Confirmation of *S*-metolachlor resistance in Palmer amaranth (*Amaranthus palmeri*)

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Abstract

S-Metolachlor is commonly used by soybean and cotton growers, especially with POST treatments for overlapping residuals, to obtain season-long control of glyphosate- and acetolactate synthase (ALS)–resistant Palmer amaranth. In Crittenden County, AR, reports of Palmer amaranth escapes following *S*-metolachlor treatment were first noted at field sites near Crawfordsville and Marion in 2016. Field and greenhouse experiments were conducted to confirm *S*-metolachlor resistance and to test for cross-resistance to other very-long-chain fatty acid (VLCFA)–inhibiting herbicides in Palmer amaranth accessions from Crawfordsville and Marion. Palmer amaranth control in the field (soil <3% organic matter) 14 d after treatment (DAT) was ≥94% with a 1× rate of acetochlor (1,472 g ai ha^{-1}; emulsifiable concentrate formulation) and dimethenamid-P (631 g ai ha^{-1}). However, *S*-metolachlor at 1,064 g ai ha^{-1} provided only 76% control, which was not significantly different from the 1/2× and 1/4× rates of dimethenamid-P and acetochlor (66% to 85%). In the greenhouse, Palmer amaranth accessions from Marion and Crawfordsville were 9.8 and 8.3 times more resistant to *S*-metolachlor compared with two susceptible accessions based on LD$_{50}$ values obtained from dose–response experiments. Two-thirds and 1.5 times *S*-metolachlor at 1,064 g ha^{-1} were the estimated rates required to obtain 90% mortality of the Crawfordsville and Marion accessions, respectively. Data collected from the field and greenhouse confirm that these accessions have evolved a low level of resistance to *S*-metolachlor. In an agar-based assay, the level of resistance in the Marion accession was significantly reduced in the presence of a glutathione S-transferase (GST) inhibitor, suggesting that GSTs are the probable resistance mechanism. With respect to other VLCFA-inhibiting herbicides, Marion and Crawfordsville accessions were not cross-resistant to acetochlor, dimethenamid-P, or pyroxasulfone. However, both accessions, based on LD$_{50}$ values obtained from greenhouse dose–response experiments, exhibited reduced sensitivity (1.5- to 3.6-fold) to the tested VLCFA-inhibiting herbicides.

Introduction

Very-long-chain fatty acid (VLCFA)–inhibiting herbicides (Group 15) are labeled for use in numerous crops for residual control of annual grasses and small-seeded broadleaves. Fatty acids with a carbon chain length of ≥20 (20 to 34 carbons) are referred to as VLCFAs and are important building blocks for cuticular waxes and sphingolipids. Sphingolipids are required for proper vesicle trafficking and membrane dynamics, especially in dividing cells (Bach et al. 2011; Jenks et al. 2002; Li-Beisson et al. 2010). VLCFAs are synthesized by a four-enzyme complex, and the substrate specificity of this complex (fatty acid ≥18 carbons) is determined by B-ketoacyl-CoA synthase, otherwise known as fatty acid elongase (FAE) (Joubes et al. 2008; Li-Beisson et al. 2010). FAEs are the molecular target of VLCFA-inhibiting herbicides. In *Arabidopsis*, the genome contains 21 FAE-like genes with overlapping expression profiles and protein substrate specificity (i.e., C22, C24, and C26 VLCFAs). This indicates that VLCFA-inhibiting herbicides must inhibit different FAE proteins and thus must have multiple target sites (Joubes et al. 2008; Li-Beisson et al. 2010; Trenkamp et al. 2004).

Several VLCFA-inhibiting herbicides exist and can be grouped into three major chemical families: chloroacetamides such as *S*-metolachlor, acetochlor, and dimethenamid-P; the pyrazole, pyroxasulfone; and the oxyacetamide, flufenacet. VLCFA-inhibiting herbicides have been used for over 60 yr, but resistance appears to be a rare event (Heap 2018). Because of the genetic redundancy of FAEs, the evolution of target-site resistance is predicted to be low and has yet to be identified in nature. To date, resistance to VLCFA-inhibiting herbicides has only been confirmed in

