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Quizalofop-resistant rice response to quizalofop when exposed to low rates of glyphosate and imazethapyr

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Research Article

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Abstract

Injury to quizalofop-resistant rice was reported in some fields following postemergence applications of quizalofop. Glyphosate-resistant (GR) corn, cotton, and soybean, and imidazolinone-resistant rice are grown near quizalofop-resistant rice. Herbicide drift from glyphosate and imazethapyr and the resulting crop injury and potential yield loss is a cause of concern for producers. Field experiments conducted near Colt, and Keiser, AR, in 2021 evaluated whether low rates of glyphosate or imazethapyr interact with sequential quizalofop applications to exacerbate injury to quizalofop-resistant rice compared to quizalofop applications alone. Herbicide treatments consisted of a low rate of glyphosate (90 g ae ha^{-1}) or imazethapyr (10.7 g ai ha^{-1}) applied 10, 7, 4, and 0 d before the 2-leaf growth stage of rice, and glyphosate or imazethapyr, at the same rate and timings, followed by quizalofop at 120 g ai ha^{-1} applied to 2-leaf rice. All plots treated with quizalofop received a subsequent application of the same herbicide and rate at the 5-leaf rice stage. At 28 d after final treatment (DAFT), glyphosate followed by quizalofop the same day to 2-leaf rice caused 77% injury compared with 58% when glyphosate was applied alone, regardless of location. Glyphosate followed by quizalofop the same day reduced rough rice grain yield by 67% compared with 33% when glyphosate was applied alone to 2-leaf rice at the Colt location. Application of imazethapyr followed by quizalofop the same day to 2-leaf rice caused more injury (63% and 19% injury at the Colt and Keiser locations, respectively) than imazethapyr alone (42% and 7% injury at the Colt and Keiser locations, respectively) at 35 DAFT. Overall, glyphosate and imazethapyr followed by quizalofop applications worsened injury compared to glyphosate, imazethapyr, and quizalofop applications alone. As the interval between exposure to a low rate of glyphosate or imazethapyr and quizalofop decreases, the detrimental effect of herbicide on rice likewise increases.

Introduction

Provisia® (BASF Corp., Research Triangle Park, NC) rice is a non-genetically modified herbicide-resistant grain developed using traditional breeding techniques. Provisia rice allows for postemergence applications of quizalofop (Provisia, BASF Corporation)and has been commercially available since 2018. Quizalofop is an acetyl coenzyme-A carboxylase (ACCase)–inhibiting herbicide used for managing acetolactate synthase–resistant barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.] and weedy rice (*Oryza sativa* L.), along with other annual and perennial grass weeds (Anonymous 2017; Guice et al. 2015). Provisia rice constituted 2.7% of the total rice hectares planted in Arkansas in the growing season of 2020. However, when evaluated in commercial rice trials (Frizzell et al. 2021, Hardke 2021), quizalofop-resistant rice has had lower yield potential resulting in reduced commercial adoption even though quizalofop is another tool for managing herbicide-resistant weedy rice (Roma-Burgos et al. 2021). A single-point mutation confers the resistance mechanism in quizalofop-resistant rice, and the presence of the resistant ACCase allele can be detected at low cost with standard molecular biology laboratory equipment. Therefore, rapid and effective development of new quizalofop-resistant varieties is feasible (Camacho et al. 2020; Pereira et al. 2019).

Conventional and quizalofop-resistant rice are often grown next to glyphosate-resistant (GR) corn, cotton, soybean, and imidazolinone-resistant rice, thereby increasing the potential for injury to conventional and quizalofop-resistant rice from herbicide drift. Conventional and quizalofop-resistant rice acreage is highly susceptible to injury from glyphosate and imidazolinone herbicides due to herbicide drift, tank contamination, or misapplication. From 2010 to

2020, out of 94 confirmed cases of herbicide drift onto conventional rice varieties in Arkansas, 35% and 14% of cases were caused by glyphosate and imazethapyr drift, respectively (personal communication with Susie Nichols of the Arkansas Department of Agriculture). In a 2019 survey representing 49% of all agronomic crop hectares in Arkansas, ground and aerial application equipment were used in almost equivalent proportions for herbicide applications, and herbicide drift was the primary concern for producers in terms of herbicide application challenges (Butts et al. 2021).

Glyphosate has been the most widely used herbicide in U.S. agronomic crops since the commercialization of GR crop technology in 1996 (Benbrook 2016). Glyphosate, a broad-spectrum, nonselective herbicide, is used preplant to control existing weeds and postemergence in genetically modified GR crops. Glyphosate inhibits enolpyruvyl shikimate-3-phosphate synthase, causing aromatic amino acid depletion by blocking the shikimic acid pathway (Amrhein et al. 1980; Shaner 2014). As a result of glyphosate application, growth ceases for sensitive plants, followed by chlorosis and necrosis symptomology, then plant death (Shaner 2014).

