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**Cite this article:** Carvalho-Moore P, Norsworthy JK, Souza MCCR, Barber LT, Boniha Piveta L, Meiners I, Porri A (2025) Resistance profile of glufosinate-resistant Palmer amaranth accessions and herbicide options. Weed Technol. **39**(e31), 1–12. doi: 10.1017/wet.2025.8

Received: 20 December 2024 Revised: 24 January 2025 Accepted: 30 January 2025

#### Associate Editor:

Amit Jhala, University of Nebraska, Lincoln

#### Nomenclature:

2,4-D; atrazine; dicamba; diuron; fomesafen; glyphosate; imazethapyr; mesotrione; paraquat; pyroxasulfone; trifludimoxazin; Palmer amaranth; *Amaranthus palmeri* S. Watson

#### Keywords:

Multiple herbicide resistance; limited chemical control; seven-way postemergence resistance

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# Resistance profile of glufosinate-resistant Palmer amaranth accessions and herbicide options

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## Abstract

Glufosinate resistance was previously confirmed in three Palmer amaranth accessions from Arkansas (MSR1, MSR2, and CCR). Greenhouse screening results suggested the presence of multiple herbicide resistance. Therefore, this study aimed to determine the postemergence resistance profile of these three glufosinate-resistant Palmer amaranth accessions. Field experiments were also conducted to assess preemergence and postemergence herbicide options to control the accession with the highest glufosinate resistance level (MSR2). A dose-response assay with the three resistant accessions and two susceptible standards was conducted with the herbicides 2,4-D, atrazine, dicamba, diuron, fomesafen, glyphosate, imazethapyr, and mesotrione. The preemergence and postemergence field experiments with MSR2 evaluated 15 and 16 single active ingredients, respectively. The Palmer amaranth accessions that carried glufosinate resistance were also confirmed to be resistant to six other postemergence herbicides: 2,4-D, diuron, fomesafen, glyphosate, imazethapyr, and mesotrione. CCR is also resistant to dicamba. Therefore, accessions MSR1, MSR2, and CCR have evolved resistance to postemergence herbicides pertaining to seven sites of action. A shift toward increased tolerance to atrazine has also been observed among all resistant accessions. Overall, field preemergence treatments with atrazine, pyroxasulfone, or trifludimoxazin obtained the highest MSR2 control levels at all evaluation times and the lowest number of seedlings emerging at 3 and 6 wk after treatment. In the postemergence experiment, only paraquat obtained MSR2 control levels above 90% at all ratings. The lowest number of alive MSR2 plants was observed after postemergence treatments with paraquat or trifludimoxazin. Fields near where glufosinate resistance has been confirmed in Palmer amaranth will likely demand a more diverse and proactive management strategy that relies on combinations of chemical, cultural, and mechanical control tactics. Future efforts should focus on sequential applications and mixture, the elucidation of all resistance mechanisms in the evaluated accessions, and soil-applied dose-response.

## Introduction

Up to the early 2000s, Palmer amaranth was only occasionally mentioned in scientific manuscripts. With the report of a Palmer amaranth population from Georgia found to be resistant to glyphosate in 2005, this species quickly became one of the most challenging weeds in row crops in the United States and is now frequently mentioned in the weed science literature (Culpepper et al. 2006; Heap 2024; Van Wychen 2022). A native plant from the desert regions of Mexico and the southwestern United States, Palmer amaranth is a formidable and extensively studied species that has been resiliently adapting to new environments through its prolific seed production, rapid growth rates, obligated outcrossing reproductive behavior, high genetic diversity transmitted through generations, and facility to evolve herbicide resistance (Chandi et al. 2013; Heap 2024; Horak and Loughin 2000; Keeley et al. 1987; Oliveira et al. 2022; Sauer 1957; Sellers et al. 2003; Ward et al. 2013; Wetzel et al. 1999). Unsurprisingly, yield reductions in crops are highly associated with Palmer amaranth interference (Burke et al. 2007; Klingaman and Oliver 1994; Massinga et al. 2001; Morgan et al. 2001).

Resistance to herbicides belonging to nine distinct sites of action (SOAs) has been confirmed in Palmer amaranth accessions across the United States (Heap 2024). The SOAs are categorized by the Weed Science Society of America (WSSA) and Herbicide Resistance Action Committee



(HRAC) as belonging to Group 2 (acetolactate synthase), Group 3 (microtubule assembly inhibitors), Group 4 (synthetic auxins), Group 5 (photosystem II inhibitors), Group 9 (5-enolpyruvylshikimate-3-phosphate synthase inhibitor), Group 10 (glutamine synthetase inhibitor), Group 14 [protoporphyrinogen oxidase (PPO) inhibitor], Group 15 (very long-chain fatty acid elongase synthesis inhibitors), and Group 27 (4-hydroxyphenylpyruvate dioxygenase inhibitors). Upon identification of resistant weeds in an area, chemical control will often rely on a herbicide with a different SOA. Continued use of single chemistries allied with high genetic variation and obligated outcrossing habits may select Palmer amaranth plants with additional resistance (Chandi et al. 2013; Sauer 1957; Wetzel et al. 1999; Zimdahl and Basinger 2024). In fact, Palmer amaranth accessions carrying five- or six-way resistance have been previously identified (Kumar et al. 2019; Shyam et al. 2021). The presence of accessions carrying multiple resistance complicates weed management by reducing the already limited herbicide options.