flufenacet-resistant blackgrass (*Alopecurus myosuroides* Huds.) (Heap 2018) and Italian ryegrass [*Lolium perenne* subsp. *multiflorum* (Lam.) Husnot] (Liu et al. 2016), pyroxasulfone-resistant wild oat (*Avena fatua* L.) (Mangin et al. 2017), butachlor-resistant barnyardgrass (*Echinochloa crus-galli* L. Beauv.) (Juliano et al. 2010), and chloroacetamide- and pyroxasulfone-resistant rigid ryegrass (*Lolium rigidum* Gaud.) (Burnet et al. 1994; Busi et al. 2014). Interestingly, in the aforementioned weed species, resistance was found only in multiple-herbicide–resistant accessions, and resistance was not always the result of selection from VLCFA-inhibiting herbicide use. In these cases, resistance to VLCFA-inhibiting herbicides, just like the tolerance mechanism found in corn (*Zea mays* L.) and sorghum (*Sorghum bicolor* L.), was hypothesized to be conferred by glutathione S-transferases (GST) (Busi et al. 2014; Deng and Hatzios 2002; Dixon et al. 1997). Busi et al. (2018) found that pyroxasulfone was rapidly detoxified within 24 h in resistant rigid ryegrass plants by GSTs, and they furthermore identified increased GST expression that correlated with resistance.

Palmer amaranth is one of the most troublesome weeds in row crops found throughout the United States (Van Wychen 2016). Seasonal and long-term control of Palmer amaranth is difficult, because it has a season-long emergence pattern, vigorous seedling growth, ability to rapidly replenish the soil seed bank, and a tendency to evolve resistance to herbicides (Heap 2018; Horak and Loughin 2000; Jha and Norsworthy 2009; Keeley et al. 1987; Norsworthy et al. 2014). A proactive weed management program that prevents weeds from reaching maturity and replenishing the soil seed bank, referred to as a "zero-tolerance" threshold, should be adopted for problematic weeds like Palmer amaranth (Bagavathiannan and Norsworthy 2012; Neve et al. 2011; Norris 1999; Norsworthy et al. 2014).

In grower fields where glyphosate- and ALS-resistant Palmer amaranth are prevalent, the use of residual herbicides at planting and with POST applications to overlap residuals are foundational steps for season-long control (Culpepper et al. 2009; Norsworthy et al. 2012; Riar et al. 2013). In soybean and cotton, VLCFA- and protoporphyrinogen oxidase (PPO)–inhibiting herbicides with residual activity (Group 14) are commonly used PRE herbicides. For example, 40% and 56% of the treated 2017 soybean hectares in the United States were treated with either VLCFA- or PPO-inhibiting herbicides (USDA 2018). However, in the Mid-South, the evolution and subsequent spread of resistance to PPO-inhibiting herbicides in Palmer amaranth (Copeland et al. 2018; Varanasi et al. 2018) will undoubtedly increase the use of VLCFA-inhibiting herbicides and consequently increase the probability of selecting for resistance to Group 15 herbicides.

In the 2016 and 2017 growing seasons, Palmer amaranth escapes following *S*-metolachlor were observed within Crittenden County, AR, at field locations near Crawfordsville and Marion. Resistance to *S*-metolachlor is highly suspected at those field locations (JK Norsworthy and T Barber, unpublished data). Therefore, this research had two objectives: to confirm and quantify the level of resistance to *S*-metolachlor in the field and greenhouse, and to test for cross-resistance to other VLCFA-inhibiting herbicides.

Materials and Methods

Field Study

In 2018, two chloroacetamide efficacy experiments were established at field sites near Crawfordsville and Marion, AR. Research at Crawfordsville was conducted on a Dundee silt loam soil with a 1.95% organic matter content and a pH of 5.39. At Marion, the soil was a Dubbs sandy loam with a 1.4% organic matter content and a pH of 5.76. At each site, experiments were a randomized complete block design with a factorial arrangement of chloroacetamides and herbicide rate. The three chloroacetamides used were *S*-metolachlor (Dual Magnum; Syngenta Crop Protection, LLC, Greensboro, NC), acetochlor (Harness; Monsanto Co., St. Louis, MO), and dimethenamid-P (Outlook; BASF, Research Triangle Park, NC). Each herbicide was applied at 1/4×, 1/2×, and 1× of their respective field use rate. The field use rate for *S*-metolachlor was 1,064 g ai ha^{-1}, acetochlor at 1,472 g ha^{-1}, and dimethenamid-P at 631 g ha^{-1}. The labeled field use rates in this study are congruent with fields with <3% organic matter and a coarse to medium soil texture. Furthermore, these rates were chosen to simulate what a typical Mid-South grower would apply PRE and/or POST. Each experiment had four replications, and plots were 4 by 8 m long. At Marion, cotton was planted before treatments were applied, whereas Crawfordsville was fallow. Fields at each location were treated with glufosinate (595 g ai ha^{-1}) and glyphosate (860 g ae ha^{-1}) to control existing vegetation before experiments were initiated.