Glyphosate at sublethal rates caused 14% injury and 32% yield reduction of conventional rice when applied at the 3- to 4-leaf stage (Davis et al. 2011). In addition, sublethal glyphosate rates are detrimental to rice and have been shown to cause substantial injury and yield reductions ranging from 18% to 89% when plants were exposed to a 140 g ae ha^{-1} (1/8× rate of labeled use rate of 1,120 g ae ha^{-1}) at the 3- to 4-leaf to the booting rice stages (Kurtz and Street 2003). In Arkansas, a survey conducted in 2020 showed that glyphosate was used as a preplant option individually or in combination with protoporphyrinogen oxidase inhibitors, synthetic auxins, and diterpene biosynthesis inhibiting herbicides on 60% of total rice fields surveyed (Roma-Burgos et al. 2021). Therefore, glyphosate applied preplant could potentially cause injury to nearby emerged conventional and quizalofop-resistant rice fields in early rice growth stages.

Clearfield® (HorizonAg, LLC, Memphis, TN) rice is a nontransgenic, imidazolinone-resistant suite of rice cultivars developed using conventional plant-breeding methods (Croughan 2003). Additionally, FullPage® (RiceTec Inc., Alvin, TX) rice technology was commercialized in 2019. FullPage® rice is also an imidazolinone-resistant, non-genetically modified rice that allows for applications of imazethapyr (Preface™) and imazamox (Postscript™; Anonymous 2019). The same active ingredients are enabled in Clearfield rice under the trade names Newpath® and Beyond®. In the imidazolinone-resistant rice systems, imazethapyr is essential for preemergence (PRE) and postemergence (POST) weed control. Imazethapyr provides broad-spectrum weed control of sedges (*Cyperus* spp.), annual grasses, and broadleaf weeds (Barber et al. 2022). Imazethapyr obstructs the synthesis of the branched-chain amino acids leucine, isoleucine, and valine by inhibiting ALS or acetohydroxy acid synthase (Shaner 2014). Imazethapyr reduces plant growth within hours of herbicide application. The primary herbicide symptoms at 7 to 14 d after herbicide application are chlorosis in the meristematic region of the plant and necrosis throughout the plant (Shaner 2014).

The Clearfield rice technology was brought to market in 2002 and quickly adopted by rice producers. By 2013, 43% of the total U.S. rice hectares were planted with Clearfield rice cultivars (Nathan et al. 2020). In Arkansas, 37% of the total rice was planted with imidazolinone-resistant rice cultivars (30.6% and 6.4% with Clearfield and FullPage rice technology, respectively) in 2020 (Hardke 2021). In 2020, a survey conducted across Arkansas and adjacent states in the mid-South revealed that imazethapyr was applied as a POST option on 48% of rice fields (Roma-Burgos et al. 2021). After the large-scale adoption of imidazolinone-resistant rice, the risk for imazethapyr pre-exposure to conventional rice fields also escalated. Lower-than-labeled rates of imazethapyr cause severe damage to conventional rice. Higher crop injury occurs in one-tiller rice, and the highest reduction in rice yield follows exposure to a sublethal rate of imazethapyr at the boot stage (Hensley et al. 2012). The simulated drift of imazethapyr and imazapyr premix at low rates caused higher rice injury when applied at early rice stages than at later stages (Bond et al. 2006). In addition, an imazethapyr and imazapyr premix applied at the 2- to 3-leaf rice growth stage caused more reduction in rice plant height and yield potential compared to late-POST application at the panicle differentiation stage (Bond et al. 2006).

Widespread glyphosate application in GR crops and imazethapyr application in imidazolinone-resistant rice can drift glyphosate and imazethapyr onto neighboring fields planted with conventional or quizalofop-resistant rice cultivars (Davis et al. 2011; Koger et al. 2005; Martin et al. 2018). The extent of injury to rice can be estimated by assessing chlorophyll content and glyphosate, or imazethapyr concentrations in plant tissue of treated plants (Ding et al. 2011; Ellis et al. 2003; Reddy et al. 2010). Camacho et al. (2020) have shown that quizalofop applied at 120 g ai ha^{-1} caused up to 26% injury on quizalofop-resistant rice, resulting in slight chlorosis and necrosis symptoms, but plants generally recovered from injury at later stages. Growers will likely adopt better-yielding quizalofop-resistant cultivars in the coming years due to the continuous evolution of herbicide-resistant weeds in rice production systems. As this technology's adoption increases, so does the risk for sublethal drift of glyphosate or imazethapyr onto quizalofop-resistant rice. Therefore, we hypothesized that glyphosate or imazethapyr would intensify the injury to quizalofop-resistant cultivars that can result from sequential quizalofop applications. Thus, the objective of this research was to determine whether low rates of glyphosate and imazethapyr interact with sequential quizalofop applications to increase the risk for injury to quizalofop-resistant rice over applications of quizalofop alone.

