ha^{-1} was applied POST at the 2-leaf growth stage. Each experiment was organized as a completely randomized design with four replications. In the PRE experiment, buckets of soil were brought to and maintained at the desired percent saturation after applying oxyfluorfen. In the POST experiment, buckets of soil were watered to 40% saturation before seeding to prevent the seeds from floating to the surface, then seeded. At the 1-leaf rice growth stage, the buckets of soil were watered to the desired saturation level after weighing every 24 h, throughout the duration of the trial.

Herbicide applications were made in a spray chamber at 187 L ha^{-1} and 1.6 km h^{-1} with 1100067 flat-fan nozzles. Additionally, a nontreated control was included with both experiments for comparison. Visible rice injury was rated on a scale of 0% to 100% every 7 d until 28 d after the PRE application and every 7 d until 21 d after the POST application (Frans and Talbert 1977). Overhead images were collected 61 cm above the canopy at each evaluation timing and later used to calculate groundcover. The percent groundcover in each image was determined using FieldAnalyzer (Green Research Services, Fayetteville, AR, USA). All aboveground biomass was collected 28 d and 21 d after the PRE and POST applications, respectively. The biomass samples were dried at 66 C for 3 d to constant mass, weighed, and made relative to the nontreated control.

Data Analysis

Data were analyzed using JMP Pro version 16.0 and SAS version 9.4 (SAS Institute, Cary, NC, USA). Data distribution was analyzed as continuous using the Fit Model function, and the Akaike information criterion was used to select the best fit for the data. All data were subjected to an analysis of variance. Main effects were separated using Fisher's protected least significant difference ($\alpha = 0.05$), and interactions were separated using Tukey's honestly significant difference ($\alpha = 0.05$) to minimize Type I error.

Barnyardgrass, Weedy Rice, and Oxyfluorfen-Resistant Rice Response to Oxyfluorfen

All data were normally distributed for the field and greenhouse experiments. In the greenhouse, the experimental run did not interact with timing or rate in most instances; therefore data were averaged over site-year, where site-year was considered random. A Pearson's correlation coefficient was used to evaluate the relationship between the level of injury to oxyfluorfen-resistant rice and weedy rice control at 7 and 28 DAA.

Soil Moisture Influence on Tolerance

All data evaluated in the PRE and POST trials best fit a gamma distribution, except for groundcover in the POST trial, which was normally distributed. Normally distributed data were analyzed using the Fit Model in JMP Pro, while injury data were analyzed using the GLIMMIX procedure in SAS (Gbur et al. 2012). Application timing averaged by site-year was significant.

Results and Discussion

Barnyardgrass, Weedy Rice, and Oxyfluorfen-Resistant Rice Response to Oxyfluorfen in the Greenhouse

Barnyardgrass

Barnyardgrass control with oxyfluorfen ranged from 86% to 99% at 7 DAA over the application timings evaluated, with control lowest when applications were delayed until the 3-leaf stage of barnyardgrass (Table 1). By 28 DAA, there was a difference in barnyardgrass control among application timings averaged over rate, but control at all timings averaged ≥98%. The 1- and 2-leaf stage applications (99% and 97% control, respectively) of oxyfluorfen resulted in greater barnyardgrass control than the 3-leaf application timing (86% control). Chauhan and Abugho (2012) found that WSSA herbicide Groups 1 and 2 effectively control smaller barnyardgrass plants. Oxyfluorfen applied at 560 g ai ha^{-1} resulted in barnyardgrass control of 98%, 97%, and 86%, respectively, at the 1-, 2-, and 3-leaf growth stage, 7 DAA. The barnyardgrass did not regrow; control was 100%, 100%, and 98% for the 1-, 2-, and 3-leaf growth stage application timing at 28 DAA. Oxyfluorfen applied before flooding in a water-seeded rice system provided 93% control of *Echinochloa* spp. (Galvin et al. 2022).

