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Research Article

Resistance of *Echinochloa crus-galli* Populations to Acetolactate Synthase-Inhibiting Herbicides

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Three *Echinochloa crus-galli* (barnyardgrass) populations from rice fields in Arkansas (AR1 and AR2) and Mississippi (MS1), USA, were recently confirmed to be resistant to imazethapyr. Experiments were conducted to characterize cross-resistance to acetolactate synthase- (ALS-) inhibiting herbicides and determine if malathion, a known cytochrome P450 monooxygenase (CYP) inhibitor, would overcome resistance. The AR1 and MS1 populations were cross-resistant to bispyribac-sodium; however, AR2 was sensitive to bispyribac-sodium. The AR1, AR2, and MS1 populations were >94, >94, and 3.3 times, respectively, more resistant to imazamox; >94, 30, and 9.4 times, respectively, more resistant to penoxsulam; and 15, 0.9, and 7.2 times, respectively, more resistant to bispyribac-sodium compared to a susceptible population. Addition of malathion to penoxsulam reduced dry weight of all populations and increased mortality of AR2 and MS1 populations compared to penoxsulam alone. Addition of malathion to imazethapyr and bispyribac-sodium increased the mortality of MS1 population in mixture with imazethapyr and AR1 population in mixture with bispyribac-sodium compared to treatments with imazethapyr and bispyribac-sodium applied alone. Synergism of ALS-inhibiting herbicides with malathion indicates increased herbicide degradation by CYP as partial mechanism of resistance to penoxsulam in all resistant populations and probably to imazethapyr in MS1 and bispyribac-sodium in AR1 populations.

1. Introduction

Echinochloa crus-galli L. (barnyardgrass), native to Europe and Asia, is a cosmopolitan annual weed infesting 36 crops in 61 countries [1]. It is a troublesome weed in rice (*Oryza sativa* L.) fields of North America and is the sixth most important herbicide-resistant weed species worldwide [2]. In a survey conducted in the fall of 2011, *E. crus-galli* was listed by crop consultants as the most problematic weed of rice in Arkansas and Mississippi states of USA [3, 4]. In Arkansas, which produces almost half of the USA rice, *E. crus-galli* is considered a noxious weed [5]. Season-long interference of *E. crus-galli* at a density of even one plant m^{-2} can reduce rice yield up to 257 kg ha^{-1} [6].

Echinochloa crus-galli biotypes resistant to acetyl-CoA carboxylase- (ACCase-) inhibiting, acetolactate synthase- (ALS-) inhibiting, chloroacetamide, dinitroaniline, isoxazolidine, photosystem II-inhibitor, synthetic auxin, thiocarbamate, or urea and amide herbicides have been reported in sixteen countries [2]. In Arkansas, reduced rotation of rice with other crops along with frequent use of propanil, quinclorac, and clomazone has led to the evolution of *E. crus-galli* biotypes resistant to propanil [7], quinclorac [8], and clomazone [9]. Similarly, studies from 2007 to 2010 revealed resistance to propanil in 45%, quinclorac in 20%, and both propanil and quinclorac in15% of *E. crus-galli* samples collected from Mississippi rice fields [10].

ACCase-inhibiting (fenoxaprop and cyhalofop) and ALS-inhibiting herbicides (bispyribac-sodium and penoxsulam for conventional rice and imazethapyr and imazamox for imidazolinone-resistant rice) are commonly used to control herbicide-resistant *E. crus-galli* biotypes [11]. Bispyribac-sodium, imazamox and imazethapyr, and penoxsulam belong to pyrimidinylthiobenzoate (PTB), imidazolinone (IMI), and triazolopyrimidine (TP) chemical families, respectively. After the commercialization of imidazolinone-resistant (Clearfield) rice in 2002, the evolution of ALS-resistant *E. crus-galli* biotypes was of high risk because of the extensive use of ALS-inhibiting herbicides, especially imazethapyr and imazamox. *Echinochloa crus-galli* populations, AR1 and AR2, were found in rice fields from northeast Arkansas in 2008 and 2009, respectively, and were later confirmed to be resistant to imazethapyr (70 g ai ha^{-1}) in trials conducted at the University of Arkansas, Fayetteville, AR, USA [12]. Another imazethapyr-resistant *E. crus-galli* population was found in a rice field from Sunflower County, Mississippi, in 2010.