Materials and Methods

Field experiments were conducted in summer 2021 on a Calloway silt loam (fine-silty, mixed, active, thermic Aquic Flaglossudalfs), pH 7.8, and with 1.7% organic matter at the Pine Tree Research Station (PTRS), in Colt, AR (35.12499°N, 90.93124°W); and on a Sharkey clay loam (very-fine, smectitic, thermic Chromic Epiaquerts), pH 6.7, with 1.7% organic matter at the Northeast Research and Extension Center (NEREC), in Keiser, AR (35.67659°N, 90.08684°W). Separate experiments were conducted to evaluate a low rate of glyphosate on quizalofop-resistant rice and a low rate of imazethapyr on quizalofop-resistant rice. Experiments were implemented as a split-plot arrangement with a randomized complete block design, replicated four times. The site was considered a whole-plot factor, while herbicide treatment was considered a split-plot factor. Previously published studies already demonstrated the effect of simulated drift of sublethal rates of glyphosate and imazethapyr on rice (Bond et al. 2006; Davis et al. 2011; Ellis et al. 2003; Hensley et al. 2012; Koger et al. 2005; Kurtz and Street 2003); the lowest possible rates were selected to evaluate whether preexposure to a sublethal rate of glyphosate and imazethapyr intensifies the risk for injury to quizalofop-resistant rice.

Table 1. Analysis of variance for rice injury and relative groundcover.

	P-value[a]			
	Injury		Relative groundcover	
Factor	Glyphosate	Imazethapyr	Glyphosate	Imazethapyr
Location	0.0059*	0.0420*	0.0753	0.5027
Herbicide	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Rating timing	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Location × herbicide	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Location × rating timing	<0.0001*	<0.0001*	<0.0001*	0.0001*
Herbicide × rating timing	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Location × herbicide × rating timing	0.1298	<0.0001*	<0.0001*	0.0291*

[a]Asterisks (*) indicate significance of treatments effects.

Table 2. Analysis of variance for relative heading and relative yield of Provisia® rice.

	Relative heading		Relative yield	
Factor	Glyphosate	Imazethapyr	Glyphosate	Imazethapyr
	P-value[a]			
Location	0.0185*	0.0733	0.4888	0.8711
Herbicide	<0.0001*	<0.0001*	<0.0001*	0.1362
Location × herbicide	0.0002*	<0.0001*	<0.0001*	0.5473

[a]Asterisks (*) indicate significance of treatments effects.

Table 3. Injury to "PVL02," a quizalofop-resistant cultivar, caused by preexposure to glyphosate at 90 g ae ha^{-1} at different rating dates averaged over both experiment locations.[a,b]

	Injury				
Herbicide	7 DAFT	14 DAFT	21 DAFT	28 DAFT	35 DAFT
	%				
G alone at 10 d	26 e-i	29 efg	21 e-l	19 f-l	17 h-n
G alone at 7 d	9 n-r	13 j-o	8 o-s	6 p-s	4 st
G alone at 4 d	17 i-n	17 g-n	17 i-n	11 l-p	10 m-q
G alone at 0 d	29 e-h	67 bc	71 B	58 cd	52 d
GFQ at 10 d	26 e-i	31 ef	17 h-n	23 e-j	18 g-m
GFQ at 7 d	12 k-p	12 k-p	4 q-t	10 m-q	5 q-t
GFQ at 4 d	23 e-j	25 e-i	25 e-i	21 e-k	15 i-o
GFQ at 0 d	33 e	78 ab	86 a	77 b	71 bc
SQ	4 rst	5 q-t	2 tu	2 u	1 u

[a]Means followed by the same letter are not different based on Fisher's protected LSD test at α = 0.05.
[b]Abbreviations: G, glyphosate; GFQ, glyphosate followed by quizalofop (2-leaf rice stage); SQ, sequential quizalofop (2-leaf stage followed by 5-leaf rice stage quizalofop application); DAFT, days after final treatment.

Exposure to Low Glyphosate Rate

Glyphosate (Roundup PowerMax®; Monsanto Company, St. Louis, MO) at 90 g ae ha^{-1}, which represents 1/12.5 of the labeled use rate (1,120 g ae ha^{-1}) for soybean, was applied 10, 7, 4, and 0 d before the 2-leaf growth stage of rice. Glyphosate at the same rate and timings was applied to 2-leaf rice followed by quizalofop (Provisia®) at 120 g ha^{-1}. Quizalofop was applied immediately following glyphosate application at the 0-d timing separately and not as a tank mix partner with glyphosate. All plots treated with quizalofop received a subsequent herbicide application at the same rate at the 5-leaf stage of rice. Quizalofop treatments were applied with 1% vol/vol crop oil concentrate (Agri-Dex®; Helena Chemical Company, Collierville, TN). A weed-free check and sequential quizalofop (no glyphosate or imazethapyr exposure) treatments were also included in the experiments for a total of 10 treatments. All application timings were based on the size of the rice in the weed-free check plots, and herbicide applications for the 10 d before 2-leaf rice growth stage were initiated when rice reached the 1-leaf growth stage. A 10-d interval was observed between the 1- and 2-leaf rice growth stages at both locations.