Applying oxyfluorfen reduced barnyardgrass biomass by ≥98% in all application timings and oxyfluorfen rates compared to the nontreated control (Table 1). Biomass reduction should be expected because a high percentage of barnyardgrass control was observed at 28 DAA. Averaging barnyardgrass control over the rate of oxyfluorfen resulted in a 99%, 100%, and 98% reduction in biomass, respectively. Oxyfluorfen is labeled to control barnyard-grass and other grass species, further suggesting that the herbicide will reduce biomass (Anonymous 2014). In a study conducted by

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Table 1. Visible barnyardgrass control and percent biomass reduction observed after an application of oxyfluorfen as influenced by the rate and timing of application in a greenhouse study conducted at the Milo J. Shult Agricultural Research and Extension Center in 2021 and 2022.[a,b,c]

Factor	Timing	Rate	Barnyardgrass control 7 DAA	Barnyardgrass control 28 DAA	Biomass reduction
		g ai ha^{-1}	%	%	%
Rate		560	94	99	99
		1,120	94	99	100
		1,680	94	99	99
P-value			0.9242	0.8623	0.5809
Timing	1-leaf		99 a	100 a	99
	2-leaf		97 a	100 a	100
	3-leaf		86 b	98 b	98
P-value			<0.0001	0.0087	0.1405
Timing × Rate	1-leaf	560	98	100	99
		1,120	100	100	100
		1,680	100	100	98
	2-leaf	560	97	100	100
		1,120	96	100	100
		1,680	97	100	100
	3-leaf	560	86	97	98
		1,120	86	99	99
		1,680	85	98	98
P-value			0.9683	0.9078	0.9145

[a]Analyses were conducted using the Fit Model in JMP Pro.
[b]Abbreviation: DAA, days after treatment.
[c]Means within the same column for rate or timing followed by the same letter are not significantly different according to Fisher's protected least significant difference (α = 0.05). Means within the same column for Timing × Rate followed by the same letter are not significantly different according to Tukey's honestly significant difference (α = 0.05).

Table 2. Visible weedy rice control and percent biomass reduction observed after an application of oxyfluorfen by rate and timing from a greenhouse study conducted at the Milo J. Shult Agricultural Research and Extension Center in 2021 and 2022.[a,b,c]

Factor	Timing	Rate	Weedy rice control 7 DAA	Weedy rice control 28 DAA	Biomass reduction
		g ai ha^{-1}	%	%	%
Rate		560	65 b	81	75 b
		1,120	79 a	94	91 a
		1,680	80 a	94	90 a
P-value			<0.0001	<0.0001	0.0004
Timing	1-leaf		82 a	97	84 ab
	2-leaf		72 b	93	92 a
	3-leaf		70 b	79	80 b
P-value			0.0006	<0.0001	0.0177
Timing × Rate	1-leaf	560	73	96 a	72
		1,120	84	99 a	92
		1,680	88	98 a	87
	2-leaf	560	58	84 a	86
		1,120	79	98 a	95
		1,680	80	96 a	96
	3-leaf	560	64	63 b	65
		1,120	74	86 a	87
		1,680	73	88 a	87
P-value			0.2740	0.0108	0.6756

[a]Analyses were conducted using the Fit Model in JMP Pro.
[b]Abbreviation: DAA, days after treatment.
[c]Means within the same column for rate or timing followed by the same letter are not significantly different according to Fisher's protected least significant difference (α = 0.05). Means within the same column for Timing × Rate followed by the same letter are not significantly different according to Tukey's honestly significant difference (α = 0.05).

Chauhan and Abugho (2012), a decrease in the biomass of barnyardgrass and junglerice [*Echinochloa colona* (L.) Link] was observed when herbicides were applied at the 4-leaf growth stage of the weeds.

Weedy Rice

Weedy rice control at 7 DAA ranged from 58% to 88% for the application timings and oxyfluorfen rates evaluated (Table 2). However, only both main effects of application timing and oxyfluorfen rate were significant at 7 DAA for weedy rice control (Table 2). The lowest rate of oxyfluorfen (560 g ha^{-1}) was less effective at controlling weedy rice than the two higher rates. Weedy rice control also declined when oxyfluorfen was applied beyond the 1-leaf stage, averaged over rates. By 28 DAA, weedy control ranged from 63% to 99%, with the lowest control provided by oxyfluorfen at 560 g ha^{-1} applied to 3-leaf weedy rice. Oxyfluorfen is currently not labeled to control weedy rice, possibly due to the moderate to good control provided when applied to vegetative weedy rice plants (Anonymous 2014; Frans and Talbert 1977). The trend of decreasing weedy rice control as application time is delayed should be expected when using oxyfluorfen because weeds are more difficult to control as size increases (Anonymous 2014; Sosnoskie 2017). An increase in the rate of oxyfluorfen should cause an increase in weedy rice control, as the increased amount of herbicide will be more difficult for the plant to metabolize (Singh and Singh 2004).