Treatments were applied with a CO_2-pressurized backpack sprayer using AIXR110015 nozzles (Teejet, Wheaton, IL) calibrated for an output of 140 L ha^{-1} at 4.83 km h^{-1}. PRE treatments were applied when a rain event was forecasted in the subsequent days. Rainfall data are summarized in Figure 1. At each location, visible weed control ratings were taken at 14 and 28 DAT on a scale from 0 to 100%, with 0 being no control and 100% being complete control. Control ratings were estimated by determining the reduction of weed emergence and biomass accumulation observed in treated plots in comparison to nontreated plots. At Crawfordsville, two counts of Palmer amaranth plants per square meter were recorded per plot at 14 and 28 DAT. To test for microbial breakdown of *S*-metolachlor, barnyardgrass density and visible control ratings at Crawfordsville were taken at 14 and 28 DAT. Weed density counts were converted to percent reduction from the nontreated control. Before analysis, data were tested for homoscedasticity using a Bartlett's test. No site-by-treatment interactions were detected, and thus visible control data were pooled over sites. Data were subjected to ANOVA with site and replication set as random effects. Means were separated using Fisher's protected LSD ($\alpha = 0.05$) in JMP Pro 12 (SAS institute Inc. Cary, NC).

Greenhouse Studies

Greenhouse experiments were conducted to complement the field study and to characterize the level of resistance to VLCFA-inhibiting herbicides. Experiments were conducted at the Altheimer Laboratory at the University of Arkansas, Fayetteville. In the fall of 2017, inflorescences from Palmer amaranth plants at Crawfordsville and Marion were collected, threshed, and cleaned to generate a seed source for further experimentation. Two susceptible accessions that had been originally collected in 2001 by Bond et al. (2006) were used in all experiments. The susceptible accessions were collected in Miller and Jefferson counties and will be referred to as ARSE-1 and ARE-8, respectively. All accessions had >50% germination.

Dose–response experiments were conducted to determine difference in sensitivity between susceptible and resistant accessions to *S*-metolachlor (Dual Magnum), acetochlor (Harness), dimethenamid-P (Outlook), and pyroxasulfone (Zidua SC; BASF, Research Triangle Park, NC). Similar to the field study, the 1× rates were *S*-metolachlor at 1,064 g ha^{-1}, acetochlor at
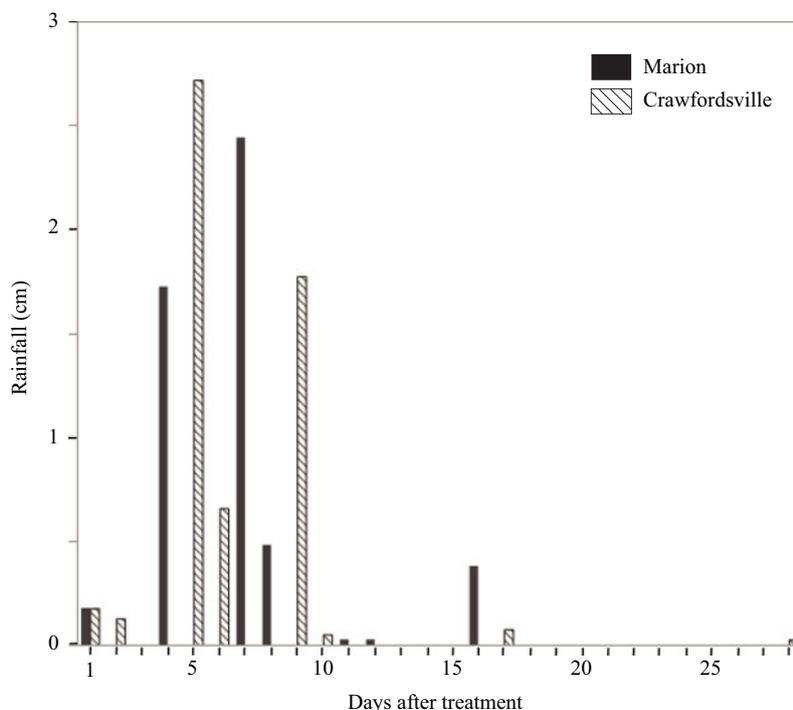
Figure 1. Rainfall (cm) at Marion and Crawfordsville locations from the initiation to the termination of trial.