Table 4. Injury to "PVL02," a quizalofop-resistant cultivar, caused by preexposure to glyphosate at 90 g ae ha^{-1}, averaged over ratings of 7, 14, 21, 28, and 35 d after final treatment.[a,b]

	Injury	
Herbicide	NEREC	PTRS
	%	
G alone at 10 d	37 cd	12 efg
G alone at 7 d	12 efg	5 hi
G alone at 4 d	18 efg	11 fgh
G alone at 0 d	41 c	69 b
GFQ at 10 d	44 c	10 gh
GFQ at 7 d	15 efg	4 ij
GFQ at 4 d	23 de	20 ef
GFQ at 0 d	45 c	88 a
SQ	3 ij	2 j

[a]Means followed by same letter are not different based on Fisher's protected least significant difference test at α = 0.05.
[b]Abbreviations: G, glyphosate; GFQ, glyphosate followed by quizalofop (2-leaf stage); SQ, sequential quizalofop treatment (2-leaf stage followed by 5-leaf rice stage quizalofop application); NEREC, Northeast Research and Extension Center; PTRS, Pine Tree Research Station.

Exposure to Low Imazethapyr Rate

Imazethapyr (Newpath®; BASF Corporation, Research Triangle Park, NC) was applied at 10.7 g ai ha^{-1}, or 1/10 of the recommended use rate, in imidazolinone-resistant rice at 10, 7, 4, and 0 d prior to the 2-leaf growth stage. An imazethapyr application at the same rate and timings was followed by quizalofop (Provisia®) at the 2-leaf growth stage of rice. Quizalofop was applied immediately after imazethapyr application at the 0-d interval to the 2-leaf rice. All plots treated with quizalofop received a subsequent quizalofop application at the 5-leaf stage. All quizalofop applications were made at the labeled rate of 120 g ai ha^{-1}. A 0.25% vol/vol nonionic surfactant (Induce®; Helena Chemical Company) was added to each imazethapyr application, and 1% vol/vol crop oil concentrate (Agri-Dex®) was added to each quizalofop application. Experiments also included a weed-free check and sequential quizalofop (no glyphosate or imazethapyr exposure) treatment, with the initial application at the 2-leaf rice stage followed by another quizalofop application at the 5-leaf rice stage for a total of 10 treatments. The size of rice in weed-free check plots was used for application timings.

Methods Common to Both Studies

Experiments at the NEREC and PTRS were planted on May 20, 2021, and May 21, 2021, respectively. A quizalofop-resistant

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Table 5. Relative rice groundcover after preexposure to glyphosate compared to the weed-free check of "PVL02" rice cultivar at different rating timings.[a,b]

Herbicide	Relative groundcover									
	NEREC					PTRS				
	7 DAFT	14 DAFT	21 DAFT	28 DAFT	35 DAFT	7 DAFT	14 DAFT	21 DAFT	28 DAFT	35 DAFT
	%									
G alone at 10 d	23 o-n	40 f-n	89 a-h	97 a-f	95 a-f	46 c-n	45 d-n	110 a-d	81 a-i	106 a-e
G alone at 7 d	58 a-k	74 a-i	98 a-f	98 a-f	102 a-e	82 a-i	95 a-f	136 a	87 a-i	91 a-h
G alone at 4 d	74 a-i	53 b-m	96 a-f	103 a-e	104 a-e	64 a-j	79 a-i	110 a-d	73 a-i	90 a-h
G alone at 0 d	98 a-f	26 k-o	67 a-j	97 a-f	98 a-f	71 a-i	22 mno	27 j-o	7 pq	44 e-n
GFQ at 10 d	11 op	22 No	76 a-i	93 a-g	98 a-f	65 a-j	77 a-i	117 ab	89 a-h	103 a-e
GFQ at 7 d	60 a-k	64 a-j	101 a-e	102 a-e	101 a-e	88 a-i	84 a-i	112 abc	98 a-f	94 a-f
GFQ at 4 d	87 a-i	36 i-n	99 a-e	107 a-e	105 a-e	55 b-l	38 g-n	95 a-f	81 a-i	107 a-e
GFQ at 0 d	109 a-e	37 h-n	51 b-n	99 a-e	108 a-e	100 a-e	69 a-i	4 qr	1 s	2 r
SQ	114 abc	88 a-i	99 a-e	103 a-e	103 a-e	84 a-i	88 a-i	124 ab	86 a-i	100 a-e

[a]Means followed by same letter are not different based on Fisher's protected LSD test at α = 0.05.
[b]Abbreviations: G, glyphosate; GFQ, glyphosate followed by quizalofop (2-leaf rice stage); SQ, sequential quizalofop (2-leaf followed by 5-leaf rice stage application); DAFT, days after final treatment; NEREC, Northeast Research and Extension Center; PTRS, Pine Tree Research Station.