Weedy rice biomass reductions reflected control ratings. Weedy rice biomass was reduced by 65% to 96% in all application timings and at all rates of oxyfluorfen application (Table 2). Averaged over timings, oxyfluorfen at 560 g ha^{-1} reduced biomass by 75%, which was less than the 90% to 91% reduction resulting from the two higher rates. Averaged over oxyfluorfen rates, delaying application of the herbicide until the 3-leaf stage of weedy rice reduced control compared to a 2-leaf application. On the basis of these findings, weedy rice control is not likely to be achieved with oxyfluorfen alone; therefore multiple herbicide MOAs should be used to prevent escapes after an application of oxyfluorfen (Norsworthy et al. 2012).

Oxyfluorfen-Resistant Rice

As much as 58% injury to oxyfluorfen-resistant rice occurred at 7 DAA of oxyfluorfen at 560 g ha^{-1} to 1-leaf rice (Table 3). Averaged over application timings, injury to oxyfluorfen-resistant rice generally increased with oxyfluorfen rate at 7 and 28 DAA. When averaged over oxyfluorfen rates, less injury to the crop occurred when applications were delayed until the 3-leaf growth stage than occurred at the 1- or 2-leaf growth stage at 7 and 28 DAA. Similarly, reductions in biomass of oxyfluorfen-resistant rice followed trends to injury assessments, with a general increase in tolerance at lower rates of the herbicide and application to larger plants. The earlier growth stages would be expected to have the greatest injury due to the small plants' inability to metabolize herbicide (Chauhan and Abugho 2012). Saflufenacil and carfentrazone, alternative PPO-inhibiting herbicides, have shown the potential to cause injury to rice cultivars (Montgomery et al. 2014).

Oxyfluorfen-Resistant Rice Sensitivity and Weedy Rice Control with Oxyfluorfen in the Field

Oxyfluorfen-Resistant Rice

In 2021, the main effect of application timing was significant, whereas both main effects (application timing and oxyfluorfen

Table 3. Visible oxyfluorfen-resistant rice injury and percent biomass reduction observed after an application of oxyfluorfen by rate and timing from a greenhouse study conducted at the Milo J. Shult Agricultural Research and Extension Center in 2021 and 2022.[a,b,c]

Factor	Timing	Rate	Oxyfluorfen-resistant rice injury 7 DAA	Oxyfluorfen-resistant rice injury 28 DAA	Biomass reduction
		g ai ha^{-1}	%		
Rate		560	35 c	34 b	45 b
		1,120	43 b	37 ab	50 ab
		1,680	49 a	45 a	58 a
P-value			<0.0001	0.0346	0.0405
Timing	1-leaf		46 a	48 a	58 a
	2-leaf		49 a	41 a	53 a
	3-leaf		31 b	28 b	42 b
P-value			<0.0001	0.0001	0.0057
Timing × Rate	1-leaf	560	39	41	52
		1,120	43	43	56
		1,680	58	59	67
	2-leaf	560	41	30	42
		1,120	54	39	52
		1,680	54	54	64
	3-leaf	560	26	31	41
		1,120	31	29	44
		1,680	34	24	42
P-value			0.0775	0.0537	0.4147

[a]Analyses were conducted using the Fit Model in JMP Pro.
[b]Abbreviation: DAA, days after treatment.
[c]Means within the same column for rate or timing followed by the same letter are not significantly different according to Fisher's protected least significant difference ($\alpha = 0.05$). Means within the same column for Timing × Rate followed by the same letter are not significantly different according to Tukey's honestly significant difference ($\alpha = 0.05$).

rate) were significant in 2022 for injury to oxyfluorfen-resistant rice in the field (Table 4). Like the greenhouse trial, injury to oxyfluorfen-resistant rice generally increased with rate, whereas an application to smaller rice had a tendency to increase the risk for injury. For instance, averaged over application timings, oxyfluorfen at 1,680 g ha^{-1} caused 79% injury to rice at 7 DAA in 2021. This level of injury would likely cause alarm to a grower. When averaged over rate, oxyfluorfen-resistant rice was injured the least at 7 DAA when applications were delayed until the tillering growth stage (Table 4).

Although direct statistical comparisons cannot be made between 7 and 28 DAA injury ratings, oxyfluorfen-resistant rice generally appeared to be recovering from initial damage by 28 DAA (Table 4). Numerically, the injury was greater in 2021 than in 2002, which may be a result of 21.5 cm of rainfall over a 4-d period in 2021, which resulted in the rice being underwater for multiple days between the 2- and 3-leaf growth stages, reducing the amount of herbicide that could be metabolized (USDA-ARS 2022). In severe cases of flooding, rice leaf and stem dry weight and the growth rate decrease following a flood event (Singh et al. 2014).