The frequency of occurrence of resistance to ALS-inhibiting herbicides is high compared with other modes of action [2]. At present, 126 weed species across 35 countries have been reported to be resistant to one or more ALS-inhibiting herbicides [2]. The mechanism of ALS resistance in almost all of the known resistant weeds species is either an altered ALS gene or enhanced metabolism by cytochrome P450 monooxygenases (CYP) [13]. Organophosphate insecticides such as malathion have been shown to inhibit herbicide detoxification catalyzed by CYP [14, 15]. Non-target-site resistance, mainly because of increased metabolism of ALS-inhibiting herbicides by CYP, has been confirmed in *Alopecurus myosuroides* Huds. (blackgrass) [16], *Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot (Italian ryegrass) [17], *E. phyllopogon* (Stapf.) Koss. (late watergrass) [18], *Phalaris minor* Retz. (littleseed canarygrass) [19], *L. rigidum* Gaud. (rigid ryegrass) [14], and *Sinapis arvensis* L. (wild mustard) [20].

Experiments were conducted to (a) characterize cross-resistance to the ALS-inhibiting herbicides bispyribac-sodium, imazamox, imazethapyr, and penoxsulam; (b) determine if increased metabolism of ALS-inhibiting herbicides by CYP is the mechanism of resistance in two imazethapyr-resistant *E. crus-galli* populations from Arkansas and one from Mississippi.

2. Materials and Methods

2.1. Plant Material and Growth Conditions. Seeds of three putative imazethapyr-resistant populations were collected from rice fields in Arkansas (herein referred to as AR1 (from Greene County) and AR2 (from Prairie County)) and Mississippi (herein referred to as MS1 (from Sunflower County)). These rice fields from Arkansas and Mississippi were under continuous IMI-resistant (Clearfield) rice for the last three years, with sequential applications of imazethapyr (70 to 105 g ai ha^{-1}) applied annually. Seeds of a susceptible *E. crus-galli* population were collected from a field in Fayetteville, AR, USA with no ALS-inhibiting herbicide history. Seeds of all populations were planted in 55.5 by 26.5 by 5.5 cm^3 plastic trays using commercial potting media (Professional GrowingMix, LC1 Mix, Sun Gro Horticulture Distribution Inc., Bellevue, WA, USA). All plants were kept in the greenhouse under conditions of 30/20 ± 3 C day/night temperature and 16 h photoperiod.

2.2. Confirmation of Cross-Resistance to ALS-Inhibiting Herbicides. Four plants of each population at the two-leaf stage were transplanted to 15 cm diam plastic pots filled with potting media. Plants at the three- to four-leaf stage were treated with the field application rate of bispyribac-sodium (Regiment, Valent U.S.A. Corp., Walnut Creek, CA, USA), imazamox (Beyond, BASF Corp., Research Triangle, NC, USA), imazethapyr (Newpath, BASF Corp., Research Triangle, NC, USA), and penoxsulam (Grasp SC, Dow AgroSciences LLC, Indianapolis, IN, USA) at 30, 35, 70, and 35 g ai ha^{-1}, respectively. A nonionic spray adjuvant and deposition aid (Dyne-A-Pak, Helena Chemical Co., Collierville, TN, USA) at 2.5% v/v was added to bispyribac-sodium treatments, and a nonionic surfactant (NIS) at 0.25% v/v (Induce, Helena Chemical Co., Collierville, TN, USA) was added to all other herbicide treatments in accordance with label recommendations.

Herbicide applications were made using an automated spray chamber with a boom containing two flat fan 800067 nozzles (TeeJet Technologies, Springfield, IL, USA) calibrated to deliver 187 L ha^{-1}. A control treatment sprayed with NIS at 0.25% was also included for each population. Plants were watered daily and once weekly with a water-soluble fertilizer (Miracle-Gro Water Soluble All Purpose Plant Food, Scotts Miracle-Gro Products, Inc., Marysville, OH, USA).

The experiment was conducted in a randomized complete block design with four replications (sixteen plants per treatment with four replications). *Echinochloa crus-galli* control was visually estimated at 21 d after treatment (DAT) on a scale of 0 to 100, where 0 represented no control, and 100 represented complete control of all plants. After recording *E. crus-galli* control, plants were harvested at ground level 21 DAT, dried at 60 C for 48 h, and weighed. Based on the dry weight of the nontreated control, dry weight data of each population were converted to percent dry weight reduction.