Table 6. Relative heading and relative yield after preexposure to glyphosate compared to the weed-free check of quizalofop-resistant rice cultivar "PVL02."[ab]

Location	Herbicide	Relative heading[c]	Relative yield[d]
		d	%
NEREC			
	G alone at 10 d	5 bc	90 ab
	G alone at 7 d	1 efg	94 ab
	G alone at 4 d	3 c-f	89 ab
	G alone at 0 d	8 a	81 ab
	GFQ at 10 d	6 ab	90 ab
	GFQ at 7 d	3 def	104 ab
	GFQ at 4 d	5 bc	104 ab
	GFQ at 0 d	8 a	93 ab
	SQ	1 efg	93 ab
	Weed-free check	———	———
PTRS			
	G alone at 10 d	−1 g	91 ab
	G alone at 7 d	0 g	92 ab
	G alone at 4 d	1 fg	108 a
	G alone at 0 d	4 b-e	67 b
	GFQ at 10 d	−1 g	77 b
	GFQ at 7 d	0 g	104 a
	GFQ at 4 d	1 fg	94 ab
	GFQ at 0 d	5 bcd	33 c
	SQ	0 g	106 a
	Weed-free check	———	———

[a]Means followed by same letter within same column are not different based on Fisher's protected LSD test at α = 0.05.
[b]Abbreviations: G, glyphosate; GFQ, glyphosate followed by quizalofop (2-leaf stage); SQ, sequential quizalofop (2-leaf stage followed by 5-leaf rice stage application); NEREC, Northeast Research and Extension Center; PTRS, Pine Tree Research Station.
[c]Days delay to 50% heading stage compared to the weed-free check of "PVL02" rice.
[d]"PVL02" cultivar yields for weed-free check plots were 9,742 kg ha^{-1} and 7,830 kg ha^{-1} for NEREC and PTRS, respectively.

cultivar, "PVL02" (Horizon Ag, LLC, Memphis, TN), was planted at a 1.3-cm depth at seeding rate of 72 seeds m^{-1} row into 1.8-m-wide by 5.2-m-long plots. Each plot consisted of 9 drill rows spaced 19 cm apart. All plots were maintained using practices recommended by the University of Arkansas System Division of Agriculture Cooperative Extension Service for proper stand establishment, fertilization, and pest management (Hardke et al. 2022). Plots were kept weed-free with labeled herbicides. Clomazone (Command 3ME; FMC Corporation, Philadelphia, PA) was applied preemergence at 560 and 336 g ai ha^{-1} at NEREC and PTRS, respectively. Halosulfuron (Permit®; Gowan Corporation, Yuma, AZ) and quinclorac (Facet L™ herbicide; BASF Corporation, Florham Park, NJ) combined with 1% vol/vol crop oil concentrate were applied to 4-leaf rice at 40 g ai ha^{-1} and 280 g ai ha^{-1}, respectively. All herbicide treatments were applied using a CO_2-pressurized backpack sprayer calibrated to deliver 94 L ha^{-1} at 276 kPa. A four-nozzle, 1.5-m-wide spray boom equipped with AIXR 110015 nozzles (TeeJet Technologies; Spraying Systems Co., Glendale Heights, IL) was used. Plots were fertilized with urea (46-0-0) at 350 kg ha^{-1} and 280 kg ha^{-1} at NEREC and PTRS, respectively, when rice reached the 5-leaf stage, and was flooded until rice reached maturity. Experimental sites were drained 2 wk before harvesting.

Visual injury estimates were rated on a 0 to 100 scale, with 0 being no injury and 100 being crop death compared to the weed-free check at 7, 14, 21, 28, and 35 d after final treatment (DAFT) with glyphosate or imazethapyr alone, or glyphosate or imazethapyr followed by the 2-leaf rice stage quizalofop application. Overall visual injury was rated for glyphosate, imazethapyr, and quizalofop based on chlorosis, necrosis, and stunting of the rice plants, and injury symptomology were not evaluated individually for each herbicide. Groundcover was assessed by taking photographs from a DJI Phantom quadcopter small, unmanned aerial system (DJI, Shenzhen, China) on a weekly basis after the 1-leaf rice stage application. Images were analyzed using Field Analyzer (Green Research Services, LLC, Fayetteville, AR) to determine the proportion of green pixels in each image to assess the amount of groundcover reduction at 7, 14, 21, 28, and 35 DAFT. The date that each plot reached the 50% heading stage was recorded. Heading dates are reported as days relative to the weed-free check reaching 50% heading. Each plot was harvested using a small-plot combine to determine the rough grain yield, and adjusted to 12% moisture. For each treatment, groundcover and yield were expressed in terms of the percentage of the corresponding weed-free check in each block.

Data Analysis

All data were analyzed using SAS software, version 9.4 (SAS Institute Inc., Cary, NC), and means were subjected to ANOVA using the GLIMMIX procedure. All ANOVA results are shown in Tables 1 and 2. The main effects of the site and herbicide

Table 7. Injury to "PVL02" rice by preexposure to imazethapyr at 10.7 g ai ha^{-1} averaged over experiments at both sites.[a,b]