1,472 g ha⁻¹, dimethenamid-P at 631 g ha⁻¹, and pyroxasulfone at 120 g ha⁻¹. The herbicide dosages used for the susceptible accessions were 0, 1/256, 1/128, 1/64, 1/32, 1/16, 1/8, 1/4, 1/2, and 1×. For the resistant accessions the rates were 0, 1/128, 1/64, 1/32, 1/16, 1/8, 1/4, 1/2, and 1×, except for *S*-metolachlor, where 2× and 4× rates were included instead of the 1/128× rate. Studies were conducted as a completely randomized experiment with three replications per treatment and two total runs, except for *S*-metolachlor, which had three total runs. A replication consisted of 12.2- by 9.5- by 5.7-cm flats (Insert TO standard; Hummert International, Earth City, MO) filled with sieved soil. A silt loam soil collected from the University of Arkansas Agriculture Research and Extension Center in Fayetteville with a pH of 6.6 and 2.4% organic matter content was used in all experiments. Prior to applications, flats were presoaked, allowed to drain, and, if needed, soil was added to reach the brim of flats. Afterwards, 100 seeds were scattered over the soil surface, and seeds were lightly covered with soil. Treatments were applied using a research track sprayer equipped with 1100067 nozzles (Teejet, Wheaton, IL) calibrated to deliver 187 L ha⁻¹ at 1.6 km h⁻¹. Afterwards, flats were watered over the top to incorporate the PRE herbicides into the soil solution. For the first 10 d of an experiment, flats were watered over the top; after that time they were placed in trays and watered from below.

At 21 DAT, live counts were recorded, and only plants with a true leaf were considered alive. For each experimental run, live counts were converted to percent survival based on the number of seedlings in the nontreated controls. There were no significant interactions between runs; therefore, data were pooled accordingly (P > 0.05). Dose–response curves were generated in R using a four-parameter log-logistic model described as in Equation 1:

$$Y = C + \frac{D-C}{1+\exp\{b[\log(x)]-\log(\mathrm{LD50})\}} \qquad (1)$$

where Y is the response variable, C is the lower limit of Y, D is the upper limit of Y, b is the slope of the curve around the LD_{50} (lethal dose causing 50% mortality), and x is the herbicide dose (Ritz et al. 2015). Estimated LD_{50} and LD_{90} values were derived from equations. Fold-change in sensitivity to each herbicide was determined by dividing the LD_{50} value of the resistant accession by the average LD_{50} value of the two susceptible accessions.

Metabolic Resistance Study

To determine if *S*-metolachlor resistance could be GST-mediated, seeds of Marion and ARSE-1 accessions (not surface sterilized) were placed on agar plates containing *S*-metolachlor with or without the GST inhibitor 4-chloro-7-nitrobenzofurazan (NBD-Cl; Sigma-Aldrich Ltd., St. Louis, MO). Preliminary experiments were conducted to determine appropriate rates of *S*-metolachlor and NBD-Cl. Medium was made by autoclaving a 1-L solution containing 2.2 g of Murashige and Skoog (MS) Basal Salt Mixture (Sigma-Aldrich) and 11 g agar. The pH of the solution was 5.7 to 5.8. The plates used in all experiments were sterile square Petri dishes with grids (100 by 100 by 15 mm; Simport). The *S*-metolachlor rates in the agar plates were 0.5 and 1 μM, and the NBD-Cl rate was 50 nM. To obtain the appropriate *S*-metolachlor rates, Dual II Magnum was diluted in DMSO, and NBD-CL was solubilized and diluted in methanol. DMSO concentration did not exceed 0.05% v/v. Thirty seeds were plated on each treatment plate for each accession. After plating seeds, agar plates were wrapped in surgical tape and placed vertically in growth chambers (22 C and a 16-h photoperiod). The day after plating was considered 1 DAT.

At 12 DAT, agar plates were photographed, and ImageJ (Schneider et al. 2012) was used to measure root lengths. Pixel number was converted to centimeters using the grid pattern on square plates, which have a height of 1.5 cm. Root lengths are expressed as percent reduction from the nontreated. Three runs

Table 1. Visible control ratings of Palmer amaranth at Marion and Crawfordsville, AR, with suspected resistance to VLCFA-inhibiting herbicides and density reduction of Palmer amaranth and barnyardgrass (BYG) at Crawfordsville only.