Percent control and dry weight reduction data were tested for normality using PROC UNIVARIATE in SAS (Version 9.1.3., SAS Institute Inc., Cary, NC, USA). Data for percent control and dry weight reduction were arcsine square root transformed before analyses. Transformed data were subjected to ANOVA using PROC MIXED in SAS to evaluate the effect of different herbicides on control and dry weight reduction of all four *E. crus-galli* populations. The experiment was repeated, and data from the two experiments were pooled because there were no treatment-by-experiment interactions. Data for each herbicide were analyzed separately to determine if populations differed in herbicide response. Means were separated using Fisher's protected LSD at $\alpha = 0.05$. Nontransformed means for control and dry weight reduction are reported for clarity with significance levels determined using transformed values.

2.3. Characterization of Resistance to ALS-Inhibiting Herbicides. Individual plants of all four populations at the two-leaf stage were transplanted to 15 cm diam plastic pots filled with potting media. Based on the results of the cross-resistance confirmation experiment, plants of all four populations at the three- to four-leaf stage were sprayed with eight doses (including recommended field application rate (1X rate) and doses above and below recommended field application rate) of bispyribac-sodium, imazamox, and penoxsulam, using the same sprayer configuration utilized for the cross-resistance confirmation experiment. Field application (1X) rates of all herbicides were similar to the ALS cross resistance confirmation experiment; however, for bispyribac-sodium, 22.5 g ha^{-1} represented the field application rate. NIS at 0.25% v/v was added to imazamox- and penoxsulam-containing treatments, and a nonionic spray adjuvant and deposition aid at 2.5% v/v was added to bispyribac-sodium-containing treatments.

Susceptible plants were treated with imazamox, penoxsulam, and bispyribac-sodium at 0, 1/64, 1/32, 1/16, 1/8, 1/4, 1/2, 1, and 2 times the field application rates. The AR1 plants were treated with imazamox and penoxsulam at 0, 1/4, 1/2, 1, 2, 4, 8, 16, and 32 times, and bispyribac-sodium at 0, 1/16, 1/8, 1/4, 1/2, 1, 2, 4, and 8 times the field application rate. The AR2 plants were treated with imazamox and penoxsulam at 0, 1/4, 1/2, 1, 2, 4, 8, 16, and 32 times, and bispyribac-sodium at 0, 1/32, 1/16, 1/8, 1/4, 1/2, 1, 2, and 4 times the field application rate. The MS1 plants were treated with imazamox at 0, 1/32, 1/16, 1/8, 1/4, 1/2, 1, 2, and 4 times, penoxsulam at 0, 1/4, 1/2, 1, 2, 4, 8, 16, and 32 times, and bispyribac-sodium at 0, 1/16, 1/8, 1/4, 1/2, 1, 2, 4, and 8 times the field application rate. Herbicide treatments were applied using the same sprayer configuration used for the cross resistance experiment. Growth conditions for plants were also similar to the cross resistance study. After treatment, plants were returned to the greenhouse. Treatment effect with regard to plant mortality was recorded at 21 DAT.

The experimental layout was a completely randomized design with twenty replications per herbicide dose treatment, and the experiment was repeated. Mortality data were subjected to probit analysis using PROC PROBIT in SAS to determine the lethal dose needed to kill 50% (LD_{50}) and 90% (LD_{90}) of the treated plants of each population, and confidence intervals (95%) were calculated to determine whether the populations differed from each other. To determine the level of resistance, a resistance index for all resistant populations for each herbicide was determined by dividing LD_{50} or LD_{90} of resistant population by LD_{50} or LD_{90} of susceptible population.

2.4. CYP Inhibition by Malathion. Ten plants of each resistant and susceptible population planted individually in 15 cm diam pots were treated with malathion (Prentox, Prentiss Inc., Floral Park, NY, USA) at the three- to four-leaf stage at 1000 g ai ha^{-1}, bispyribac-sodium at 30 g ha^{-1} alone or in mixture with malathion at 1000 g ha^{-1}, imazethapyr at 105 g ha^{-1} alone or in mixture with malathion at 1000 g ha^{-1}, and penoxsulam at 35 g ha^{-1} alone or in mixture with malathion at 1000 g ha^{-1}. Spray adjuvants were added to treatments containing bispyribac-sodium, imazethapyr, and penoxsulam as in the cross resistance experiments. A nontreated control for each population was also included. Sprayer configuration and growth conditions were similar to cross resistance experiments. Plants were harvested at ground level after recording mortality at 21 DAT, dried at 60 C for 48 h, and weighed. Dry weight data for each population were converted to percent of the nontreated control for each population.