	Injury									
	NEREC					PTRS				
Herbicide	7 DAFT	14 DAFT	21 DAFT	28 DAFT	35 DAFT	7 DAFT	14 DAFT	21 DAFT	28 DAFT	35 DAFT
	%									
I alone at 10 d	70 Ab	70 ab	69 abc	63 a-e	48 d-l	8 y-e	13 w-c	9 x-d	25 p-w	20 r-w
I alone at 7 d	36 j-q	45 e-m	41 g-p	39 g-q	19 r-w	7 a-e	16 t-a	24 q-w	58 a-g	46 d-m
I alone at 4 d	25 n-v	25 n-v	13 w-2	3 e-h	1 h	18 s-x	24 q-w	52 b-j	48 d-k	38 i-q
I alone at 0 d	24 q-w	55 a-i	39 g-q	17 t-x	7 a-e	7 a-e	14 v-a	58 a-g	45 e-m	42 f-o
IFQ at 10 d	70 Ab	71 a	70 ab	65 a-d	51 c-j	7 a-e	15 u-a	16 u-a	42 f-n	38 h-q
IFQ at 7 d	32 k-s	44 e-m	43 f-n	34 j-r	19 s-x	7 a-e	20 r-w	29 l-t	51 c-j	43 f-n
IFQ at 4 d	23 q-w	19 r-w	16 u-a	6 c-g	2 gh	17 t-y	20 r-w	69 abc	61 a-f	51 c-j
IFQ at 0 d	25 o-w	58 a-h	46 d-l	29 m-u	19 s-x	14 v-b	17 t-y	71 a	67 abc	63 a-e
SQ	8 y-e	2 gh	3 e-h	3 e-h	1 h	2 gh	2 gh	3 e-h	5 d-g	3 e-h

[a]Means followed by same lowercase and uppercase letter are not different based on Fisher's protected LSD test at α = 0.05.
[b]Abbreviations: I, imazethapyr; IFQ, imazethapyr followed by quizalofop (2-leaf rice stage); SQ, sequential quizalofop (2-leaf followed by 5-leaf rice stage quizalofop application); DAFT, days after final treatment; NEREC, Northeast Research and Extension Center; PTRS, Pine Tree Research Station.

Table 8. Rice relative groundcover compared to the weed-free check of rice cultivar "PVL02" after preexposure to imazethapyr at different rating timings at both experiment locations.[a,b]

	Relative groundcover									
	NEREC					PTRS				
Herbicide	7 DAFT	14 DAFT	21 DAFT	28 DAFT	35 DAFT	7 DAFT	14 DAFT	21 DAFT	28 DAFT	35 DAFT
	%									
I alone at 10 d	7 s	8 rs	28 n-q	67 a-m	69 a-k	25 pq	14 qr	83 a-i	85 a-g	80 a-j
I alone at 7 d	27 opq	40 i-p	78 a-j	99 a-d	95 a-e	76 a-j	15 qr	56 b-o	51 d-o	76 a-j
I alone at 4 d	79 a-j	84 a-h	93 a-f	100 a-d	95 a-e	101 a-d	39 j-p	31 m-p	41 h-p	87 a-g
I alone at 0 d	100 a-d	73 a-k	80 a-j	105 a-d	101 a-d	102 a-d	23 pq	4 g-p	68 a-l	93 a-f
IFQ at 10 d	6 s	10 rs	33 l-p	71 a-k	80 a-j	10 rs	35 l-p	58 b-n	66 a-m	100 a-d
IFQ at 7 d	26 opq	41 g-p	79 a-j	101 a-d	100 a-d	44 f-p	23 opq	43 g-p	73 a-k	107 abc
IFQ at 4 d	99 a-d	106 a-d	104 a-d	100 a-d	109 abc	68 a-l	64 a-m	46 e-p	72 a-k	98 a-d
IFQ at 0 d	92 a-f	55 c-o	72 a-k	95 a-e	102 a-d	100 a-d	24 pq	38 j-p	60 b-n	90 a-f
SQ	115 ab	111 ab	99 a-d	100 a-d	99 a-d	81 a-i	127 a	106 a-d	105 a-d	91 a-f

[a]Means followed by same letter are not different based on Fisher's protected least significant difference test at α = 0.05.
[b]Abbreviations: I, imazethapyr; IFIQ, imazethapyr followed by quizalofop; SQ, sequential quizalofop (2-leaf stage followed by 5-leaf rice stage quizalofop application); DAFT, days after final treatment; NEREC, Northeast Research and Extension Center; PTRS, Pine Tree Research Station.

treatment and their interaction were treated as fixed effects. Blocks nested within site and herbicide treatment nested within site were treated as random effects. Beta distribution was assumed for rice injury, and gamma distribution was used for relative groundcover and relative yield (Gbur et al. 2012). Normal distribution was used for days until heading relative to the weed-free check. Means were separated using Fisher's protected LSD test (α = 0.05), and the Kenward-Roger degree-of-freedom approximation was used. For injury and groundcover response variables, rating timing was considered a repeated-measure variable that allowed for comparisons across ratings and included in the treatment structure as a fixed effect. Correlations across ratings for the fixed effects and residuals were modeled using an independence covariance structure for injury and groundcover. There was no correlation between rating timings when residuals were evaluated qualitatively (Gbur et al. 2012).