Herbicide	Rate	Palmer amaranth[a]				BYG
		Control		Density reduction[b]		
		14 DAT	28 DAT	14 DAT	28 DAT	28 DAT
	g ai ha^{-1}	———————————— % ————————————				
S-metolachlor	1,064	76 CD[c]	34 B	71 B	74	95
	535	68 D				
	268	34 E				
Acetochlor[d]	1,472	98 A	60 A	91 A	80	94
	736	85 BC				
	368	68 D				
Dimethenamid-P	631	94 AB	57 A	95 A	89	97
	316	75 CD				
	158	66 D				
Herbicide		< 0.0001	< 0.0001	0.0008	0.1439	0.2452
Rate		< 0.0001	< 0.0001	0.0116	0.0061	0.0418
Herbicide × rate		0.0140	0.9279	0.1673	0.5522	0.7996

[a] Palmer amaranth data were pooled over Marion and Crawfordsville location (P > 0.05).
[b] Density reduction data were only recorded at Crawfordsville in 2018.
[c] Means were separated using Fishers protected LSD values at α = 0.05, and different letters within a column indicate a significant difference between treatments.
[d] An emulsifiable concentrate acetochlor formulation was used.

were conducted in time with one treatment plate per run. At least 12 roots were measured for each accession per plate. The root length of each seedling was considered a replication. The number of root lengths measured varied, either because of germination issues or because roots grew into the agar and thus were not measured. Before analysis, data were tested for homoscedasticity using a Bartlett's test. There was no three-way interaction between run, accession, and treatment (P = 0.18); thus, data were pooled over runs. After pooling over runs, the total number of root lengths measured from the Marion accession were 46, 39, 49, 44, and 51 and 38, 40, 45, 52, and 53 for ARSE-1 for the following treatments, respectively: NBD-CL only, *S*-metolachlor (0.5 µM), *S*-metolachlor (0.5 µM) + NBD-Cl, *S*-metolachlor (1 µM), and *S*-metolachlor (1 µM) + NBD-Cl. Data were subjected to ANOVA with run set as random. Means were separated using Tukey's HSD (α = 0.05) in SAS 9.4 (SAS Institute Inc., Cary, NC).

Results and Discussion

Chloroacetamide Field Study

The Palmer amaranth accessions at Crawfordsville and Marion responded similarly to *S*-metolachlor, acetochlor, and dimethenamid-P treatments (Table 1). At 14 DAT, both accessions were controlled ≥94% with a 1× rate of acetochlor (1,472 g ha^{-1}) and dimethenamid-P (631 g ha^{-1}). Other authors have reported a similar control of *Amaranthus* spp. (≥90%) with Group 15 herbicides after a precipitation event that incorporated herbicides into soil solution (Hay et al. 2018; Meyers et al. 2010; Steckel et al. 2002; Sweat et al. 1998). However, a 1× rate of *S*-metolachlor at 1,064 g ha^{-1} provided only 76% control. Furthermore, control with the 1× rate of *S*-metolachlor was not different from the 1/2× rate (68%) or the 1/2× and 1/4× rates of dimethenamid-P and acetochlor (66% to 85%). By 28 DAT, the chloroacetamide used was significant (P = <0.0001) but the rate interaction was not (P = 0.93). The residual activity of acetochlor and dimethenamid-P, averaged over rates and herbicides, provided 59% control of both accessions. Although poor, these herbicides still provided 25% greater control than *S*-metolachlor (34%).

At Crawfordsville only, Palmer amaranth density was recorded, and both density reduction and visible control data followed a similar pattern. For example, acetochlor and dimethenamid-P, averaged over rates and herbicides at 14 DAT, reduced Palmer amaranth density by 93%, whereas *S*-metolachlor only reduced density by 71% when compared to the nontreated control (49 plants m^{-2}) (Table 1). The above data highlight how the accessions from both Marion and Crawfordsville have reduced sensitivity to *S*-metolachlor but not acetochlor and dimethenamid-P. Interestingly, barnyardgrass control (data not shown) and density data were also recorded at Crawfordsville, and by 28 DAT, all three chloroacetamides regardless of rate reduced barnyardgrass density by ≥94% when compared to the nontreated control (16 plants m^{-2}). The fact that *S*-metolachlor still controlled barnyardgrass, but not Palmer amaranth, suggests that increased microbial breakdown is probably not a major factor in the low *S*-metolachlor activity observed on these accessions.