The experiment was arranged in a completely random design and was repeated. Data were tested for normality using PROC UNIVARIATE in SAS. Percentage dry weight data were arcsine-square root transformed and subjected to ANOVA using PROC MIXED in SAS. There were no treatment-by-experiment interactions; thus, data were pooled for two experimental runs. Means were separated using Fisher's protected LSD at $\alpha = 0.05$. For each population, t-tests were conducted between treatments with bispyribac-sodium, imazethapyr, or penoxsulam alone versus bispyribac-sodium, imazethapyr, or penoxsulam in mixture with malathion, respectively, to evaluate if malathion synergizes the control of each resistant *E. crus-galli* population. Additionally, a chi-square test was conducted using PROC FREQ in SAS to determine if the addition of malathion to each herbicide increased mortality.

3. Results

3.1. Confirmation of Cross-Resistance to ALS-Inhibiting Herbicides. Field rate applications of bispyribac-sodium, imazamox, imazethapyr, and penoxsulam controlled the susceptible population ≥98% (Table 1). With bispyribac-sodium, control of AR2 population was 98%, but control of AR1 and MS1 populations was ≤16%. Control of all resistant populations was similar with imazamox and imazethapyr: ≤59, 6, and ≤86% for AR1, AR2, and MS1, respectively. Penoxsulam controlled AR1, AR2, and MS1 populations by 26, 51, and 22%, respectively. Both AR1 and MS1 populations were resistant to bispyribac-sodium, imazamox, imazethapyr, and penoxsulam compared to the susceptible population. The AR2 population was sensitive to bispyribac-sodium but was resistant to imazamox, imazethapyr, and penoxsulam compared to susceptible population.

Dry weight reduction data followed a trend similar to the control data (Table 2). Dry weight reduction of the susceptible population (≥99%) was greater than AR1 (19 to 58%) and MS1 (25 to 82%) with all herbicide treatments, and AR2 (22 to 50%) with imazethapyr, imazamox, and penoxsulam treatments. Bispyribac-sodium reduced dry weight of AR2 (99%) similar to susceptible population. Among resistant populations, the dry weight reduction of AR2 was from 54% to 62% and from 71% to 73% less compared to AR1 and MS1 populations, respectively, with imazamox and imazethapyr treatments. In contrast, the dry weight reduction of AR1 and MS1 populations with bispyribac-sodium and penoxsulam was >74 and >58%, respectively, less compared to AR2. In general, among resistant populations, AR2 was most resistant to imazamox and imazethapyr, whereas AR1 and MS1 were most resistant to bispyribac-sodium and penoxsulam.

Table 1: Control of *E. crus-galli* populations at 21 d after treatment with various ALS herbicides at recommended field rates[a,b,c].

Treatment	Rate	Control							
		Susceptible		AR1		AR2		MS1	
	g ai ha^{-1}	%							
Imazethapyr	70	100	aA	57	cA	6	dC	83	bA
Imazamox	35	100	aA	59	cA	6	dC	86	bA
Penoxsulam	35	99	aAB	26	cB	51	bB	22	cB
Bispyribac-sodium	30	98	aB	15	bC	98	aA	16	bC

[a]Means for each population within a column followed by the same uppercase letters and means for each herbicide within a row followed by the same lowercase letters are not significantly different according to Fisher's protected LSD test ($\alpha = 0.05$).
[b]All herbicide treatments except bispyribac-sodium contained nonionic surfactant at 0.25% v/v.
[c]Bispyribac-sodium treatments contained a nonionic spray adjuvant and deposition aid at 2.5% v/v.

Table 2: The above-ground dry weight reduction of ALS-resistant (AR1, AR2, and MS1) and -susceptible *E. crus-galli* populations at 21 d after treatment with various ALS-inhibiting herbicides at recommended field rates[a,b,c].

Treatment	Rate	Above-ground dry weight reduction							
		Susceptible		AR1		AR2		MS1	
	g ai ha^{-1}	% of nontreated control							
Imazethapyr	70	100	aA	52	cA	24	dC	82	bA
Imazamox	35	100	aA	58	cA	22	dC	80	bA
Penoxsulam	35	99	aAB	21	cB	50	bB	22	cB
Bispyribac-sodium	30	99	aAB	19	bB	99	aA	25	bB

[a]Means for each population within a column followed by the same uppercase letters and means for each herbicide within a row followed by the same lowercase letters are not significantly different according to Fisher's protected LSD test ($\alpha = 0.05$).
[b]All herbicide treatments except bispyribac-sodium contained nonionic surfactant at 0.25% v/v.
[c]Bispyribac-sodium treatments contained a nonionic spray adjuvant and deposition aid at 2.5% v/v.