Results and Discussion

Provisia Rice Exposure to Glyphosate

Injury to rice was generally greatest when glyphosate was applied alone at the 2-leaf stage or was followed by a quizalofop application on the same day (Table 3). Averaged over locations, injury to quizalofop-resistant rice at 7 and 14 DAFT was similar for each application timing individually when glyphosate was applied alone at a 10-, 7-, 4-, and 0-d interval prior to 2-leaf rice and glyphosate at a 10-, 7-, 4-, and 0-d interval before quizalofop applied to 2-leaf rice, respectively (Table 3). At 21 DAFT, regardless of location, quizalofop application on the same day as glyphosate at the 2-leaf stage caused 15 percentage points greater injury than glyphosate alone at the 2-leaf stage of rice (Table 3). Greater injury to rice (19 percentage points) occurred with the addition of quizalofop at 28 and 35 DAFT, and higher injury persisted through 35 DAFT (Table 3). Glyphosate followed by quizalofop at a 4-d interval caused a 10 percentage point increase in injury over glyphosate applied alone 4 d before the 2-leaf rice stage when evaluated at 28 DAFT, regardless of location. There were no differences in injury between glyphosate followed by quizalofop applications at a 7- and 10-d interval compared to glyphosate applied alone at a 7- and 10-d interval before the 2-leaf growth stage of rice at all rating timings averaged over both locations (Table 3).

Glyphosate followed by quizalofop at a 0-d interval caused 88% injury compared with 69% injury caused by glyphosate applied

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Table 9. Rice relative heading compared to the weed-free check after preexposure to imazethapyr.[a,b]

Location	Herbicide	Relative heading[c]
		d
NEREC		
	I alone at 10 d	5 a
	I alone at 7 d	3 c-f
	I alone at 4 d	1 hi
	I alone at 0 d	4 b-e
	IFQ at 10 d	5 ab
	IFQ at 7 d	4 abc
	IFQ at 4 d	1 gh
	IFQ at 0 d	4 a-d
	SQ	0 i
	Weed-free check	——
PTRS		
	I alone at 10 d	2 f-h
	I alone at 7 d	3 d-h
	I alone at 4 d	3 d-h
	I alone at 0 d	3 c-f
	IFQ at 10 d	2 e-h
	IFQ at 7 d	2 e-h
	IFQ at 4 d	3 c-g
	IFQ at 0 d	3 c-f
	SQ	1 hi
	Weed-free check	——

[a]Means followed by same letter are not different in same column based on Fisher's protected LSD test at $\alpha = 0.05$.
[b]Abbreviations: I, imazethapyr; IFQ, imazethapyr followed by quizalofop (2-leaf stage); SQ, sequential quizalofop (2-leaf stage followed by 5-leaf rice stage quizalofop application); NEREC, Northeast Research and Extension Center; PTRS, Pine Tree Research Station.
[c]Days delay to 50% heading stage compared to the weed-free check of "PVL02" rice cultivar.

alone at the same timing at PTRS, pooled over ratings (Table 4). No differences were observed in injury between glyphosate alone or glyphosate followed by quizalofop at the 0-d interval at NEREC, averaged over ratings. Additionally, pooled over ratings, <3% injury was observed from sequential quizalofop applications at both locations (Table 4). Higher air temperature during glyphosate application at NEREC probably caused greater injury at this location than at PTRS from glyphosate applied alone or glyphosate followed by quizalofop at a 10- and 7-d interval averaged over ratings. However, injury ratings did not differ between locations when glyphosate was applied alone at a 4-d interval compared to glyphosate applied 4 d prior to quizalofop, regardless of the rating dates (Table 4). Ellis et al. (2003) documented a similar finding, that variations in air temperature conditions across site years affected the crop response in terms of visual injury, plant height, and yield when sublethal rates of glyphosate were applied to rice. In contrast, greater injury to quizalofop-resistant rice was observed after glyphosate was applied alone or after an application of glyphosate followed by quizalofop at the 0-d interval, averaged over ratings at PTRS compared to NEREC due to saturated soil conditions during herbicide treatment. Previous research reported that higher soil moisture content increased the efficacy of the aryloxyphenoxypropionate herbicide diclofop on barnyardgrass and glyphosate on windmill grass (*Chloris truncata* R.Br.; Dortenzio and Norris 1980; Peerzada et al. 2021).

Sequential quizalofop applications caused a <16 percentage point reduction in relative groundcover to rice compared to the weed-free check at both locations averaged across evaluations (Table 5). Glyphosate followed by quizalofop the same day caused a reduction of 6 to 47 percentage points in relative groundcover compared with glyphosate alone at PTRS when evaluated at 14, 21, 28, and 35 DAFT (Table 5). At both locations, no differences in relative groundcover were observed between glyphosate followed by quizalofop at 4-, 7-, and 10-d interval and glyphosate alone applied 4, 7, and 10 d before the 2-leaf growth stage of rice at all evaluations (Table 5).