Confirmation of S-Metolachlor Resistance

To confirm and quantify the level of *S*-metolachlor resistance in the Marion and Crawfordsville accessions, seeds were collected from each location and used to generate dose–response curves in the greenhouse (Figure 2A and Table 2). At 21 DAT, the two susceptible accessions, ARE-8 and ARSE-1, had LD$_{50}$ values of 13 and 20 g ha^{-1}, respectively. Germinating seedlings at higher rates increasingly exhibited characteristic VLCFA-inhibitor symptomology of fused or crinkled leaf margins, and as rates exceeded 100 g ha^{-1}, shoot development was severely suppressed or completely inhibited (~267 g ha^{-1}). The LD$_{50}$ values for the Marion and Crawfordsville accessions were 156 and 133 g ha^{-1}, respectively. Based on these values, Marion and Crawfordsville were 9.8 and 8.3 times less responsive to *S*-metolachlor in comparison to the average LD$_{50}$ value of the susceptible accessions (16 g ha^{-1}). To obtain 90% mortality of the Crawfordsville and Marion accessions, it was estimated to require two-thirds and 1.5 times the 1× rate of *S*-metolachlor (1,064 g ha^{-1}) in the greenhouse. Furthermore, no seedlings from any accession survived at a 2× rate.
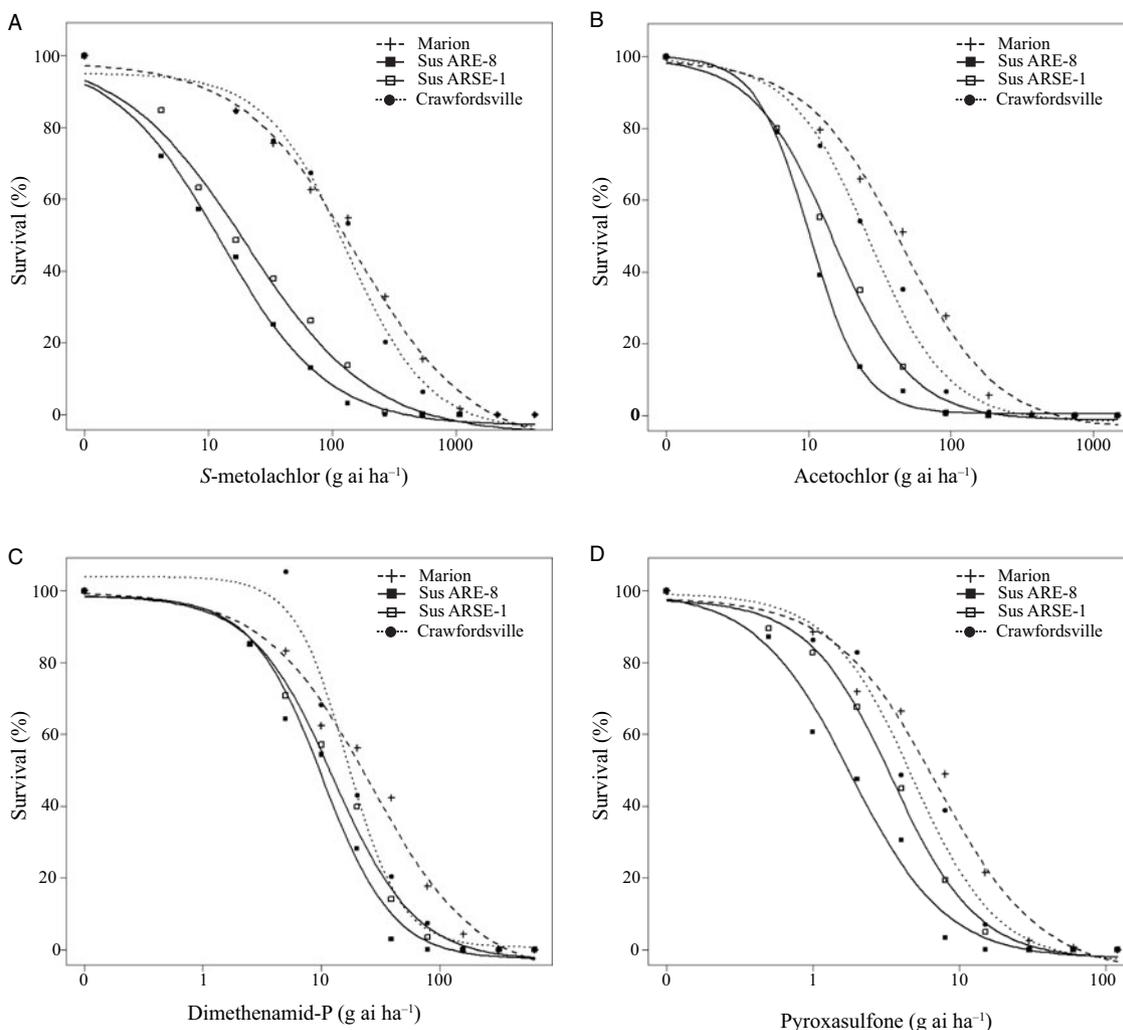
Figure 2. The percent survival of Marion, Crawfordsville, ARE-8, and ARSE-1 Palmer amaranth accessions to increasing rates of (A) *S*-metolachlor, (B) dimethenamid-P, (C) acetochlor, and (D) pyroxasulfone. The Marion and Crawfordsville accessions were suspected to have evolved resistance to VLCFA-inhibiting herbicides, and Sus ARE-8 and Sus ARSE-1 are the susceptible controls. The log-logistic parameters for each herbicide by accession and the rate that causes 50% and 90% mortality (LD$_{50}$ and LD$_{90}$) in each accession and herbicide are listed in Table 2.