Palmer amaranth accessions that survived several glufosinate applications were collected in Arkansas from cotton fields located in Crittenden County (CCR accession) and Mississippi County (MSR1 and MSR2 accessions) in 2019 and 2020. Glufosinate resistance was confirmed in all three accessions with resistance fold compared to two susceptible standards ranging from 5.1-fold to 5.9-fold in CCR, 16.9-fold to 19.7-fold in MSR1, and 23.5-fold to 27.4-fold in MSR2 (Priess et al. 2022). Initial greenhouse screening results suggested the presence of multiple resistance in these accessions due to the lack of control with different herbicides (Priess et al. 2022). Therefore, a research gap exists in studies of the chemical options available to control this problematic biotype. The objective of this study was to determine the postemergence resistance profile of three previously confirmed glufosinateresistant Palmer amaranth accessions (MSR1, MSR2, and CCR) to multiple postemergence herbicides. Additionally, field experiments were conducted to assess preemergence and postemergence herbicide options to control the accession that showed the highest resistance to glufosinate (MSR2).

### **Materials and Methods**

## Whole-Plant Postemergence Dose Response

A dose-response assay was conducted under controlled environmental conditions ( $25 \pm 5$  C and 16-h day) at greenhouse facilities located at the Milo J. Shult Agricultural Research and Extension Center in Fayetteville, AR, to obtain the resistance profile of glufosinate-resistant Palmer amaranth accessions. Seeds from glufosinate-resistant accessions were collected in 2019 (CCR) and 2020 (MSR1 and MSR2). The collected seeds were sown, plants grown to the 5- to 6-leaf stage, and then sprayed with glufosinate (Liberty\*, BASF Corporation, Research Triangle, NC) at 656 g ai ha<sup>-1</sup> (1×). Survivors were allowed to set seeds that were used in the dose-response assay. Additionally, two well-characterized susceptible standards collected in South Carolina in 1986 (SS1) and in Arkansas in 2001 (SS2) were included for comparison.

The herbicides evaluated included 2,4-D (Group 4), atrazine (Group 5), dicamba (Group 4), diuron (Group 5), fomesafen (Group 14), glyphosate (Group 9), imazethapyr (Group 2), and mesotrione (Group 27). To account for differences in sensitivity, dose structures differed across the accessions and herbicides (Table 1). Palmer amaranth seedlings were transplanted into 50-cell trays that were 28 by 54 cm, and 7.5 cm deep (SureRoots

Deep 50 Cell Plug Trays; Greenhouse Megastore, Danville, IL) filled with potting mix (Sun Gro Horticulture, Agawam, MA) and sprayed when most plants reached the 5- to 6-leaf stage (height ranging from 7 to 10 cm). The experiment was organized as a completely randomized design with two (2,4-D, atrazine, dicamba, fomesafen, glyphosate, and imazethapyr) or three (diuron and mesotrione) experimental runs. A total of 50 seedlings per accession and per herbicide dose were sprayed in each experimental run.

Herbicide treatments were delivered in a two-nozzle spray chamber equipped with 1100067 nozzles (TeeJet Technologies, Glendale Heights, IL) calibrated to deliver  $187 \text{ L} \text{ ha}^{-1}$  at  $1.6 \text{ km h}^{-1}$ . The percentage mortality was calculated using the number of plants alive counted before and 4 wk after treatment (WAT).

## Preemergence and Postemergence Experiments

Bare-ground field experiments were conducted to determine the available preemergence and postemergence herbicide options for controlling a highly glufosinate-resistant Palmer amaranth accession, MSR2. In summer 2021, MSR2 seeds were spread and incorporated with a power takeoff-driven rototiller over a 2-ha secluded field at the Milo J. Shult Agricultural Research and Extension Center in Fayetteville, AR (36.09194 N, -94.18472 W). Palmer amaranth plants were allowed to grow and were sprayed with glufosinate at 656 g ai ha<sup>-1</sup> (1×). Survivors were allowed to produce and deposit seeds to ensure the MSR2 presence in the experiments conducted in the following years. The field was previously used for pasture and had no history of Palmer amaranth. The experiments were established on Captina silt loam soil, pH 6.6, and with 2.6% organic matter. No crops were present in either experiment due to the array of products tested.