Table 3: Bispyribac-sodium, imazamox, and penoxsulam dose required to kill 50% (LD_{50}) and 90% (LD_{90}) of ALS-resistant (AR1, AR2, and MS1) and -susceptible *E. crus-galli* populations (with 95% CI in parenthesis)[a,b,c,d].

Herbicide	Population	LD_{50} (95% CI) g ai ha^{-1}		R/S ratio (LD_{50})	LD_{90} (95% CI) g ai ha^{-1}		R/S ratio (LD_{90})
Bispyribac-sodium	AR1	49	(43–56)	15	94	(78–122)	8.3
	AR2	3.0	(1.8–4.8)	0.9	8.6	(5.3–26)	0.8
	MS1	24	(14–43)	7.2	81	(44–312)	7.1
	Susceptible	3.3	(2.4–4.7)		11	(7.5–22)	
Imazamox	AR1	>1120		>94	>1120		>52
	AR2	>1120		>94	>1120		>52
	MS1	39	(34–45)	3.3	73	(61–94)	3.4
	Susceptible	12	(10–14)		21	(18–28)	
Penoxsulam	AR1	>1120		>94	>1120		>42
	AR2	358	(297–437)	30	1110	(835–1669)	41
	MS1	112	(94–133)	9.4	308	(245–422)	12
	Susceptible	12	(10 to 14)		27	(22 to 35)	

[a]LD_{50} and LD_{90} were determined by conducting Probit analysis in SAS.
[b]R/S ratio was calculated by dividing the LD_{50} and LD_{90} dose of resistant population by the LD_{50} and LD_{90} dose, respectively, of susceptible population.
[c]All imazamox and penoxsulam treatments contained a nonionic surfactant at 0.25% v/v.
[d]Bispyribac-sodium treatments contained a nonionic spray adjuvant and deposition aid at 2.5% v/v.

3.2. Characterization of Resistance to ALS-Inhibiting Herbicides. The dose-response experiments revealed that the LD_{50} of bispyribac-sodium, imazamox, and penoxsulam for the susceptible population was 3.3, 12, and 12 g ha^{-1}, respectively (Figure 1 and Table 3). The LD_{50} of bispyribac-sodium, imazamox, and penoxsulam for AR1 was 49, >1120, and >1120 g ha^{-1}, respectively; for AR2 was 3.0, >1120, and 358 g ha^{-1}, respectively; for MS1 was 24, 39, and 112 g ha^{-1},