Weedy Rice Control

In 2021, the interaction between application timing and the rate of oxyfluorfen was significant; however, in 2022, only the main effect of application timing was significant at 7 DAA. Applying oxyfluorfen to weedy rice across all rates and application timings resulted in 41% to 100% weedy rice control in 2021 and 25% to 97% in 2022 (Table 4). At 7 DAA in 2021, weedy rice control ranged from 41% at the tillering growth stage with 560 g ha^{-1} oxyfluorfen application to 99% control at the 1-leaf growth stage when oxyfluorfen was applied at 1,120 g ha^{-1}. By 28 DAA, weedy rice control ranged from 31% when 560 g ha^{-1} of oxyfluorfen was applied at the tillering growth stage to 85% control when oxyfluorfen was applied at a rate of 1,680 g ha^{-1} at the 1-leaf growth stage. Weedy rice control is a function of the size of each plant at the time of application, as the smallest weeds should have the greatest amount of control (Anonymous 2014; Sarangi and Jhala 2018). In 2022, weedy rice control ranged from 35% to 93% when the application timing was averaged over the rate of oxyfluorfen at 7 DAA. By 28 DAA, the main effect of application timing was not significant in 2022; however, the weedy rice control ranged from 12% to 83%. The differing results in 2021 and 2022 may result from a significant rainfall between the 2- and 3-leaf growth stages (USDA-ARS 2022). Because oxyfluorfen-resistant rice and weedy rice are both *Oryza* spp., a flood event could cause an increase in weedy rice control because a flood will cause increased injury to cultivated rice (Singh et al. 2014).

Relative weedy rice shoot counts collected in 2022 showed that oxyfluorfen at 560 g ha^{-1} applied at the 1-, 2-, 3-leaf, and tillering growth stages resulted in 57%, 82%, 70%, and 112% relative shoot counts, respectively (Table 4). The variation in relative weedy rice shoot counts could be caused by too small of a sample size or slight variation within the trial. Oxyfluorfen applications across all rates and timings resulted in relative weedy rice shoot counts of 11% to 112% compared to the nontreated control. Weedy rice shoots were reduced at the earliest timing because of the small plants at the time of application, leading to more injury and fewer live plants (Anonymous 2014; Sarangi and Jhala 2018).

Effect of Soil Moisture on Preemergence Oxyfluorfen Activity

Rice plants injured from the PRE application of oxyfluorfen were lighter green and smaller than the plants in the nontreated checks. Overall, the PRE application of oxyfluorfen resulted in unhealthy rice plants 7 d after emergence (DAE). At 7 DAE, 51% to 75% injury was observed across all soil moisture levels after applying oxyfluorfen (Table 5). At 14 and 21 DAE, injury to oxyfluorfen-resistant rice ranged from 43% to 68% and from 39% to 56%, respectively, across all soil moisture levels. The greatest injury observed with a PRE application of oxyfluorfen occurred when the soil moisture level was at 100% saturation, likely due to the herbicide availability under saturated conditions. By 28 DAE, injury to rice ranged from 27% to 47% across all soil moisture levels. In general, there were increasing levels of oxyfluorfen-resistant rice injury as the amount of soil moisture increased. Higher amounts of water in the soil will allow for greater uptake by the plant, resulting in a plant growing more than a drought-stressed plant (Paiman and Effendy 2019). The higher amount of injury associated with the higher soil moisture level can also be attributed to the herbicide being readily available for plant uptake (Ross and Lembi 1985). Similarly, applications of PPO-inhibiting herbicides have injured soybean [*Glycine max* (L.) Merr.] when applied under cool, wet soil conditions (Jhala 2017).

The groundcover reduction of oxyfluorfen-resistant rice was similar across all treatments because of the nature of the injury observed with oxyfluorfen applied PRE. All treatments reduced ground cover by 70% to 90% compared to the nontreated check at each soil moisture level from 7 to 21 DAE (data not shown). By 28 DAE, all ground cover was reduced by 52% to 76%. Treatments with 40% and 60% saturation soil moisture levels resulted in greater relative biomass than the treatment with a soil moisture level of 100% saturation (Table 5). All treatments produced

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Table 4. Ratings of oxyfluorfen-resistant rice injury, weedy rice control, and percent relative shoot counts observed after oxyfluorfen application at four timings and three rates in a study conducted at the Rice Research and Extension Center near Stuttgart, AR, in 2021 and at the Pine Tree Research Station near Colt, AR, in 2022.[a,b,c,d]