Cross-Resistance to VLCFA-Inhibiting Herbicides

Marion, Crawfordsville, ARE-8, and ARSE-1 accessions were subjected to other Group 15 herbicides to determine the specificity of the *S*-metolachlor–resistant mechanism(s). The average LD$_{50}$ values for the susceptible accessions to acetochlor, dimethenamid-P, and pyroxasulfone were 13, 12, and 3 g ha^{-1}, respectively (Figure 2B–D and Table 2). A 1.5- to 2.1-fold and a 2.3- to 3.6-fold increase in herbicide rate was estimated to achieve 50% mortality for the Crawfordsville and Marion accessions, respectively. All accessions were either completely killed or severely stunted with a 1/4× rate of each herbicide.

Metabolic Resistance: GST Inhibition

Crop tolerance and weed resistance to VLCFA-inhibiting herbicides are either known or suspected to be conferred by GSTs (Busi et al. 2014, 2018; Deng and Hatzios 2002; Dixon et al. 1997; Skipsey et al. 1997). Under this assumption, seeds from the Marion and the ARSE-1 accessions were germinated on agar media containing *S*-metolachlor with or without the GST inhibitor

NBD-Cl (50 nM) (Figure 3A, B). The root lengths of Marion seedlings were reduced by 20% and by 40% with *S*-metolachlor at 0.5 μM and 1 μM, respectively. The addition of NBD-Cl with *S*-metolachlor at either rate significantly reduced the root growth of Marion seedlings on average by 19% over *S*-metolachlor alone. In the presence of NBD-Cl, the root growth inhibition of Marion seedlings was similar to that of ARSE-1 seedlings at the same *S*-metolachlor rate with or without NBD-Cl. This observation suggests that GSTs play some role in conferring resistance to *S*-metolachlor in the Marion accession, but more detailed laboratory experiments are needed to ascertain this.

S-metolachlor is frequently used by soybean and cotton growers, especially for overlapping residual activity with POST treatments, to obtain season-long control of glyphosate- and ALS-resistant Palmer amaranth (Culpepper et al. 2009; Norsworthy et al. 2012; Riar et al. 2013). We report here the first case of VLCFA-inhibiting herbicide resistance in Palmer amaranth. Specifically, both Marion and Crawfordsville accessions, based on our 1× rate, exhibited low levels of resistance to *S*-metolachlor (<2-fold based on LD$_{90}$ values), but not to acetochlor, dimethenamid-P, and pyroxasulfone. The evolution of a resistance

Table 2. Dose–response parameter estimates and LD90 values of Marion, Crawfordsville, and the two susceptible accessions ARE-8 and ARSE-1 to S-metolachlor, acetochlor, dimethenamid-P, and pyroxasulfone. A four-parameter log-logistic function was used to generate dose–response curves.[a]

Herbicide	Accession	Dose–response parameters estimates				
		B	C	D	$LD_{50} \pm SE$[b]	$LD_{90} \pm SE$
S-metolachlor	ARE-8	1.0	−3.16	99.56	13 ± 1	112 ± 23
	ARSE-1	0.85	−5.52	100.8	20 ± 2	267 ± 72
	Crawfordsville	1.32	−4.46	95.14	133 ± 10*	704 ± 119
	Marion	0.93	−8.95	98.15	156 ± 17*	1,632 ± 414
Acetochlor	ARE-8	2.33	0.46	100.28	10 ± 0.4	26 ± 2
	ARSE-1	1.59	−1.27	99.81	15 ± 1	60 ± 6
	Crawfordsville	1.56	−1.84	99.4	27 ± 1*	110 ± 12
	Marion	1.3	−3.81	98.97	45 ± 3*	245 ± 32
Dimethenamid-P	ARE-8	1.44	−2.7	98.54	10 ± 1	47 ± 6
	ARSE-1	1.26	−2.92	98.59	13 ± 1	73 ± 12
	Crawfordsville	1.92	0.61	104.0	17 ± 1*	53 ± 6
	Marion	0.96	−7.7	99.69	26 ± 3*	258 ± 69
Pyroxasulfone	ARE-8	1.33	−2.48	99.79	2 ± 0.1	8 ± 1
	ARSE-1	1.5	−2.66	97.62	3 ± 0.3	13 ± 2
	Crawfordsville	1.51	−3.44	99.26	5 ± 0.3*	17 ± 1
	Marion	1.23	−6.45	98.11	7 ± 0.6*	30 ± 4