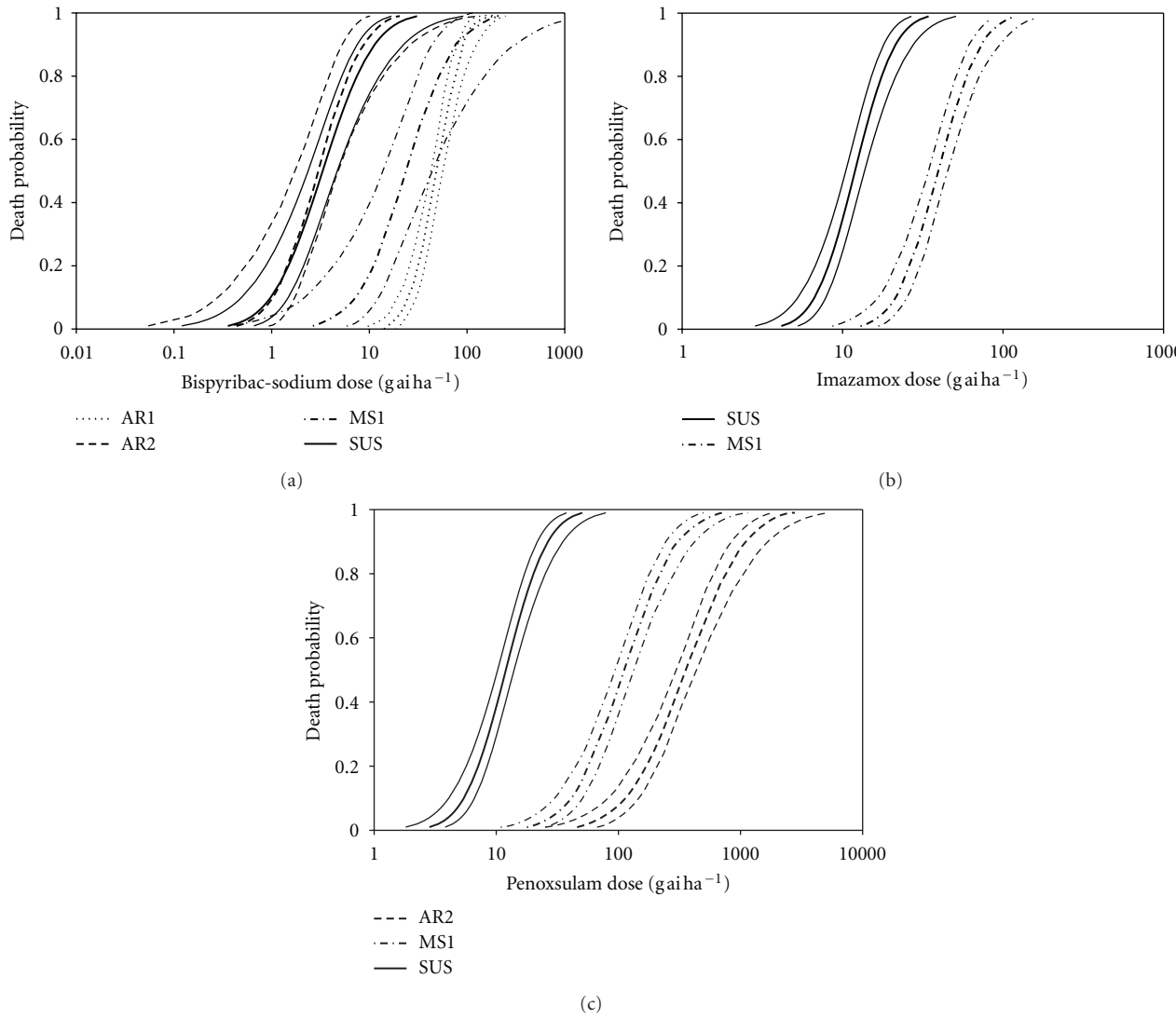

Figure 1: Probit analysis to predict the lethal dose of (a) bispyribac-sodium, (b) imazamox, and (c) penoxsulam required to kill 50% (LD_{50}) and 90% (LD_{90}) of *E. crus-galli* plants of each population (thick lines). Thin lines represent 95% confidence intervals (CIs) for each population. Lines of AR1 and AR2 for imazamox, and of AR1 for penoxsulam not shown, because the highest tested doses of imazamox (32X; 1X = 35 g ai ha^{-1}) and penoxsulam (32X; 1X = 35 g ai ha^{-1}) were not able to kill 50% of plants of these populations.

respectively. The resistance index based on LD_{50} values revealed that AR1 and MS1 populations were 15 and 7.2 times more resistant to bispyribac-sodium compared to the susceptible population; nevertheless, AR2 was very sensitive to bispyribac-sodium.

Additionally, AR1, AR2, and MS1 populations were >94, >94, and 3.3 times, respectively, more resistant to imazamox, and >94, 30, and 9.4 times, respectively, more resistant to penoxsulam compared to the susceptible population (Table 3). There was a high level of resistance to imazamox in both AR1 and AR2; nevertheless, the dry weight reduction of AR2 (22%) was less than AR1 (58%) even with field rate of imazamox (Table 2). AR1 plants treated with imazamox exhibited reduced growth and increased tillering typical of the symptoms of ALS-inhibiting herbicides, while AR2 plants continued growth without increased tillering (data not shown).

The LD_{90} values of bispyribac-sodium, imazamox, and penoxsulam were calculated to evaluate herbicide dose required for 90% mortality of all populations (Figure 1 and Table 3), a mortality level that would be needed in a production system. The LD_{90} of bispyribac-sodium was 94, 8.6, 81, and 11, imazamox was >1120, >1120, 73, and 21, and penoxsulam was >1120, 1110, 308, and 27 for AR1, AR2, MS1, and susceptible populations, respectively.

3.3. CYP Inhibition by Malathion. Bispyribac-sodium, imazethapyr, and penoxsulam with and without malathion reduced dry weight of susceptible population by 100% compared to the nontreated control treatment (Table 4). Addition of malathion to penoxsulam in comparison to penoxsulam applied alone reduced dry weight of AR1, AR2, and MS1 populations by 40, 94, and 97%, respectively. Additionally, the plant mortality of AR2 and MS1 populations followed

Table 4: The percent of above-ground dry weight (with percent mortality in parenthesis) of ALS-resistant (AR1, AR2, and MS1) and -susceptible *E. crus-galli* populations at 21 d after treatment with different ALS herbicides applied alone or in combination with malathion[a,b,c,d,e].