At both locations, no delay to 50% heading was observed in rice between glyphosate followed by quizalofop applied at 10-, 7-, 4-, and 0-d intervals compared with glyphosate alone at the same timings (Table 6). Glyphosate followed by quizalofop at the 0-d interval caused a 67% yield reduction compared with a 33% reduction when glyphosate was applied alone at the same time at PTRS compared to the weed-free check. However, at all other intervals between glyphosate followed by quizalofop, yields were comparable to those of the corresponding glyphosate-alone timings at both locations. Additionally, no yield reductions resulted from sequential quizalofop applications alone at either location (Table 6). Preexposure of quizalofop-resistant rice to a sublethal glyphosate rate, to attenuate the risk for injury, needs to be avoided in a close interval with quizalofop applications. Exposure to low rates of glyphosate affects the tolerance of quizalofop-resistant rice to quizalofop applications and increases the risk for injury to the crop over individual exposure to glyphosate or quizalofop alone. Similarly, in other research, Brown et al. (2009) reported higher injury to corn when simulated sublethal glyphosate exposure was followed by an in-crop standard herbicide program compared to sublethal glyphosate exposure and in-crop herbicides alone.

Provisia Rice Exposure to Imazethapyr

Applications of imazethapyr followed by quizalofop on the same day caused 12 percentage point greater injury than imazethapyr alone at NEREC when evaluated at 35 DAFT (Table 7). At PTRS, applications of imazethapyr followed by quizalofop the same day caused greater injury by 22 and 21 percentage points, respectively, than imazethapyr applied alone at 28 and 35 DAFT. Myers and Coble (1992) documented similar findings regarding weed control, when an application of imazethapyr followed by quizalofop the same day provided greater control of fall panicum (*Panicum dichotomiflorum* Michx) than quizalofop applied alone or imazethapyr followed by quizalofop at 5- and 3-d intervals. No differences in injury were observed at either PTRS or NEREC between imazethapyr followed by quizalofop applied at the 4- and 7-d intervals and imazethapyr applied alone at the same timings, averaged across all ratings (Table 7). At PTRS, imazethapyr followed by quizalofop at a 10-d interval caused more injury (by 17 and 18 percentage points) than imazethapyr applied alone at the same timing when evaluated at 28 and 35 DAFT. Imazethapyr caused more severe injury when rice was exposed to sublethal rates at the 1- to 2-leaf growth stage compared to 3- to 4-leaf rice (Levy et al. 2006), which could be attributed to differential metabolism at the different growth stages. A sequential application of quizalofop alone caused minimal injury (<8% at both locations regardless of evaluation; Table 7).

No differences were discerned in relative groundcover between applications of imazethapyr to 2-leaf rice followed by quizalofop at 0-, 4-, 7-d intervals and imazethapyr applied alone at the same timings at either location (Table 8). At PTRS, imazethapyr followed by quizalofop at a 10-d interval caused a reduction in relative groundcover at 7 and 14 DAFT, ranging from 15 to 21 percentage points compared with imazethapyr alone at 10 d before the 2-leaf rice stage (Table 8). A sequential quizalofop application never reduced relative groundcover at either location for any evaluations (Table 8). Camacho et al. (2020) observed that quizalofop applied at the 1× or 2× rate caused transient injury to quizalofop-resistant rice cultivars, and crop injury from quizalofop applications in the vegetative stage recovered at a later growth stage and had no impact on yield potential. No differences in reaching 50% heading were observed between applications of imazethapyr followed by an initial application of quizalofop at 0-, 4-, 7-, and 10-d intervals; nor with imazethapyr applied alone at 0-, 4-, 7-, and 10-d intervals before 2-leaf rice stage at both locations (Table 9). The sublethal rate of imazethapyr increases the risk for injury to quizalofop-resistant rice when applied at an early growth stage (1-leaf stage) or when exposure occurs near sequential quizalofop application.

Effects of exposure of nontraited rice to sublethal rates of glyphosate and imazethapyr are well documented (Bond et al. 2006; Davis et al. 2011; Ellis et al. 2003; Hensley et al. 2012; Koger et al. 2005; Kurtz and Street 2003). Preexposure of rice to low rates of glyphosate and imazethapyr poses more risk for injury to quizalofop-resistant rice when exposure occurs near the first quizalofop application. Furthermore, preexposure of quizalofop-resistant rice to low rates of imazethapyr needs to be avoided because it increases damage to rice; however, no significant reduction in yield was observed after crop exposure to sublethal rates of imazethapyr. Therefore, the hypothesis is accepted. Sublethal rates of glyphosate or imazethapyr interact with quizalofop applications to increase the likelihood of injury to quizalofop-resistant rice over glyphosate, imazethapyr, and quizalofop applications alone. These experiments show the additive response in injury caused by individual herbicide exposure events; however, the severity of injury to quizalofop-resistant rice could be increased by exposure to sublethal rates of glyphosate or imazethapyr prior to standard herbicide applications. Rice producers should avoid applying quizalofop on the same day if preexposure to a low rate of glyphosate or imazethapyr is suspected on quizalofop-resistant rice, because it intensifies the risk for injury and could reduce the yield potential. Future research needs to be conducted to evaluate the use of fertilizers to aid the recovery of quizalofop-resistant rice following exposure to low doses of glyphosate or imazethapyr followed by standard herbicide programs.

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