[a] $Y = C + D − C/(1 + \exp\{b[\log(x)] − \log(LD_{50})\}$
[b] Statistical comparison was only done with LD_{50} values, and an asterisk indicates a significant difference from both susceptible accessions values.
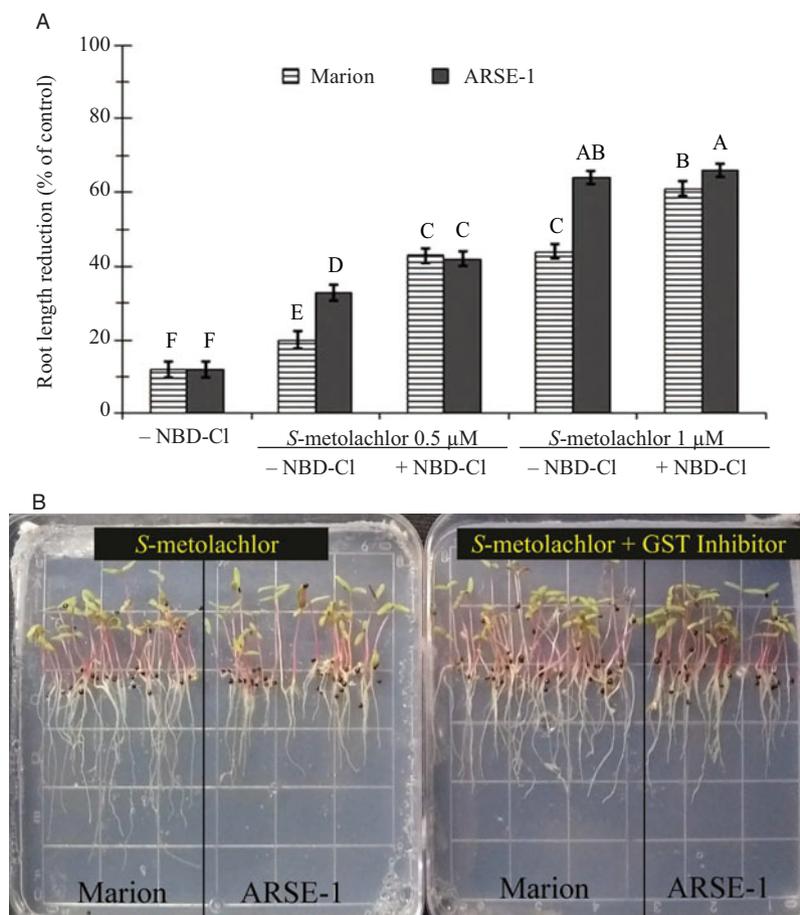
Figure 3. The response of Marion and ARSE-1 to S-metolachlor at 0.5 μM and 1 μM with or without the GST inhibitor 4-chloro-7-nitrobenzofurazan (NBD-Cl) in an agar-based assay. Marion is the resistant accession, and ARSE-1 is the susceptible control. (A) Summary of root length reductions from S-metolachlor with or without NBD-Cl treatments at 12 DAT. The error bars represent 1 ± SEM. Means were separated using Tukey's HSD values, and different letters indicate a significant difference (α = 0.05). (B) Representative response of Marion and ARSE-1 accessions to S-metolachlor at 1 μM (left) and S-metolachlor plus NBD-Cl at 50 nM (right).

mechanism(s) that confers field-level resistance to S-metolachlor, shown here to be probably mediated by GSTs, is extremely problematic.

Although the resistance mechanism did not confer cross-resistance to the other tested VLCFA-inhibiting herbicides at field-use rates, a 1.5- to 3.6-fold reduction in sensitivity based

on the susceptible LD$_{50}$ values was detected. Extrapolating this to the field, the residual activity provided by dimethenamid-P, pyroxasulfone, and acetochlor may be reduced. However, it should be noted that both acetochlor (1,472 g ha^{-1}) and dimethenamid-P (631 g ha^{-1}) in the field provided ≥94% control of both the Marion and Crawfordsville accessions at 14 DAT (Figure 1). In any case, the efficacy of all VLCFA-inhibiting herbicides on Palmer amaranth, especially the *S*-metolachlor–resistant accessions in this paper, must be managed properly. Otherwise, under our current management programs, the likelihood of selecting for resistance or reduced sensitivity to these herbicides is high.

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