Treatment	Rate	Above-ground dry weight							
		SUS		AR1		AR2		MS1	
	g ai ha^{-1}	% of control							
Malathion	1000	105	A	92 (0)	A	137 (0)	A	126 (0)	A
Bispyribac-sodium	30	0.0	A	3.6 (70)	E	0.0 (100)	D	4.8 (85)	C
Bispyribac-sodium + malathion	30 + 1000	0.0	A	0.0 (100)†	E	0.0 (100)	D	0.0 (100)	C
Imazethapyr	105	0.0	A	23 (0)	D	92 (0)	B	1.4 (80)	C
Imazethapyr + malathion	105 + 1000	0.0	A	33 (0)	C	87 (0)	B	0.0 (100)†	C
Penoxsulam	35	0.0	A	71 (0)	B	51 (0)	C	33 (10)	B
Penoxsulam + malathion	35 + 1000	0.0	A	43* (15)	C	3.2* (75)†	D	1.1* (95)†	C

[a]Means for each population within a column followed by the same letters are not significantly different according to Fisher's protected LSD test ($\alpha = 0.05$).
[b]* Represents reduced dry weight with addition of malathion to a particular herbicide treatment based on t-test ($\alpha = 0.05$).
[c]† Represents increased mortality with addition of malathion to a particular herbicide treatment based on chi-square test ($\alpha = 0.05$).
[d]Imazethapyr and penoxsulam treatments contained a nonionic surfactant at 0.25% v/v.
[e]Bispyribac-sodium treatments contained a nonionic spray adjuvant and deposition aid at 2.5% v/v.

the trend of dry weight and increased from 0 and 10% to 75 and 85%, respectively, after addition of malathion to penoxsulam. There was no effect on dry weight reduction of any population after addition of malathion to imazethapyr or bispyribac-sodium. The differences in mortality of MS1 with imazethapyr and of AR1 and MS1 populations with bispyribac-sodium, however, were obvious with and without malathion. Mortality of MS1 increased from 80 to 100% with addition of malathion to imazethapyr, and mortality of AR1 increased from 70 to 100% with addition of malathion to bispyribac-sodium.

4. Discussion

All evaluated resistant populations evolved cross resistance to imazamox and penoxsulam. In addition, AR1 and MS1 have evolved cross resistance to bispyribac-sodium. Weeds have the ability to evolve resistance over several herbicide families within a herbicide mode of action group, even if some herbicides were never used to control the weed (cross resistance). For example, an *E. phyllopogon* population from California, USA, evolved cross resistance to both bensulfuron and bispyribac-sodium; however, the rice fields where it was found were treated only with bensulfuron and never with bispyribac-sodium [21]. *Echinochloa crus-galli* populations exhibiting resistance to a broad range of ALS-inhibiting herbicides have been reported in Brazil, Italy, South Korea, Turkey, and Yugoslavia [2, 22]. Our studies present the first report of ALS cross-resistant *E. crus-galli* in the USA.

Ninety percent of the susceptible plants were killed with below labeled field rates of bispyribac-sodium (22.5 g ha^{-1}), imazamox (35 g ha^{-1}), and penoxsulam (35 g ha^{-1}). However, the LD_{90} of bispyribac-sodium, imazamox, and penoxsulam for all resistant populations (except AR2 with bispyribac-sodium) was greater than field rates, which suggests that it is no longer possible to effectively control these populations. Additionally, a high level of resistance was observed for imazamox and penoxsulam in AR1 and AR2. For instance, more than 32 times (>1120 g ha^{-1}) the field application rate of imazamox was needed to kill 90% of the treated plants of AR1 and AR2 populations. The high level of resistance to imazamox in both AR populations is indicative of an altered target site, but the differential phenotypic response along with differences in pattern of cross resistance suggests that the mechanism likely differs between these two populations. Low to moderate level of resistance to bispyribac-sodium in AR1 and MS1 and to imazamox in MS1 indicates the possibility of non-target-site-based resistance. Further research is needed to elucidate the mechanism(s) of cross resistance to ALS-inhibiting herbicides in these populations.