			Injury				Weedy rice control				Relative shoot count
			7 DAA		28 DAA		7 DAA		28 DAA		
Factor	Timing	Rate	2021	2022	2021	2022	2021	2022	2021	2022	2022
		g ai ha^{-1}	%								
Rate		560	62 b	18	47 b	6	83	61	61	31	83
		1,120	75 a	22	54 ab	9	89	68	67	35	76
		1,680	79 a	27	63 a	7	91	69	79	51	67
P-value			0.0017	0.1179	0.0046	0.5714	0.0130	0.4746	0.0019	0.0554	0.4797
Timing	1-leaf		74 a	50 a	66 a	26 a	97	93 a	74	83	33 b
	2-leaf		87 a	21 b	64 a	8 b	85	72 b	72	51	78 a
	3-leaf		79 a	20 b	61 a	4 b	92	43 c	77	17	85 a
	Tillering		42 b	8 c	27 b	3 b	61	35 c	51	12	102 a
P-value			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0335	0.05701	0.0005
Timing × Rate	1-leaf	560	56	47	51	27	94 a–c	89	69 a	71	57
		1,120	80	40	65	26	99 a	95	65 ab	87	30
		1,680	81	61	79	27	97 ab	95	85 a	89	11
	2-leaf	560	82	16	64	6	85 b–e	69	63 ab	44	82
		1,120	87	21	63	16	81 c–e	74	71 a	59	82
		1,680	90	28	79	6	87 b–e	72	81 a	48	68
	3-leaf	560	66	16	49	3	91 a–d	55	77 a	17	70
		1,120	79	22	60	4	86 b–e	36	73 a	11	102
		1,680	87	23	73	4	95 a–c	38	80 a	27	84
	Tillering	560	40	7	24	3	41 f	22	31 b	5	112
		1,120	43	10	29	3	66 ef	42	57 ab	3	90
		1,680	44	8	29	3	74 e	45	65 ab	10	105
P-value			0.3719	0.5945	0.2801	0.8167	0.0411	0.2883	0.0287	0.0601	0.6177

[a]Shoot densities were not assessed as nontreated in 2021 at 28 d after each timing. In 2022, average shoot densities at 28 DAA in the nontreated for the 1-leaf, 2-leaf, 3-leaf, and tillering timings were 26, 42, 78, and 80 plants m row^{-1}, respectively.
[b]Analyses were conducted using the GLIMMIX procedure in SAS version 9.4.
[c]Abbreviation: DAA, days after application.
[d]Means within the same column for rate or timing followed by the same letter are not significantly different according to Fisher's protected least significant difference ($\alpha = 0.05$). Means within the same column for Timing × Rate followed by the same letter are not significantly different according to Tukey's honestly significant difference ($\alpha = 0.05$).

Table 5. Soil moisture influence on oxyfluorfen-resistant rice sensitivity to oxyfluorfen applied preemergence averaged over the years.[a,b]

	Injury[c]				Relative biomass[d]
Soil moisture	7 DAE	14 DAE	21 DAE	28 DAE	28 DAE
% saturation	%				
40	53 a	45 a	39 a	27 c	51 a
60	52 a	43 a	39 a	29 bc	51 a
80	51 a	44 a	43 a	34 b	44 ab
100	75 b	68 b	56 b	47 a	34 b
P-value	<0.0001	<0.0001	<0.0001	<0.0001	0.02889

[a]Abbreviation: DAE, days after emergence.
[b]Means within the same column that do not contain the same letter are significantly different according to Fisher's protected least significant difference ($\alpha = 0.05$).
[c]P-values were generated using GLIMMIX in SAS version 9.4
[d]P-value was generated using the Fit Model in JMP Pro.

Table 6. Soil moisture influence on oxyfluorfen-resistant rice sensitivity to oxyfluorfen applied postemergence averaged over the years.[a,b,c]

	Injury			Relative biomass
Soil moisture	7 DAE	14 DAE	21 DAE	21 DAE
% saturation	%			
40	63 b	49 b	45 b	30 a
60	68 ab	66 a	60 a	16 b
80	70 a	61 a	59 a	21 b
100	63 b	62 a	61 a	15 b
P-value	0.0303	0.0001	0.0002	0.0045

[a]Abbreviation: DAE, days after emergence.
[b]Means within the same column that do not contain the same letter are significantly different according to Fisher's protected least significant difference ($\alpha = 0.05$).
[c]P-values were generated using GLIMMIX in SAS version 9.4.

biomass that was 34% to 51% of the relative biomass produced in the nontreated plots. The decrease in biomass at higher soil moisture levels results from increased injury from the herbicide due to the excess moisture in the soil (Ross and Lembi 1985).

Effect of Soil Moisture on Postemergence Oxyfluorfen Activity

In the POST trial, necrotic, irregularly shaped lesions where the droplet landed on the rice leaf surface were the observed injury from oxyfluorfen. There was additional necrotic tissue, and the plants' overall health was reduced compared to the nontreated plants. Injury ranged from 63% to 70% across all treatments at 7 DAA from POST oxyfluorfen applications (Table 6). Injury to oxyfluorfen-resistant rice ranged from 49% to 66% at 14 DAA for all soil moisture levels. Across all treatments, 45% to 61% injury was observed 21 DAA after applying oxyfluorfen. Oxyfluorfen applied at 40% soil saturation resulted in less injury to rice than when applied at all other soil moisture levels at 21 DAA. The lower injury associated with the dry conditions could be due to the plants developing a thicker cuticle, which lessens adsorption of the herbicide compared to higher soil moisture levels (Shrestha 2016).

All treatments produced 15% to 30% relative biomass of oxyfluorfen-resistant rice compared to the nontreated plots (Table 6). Rice in the bucket maintained at soil moisture of 40% saturation resulted in the greatest relative biomass due to the reduced injury associated with the application of oxyfluorfen. Rice

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in the driest treatment produced the most biomass due to the reduced injury associated with the treatment. Owing to increased plant growth, postemergence applications are most effective at soil moisture levels above 50% saturation (Cox et al. 2016). The increase in herbicide efficacy at soil moisture levels >50% saturation could be due to the plants not developing a thicker cuticle to retain moisture. Therefore herbicide entry into the plant is easier, leading to increased injury and reduced biomass.

At 7 DAA, percent groundcover reduction ranged from 82% to 94% (data not shown). At 14 DAA, groundcover was reduced by 93% to 95% (data not shown) compared to the nontreated plots, which was likely a result of the treated rice plants not beginning to regrow at this timing. PPO-inhibiting herbicides can also inhibit photosynthesis in plants; therefore, if enough leaf area becomes covered, the plant will not be able to overcome the effects of the herbicide (Rangani et al. 2019). Groundcover was reduced from 91% to 94% (data not shown) at 21 DAA across all treatments. The amount of groundcover observed at 14 and 21 DAA decreased due to the severe injury from applying oxyfluorfen POST.

Practical Implications

If labeled, oxyfluorfen use with the ROXY® rice production system would allow producers to use an additional MOA when barnyardgrass or weedy rice is present in a field. Using a Group 14 herbicide would allow producers an additional MOA to control herbicide-resistant barnyardgrass and weedy rice (Heap 2023). Application timing of oxyfluorfen is critical to the success of the herbicide for weed control and crop tolerance. Oxyfluorfen should be applied at the 1- and 2-leaf growth stages to control both barnyardgrass and weedy rice adequately. Oxyfluorfen should be used very carefully to achieve complete control of barnyardgrass and be combined with alternative MOAs to achieve complete weedy rice control; however, complete weedy rice control will be difficult without severely injuring oxyfluorfen-resistant rice.

A major concern associated with using oxyfluorfen for barnyardgrass and weedy rice control is the injury to the rice crop. Oxyfluorfen applied at all application timings and rates injured oxyfluorfen-resistant rice that may persist through the growing season (Frans and Talbert 1977). When oxyfluorfen was applied PRE, the greatest injury was observed at the 100% soil moisture level, indicating that oxyfluorfen should not be applied immediately prior to a rain event. Oxyfluorfen applications PRE could potentially injure producers' rice in the mid-southern United States due to the rainfall that occurs during the vegetative stage of rice. When oxyfluorfen was applied POST in the greenhouse, no soil moisture levels showed the ability for oxyfluorfen-resistant rice to recover from an application of oxyfluorfen.

Owing to the injury observed to oxyfluorfen-resistant rice, no recommendation could be made to achieve adequate weed control and avoid crop injury lasting through the growing season. Weed control and injury to oxyfluorfen-resistant rice could be altered due to slight changes in plant size. This could be troublesome for producers because of weather patterns common in Arkansas when rice plants are in the vegetative stages. If oxyfluorfen becomes labeled for use in rice, producers should make timely applications to achieve control of barnyardgrass and weedy rice.

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