Echinochloa phyllopogon is a closely related species to *E. crus-galli* and is also known in the literature as *E. crus-galli* (L.) Beauv. var. *oryzicola* Ohwi [21]. Two *E. phyllopogon* accessions from California, USA, were reported to be 3.8 and 5.0 times more resistant to bispyribac-sodium compared to the susceptible *E. phyllopogon* biotype [21]. Additionally, similar to our studies with *E. crus-galli*, various levels of resistance to ALS-inhibiting herbicides belonging to different chemical families were observed in *E. phyllopogon*. An *E. phyllopogon* biotype from California was 9 and >25 times more resistant to bispyribac-sodium and bensulfuron, respectively, compared to the susceptible *E. phyllopogon* biotype [23].

The addition of malathion to penoxsulam reduced *E. crus-galli* dry weights or increased mortality of AR1, AR2, and MS1 populations. More than 94% reduction in the dry weight of AR2 and MS1 populations after addition of malathion to penoxsulam suggests that CYP inhibition by malathion is likely responsible for imparting at least some level of resistance to penoxsulam in these populations. Target site mutation was not the mechanism of ALS resistance in the closely related species, *E. phyllopogon* [24]. However, addition of malathion to bispyribac-sodium reverted the bispyribac-sodium resistance in an *E. phyllopogon* population from California, suggesting that increased herbicide

metabolism by CYP was the mechanism of resistance to bispyribac-sodium and bensulfuron methyl [23]. Resistance to penoxsulam in *E. phyllopogon* has also been reported to be due to the increased herbicide metabolism by CYP [18].

There was only 40% reduction in dry weight with no significant difference in plant mortality of the AR1 population after addition of malathion to penoxsulam. In general, plants have several CYP isozymes with varying levels of herbicide specificity [25–27]. Previous studies have shown the differential enhancement of toxicity in resistant biotypes treated with herbicide in combination with different CYP inhibitors such as malathion, piperonyl butoxide, or aminobenzotriazole [14, 16, 28], which again hints toward involvement of more than one CYP isoform. Less reduction in dry weight with penoxsulam plus malathion in AR1 compared to AR2 and MS1 might be because of more than one CYP isoform in AR1 that is specific to different CYP inhibitors. Mechanisms other than metabolism by CYP may also be involved in imparting complete penoxsulam resistance to AR1 population.

Dose response experiments revealed that the LD_{50} and LD_{90} of bispyribac-sodium ranged from 49 to 94 g ha^{-1} for AR1 and from 24 to 81 g ha^{-1} for MS1 populations. Therefore, even when bispyribac-sodium was applied alone at 30 g ha^{-1}, there was substantial reduction in the dry weight of AR1 and MS1 populations, ultimately resulting in no difference with and without malathion. Similarly, because of low level of resistance of MS1 to the imidazolinone herbicides, no difference in dry weight of the MS1 population occurred when imazethapyr was applied with and without malathion. Further dose-response experiments with and without CYP inhibitors as well as metabolism studies with [^{14}C]-herbicides are needed to confirm metabolism-based resistance to imazethapyr and bispyribac-sodium in these populations.

In *E. phyllopogon*, enhanced herbicide degradation by CYP was responsible for multiple resistance to bispyribac-sodium, fenoxaprop ethyl, and thiobencarb [29]. CYP-based cross- and multiple resistance in several other weed species has been reviewed [30]. Four out of the seven ALS-resistant *E. crus-galli* biotypes listed by [2] have multiple resistance to other herbicides. Although not reported so far, the mechanism of multiple resistance in those *E. crus-galli* biotypes from Brazil, Italy, South Korea, and Turkey may also be increased metabolism by CYP that oxidizes a broad range of substrates.

Multiple non-target-site-based resistance (NTSR) genes endow resistance to multiple herbicides [31]. Herbicide selection tends to accumulate NTSR genes in resistant species [32]. There is a high probability that the ALS-resistant populations from Arkansas and Mississippi, USA, which are under continuous herbicide selection, can also have inherent multiple resistance to other herbicide groups, especially ACCase-inhibiting herbicides and photosystem II inhibitors. Future experiments are needed to determine if these herbicide-resistant populations have evolved multiple resistance to any of the other important rice herbicides as well as herbicides that would be used in rotations crop such as soybean (*Glycine max* L.). Not only in the southern central region of the USA, where *E. crus-galli* has already evolved resistance to propanil, quinclorac, or clomazone [10, 33], the evolution of metabolism-based resistance is of great concern worldwide and could have far-reaching implications on the management of *E. crus-galli* in rice and other crops. Furthermore, continued research is needed to elucidate the resistance mechanism(s) in each of these three ALS-resistant *E. crus-galli* populations from USA and multiple herbicide-resistant biotypes from other countries.

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