The preemergence experiment was organized in a randomized complete block design and spatially replicated in June 17, 2022, June 9, 2023, and May 30, 2024. Each treatment had four replications in 2022 and 2023, and three replications in 2024. Plots measured 1.8 m wide by 3 m long in 2022, and 1.8 m wide by 6.1 m long in 2023 and 2024. Before trial initiation, the entire area was mowed and tilled to ensure weed-free conditions. After tillage, 15 preemergence herbicides were applied at the recommended crop rate (Table 2), with a nontreated control included for comparison. Herbicide applications were conducted using a CO<sub>2</sub>-pressurised backpack sprayer with a four-nozzle handheld boom equipped with TeeJet 110015 AIXR nozzles calibrated to deliver 140 L ha<sup>-1</sup> at 4.8 km h<sup>-1</sup>. Rainfall data (Figure 1), which aided the incorporation of preemergence herbicides into the soil solution, were obtained from a weather station approximately 1 km from the field. In all years, the field received at least 2 cm of rain within 7 d of preemergence herbicide application.

The postemergence experiment was organized in a randomized complete block design with four replications. The experiment was spatially repeated on June 26, 2023, and June 20, 2024, with plots measuring 1.8 m wide by 3 m long in both years. Palmer amaranth plants were sprayed with 16 postemergence herbicides at the recommended burndown or crop rate (Table 3). A nontreated control was kept for comparison. Plant height at the time of application ranged from 5 to 12 cm in 2023, and 2.5 to 15 cm in 2024. The Palmer amaranth density at the time of postemergence application across the nontreated plots in 2023 averaged 161 plants m<sup>-2</sup> and 36 plants m<sup>-2</sup> in 2024. Application equipment and conditions were similar to those described for the preemergence experiment, except that TeeJet 110015 TTI nozzles were used for

Table 1.	Postemergence	herbicides	used in	the whole-p	lant dose-re	esponse	assay. <sup>a</sup>
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	WSSA/HRAC group				
Herbicide	number	Trade name	Manufacturer	Doses ( $\times$ ) used per accession and herbicide	Labeled rate $(1\times)$
					g ai or ae ha <sup>-1</sup>
2,4-D	4	Enlist One	Corteva Agriscience USA	SS1 and SS2: $0 \times$ to $2 \times (n = 9 \text{ doses})$	1,064
			-	CCR: $0 \times$ to $16 \times$ (n = 10 doses)	
				MSR1 and MSR2: $0 \times$ to $8 \times$ (n = 9 doses)	
Atrazine <sup>b</sup>	5	Aatrex	Syngenta Crop Protection, LLC	SS1 and SS2: $0 \times$ to $1 \times (n = 9 \text{ doses})$	1,680
				MSR1, MSR2 and CCR: $0 \times$ to $2 \times$ (n = 9 doses)	
Dicamba	4	XtendiMax	Bayer CropScience	SS1 and SS2: $0 \times$ to $2 \times$ (n = 9 doses)	560
				MSR2 and CCR: $0 \times$ to $8 \times$ (n = 10 doses)	
				MSR1: $0 \times$ to $4 \times$ (n = 9 doses)	
Diuron	7	Direx	ADAMA	SS1 and SS2: 0 to $2 \times (n = 9 \text{ doses})$	840
				MSR1 and MSR2: $0 \times$ to $4 \times$ (n = 10 doses)	
				CCR: $0 \times$ to $8 \times$ (n = 11 doses)	
Fomesafen	14	Flexstar	Syngenta Crop Protection, LLC	SS1 and SS2: $0 \times$ to $2 \times$ (n = 9 doses)	264
				MSR2 and CCR: $0 \times$ to $8 \times$ (n = 11 doses)	
				MSR1: $0 \times$ to $16 \times$ (n = 10 doses)	
Glyphosate	9	Roundup PowerMAX 3	Bayer CropScience	SS1 and SS2: $0 \times$ to $1 \times$ (n = 9 doses)	1,120
				MSR1, MSR2 and CCR: $0 \times$ to $64 \times$ (n = 12 doses)	
Imazethapyr	2	Pursuit	BASF Ag Products	SS1: $0 \times$ to $4 \times$ (n = 14 doses)	70.6
				SS2: $0 \times$ to $4 \times$ (n = 10 doses)	
				MSR1, MSR2 and CCR: $0 \times$ to $64 \times$ (n =12 doses)	
Mesotrione	27	Callisto	Syngenta Crop Protection, LLC	SS1 and SS2: $0 \times$ to $1 \times (n = 9 \text{ doses})$	220
				CCR: $0 \times $ to $8 \times $ (n = 10 doses)	
				MSR1 and MSR2: $0 \times$ to $4 \times$ (n = 10 doses)	

<sup>a</sup>Abbreviations: CCR, glufosinate-resistant Palmer amaranth accession collected in 2019; HRAC, Herbicide Resistance Action Committee; MSR1 and MSR2, glufosinate-resistant Palmer amaranth accession collected in 2020; SS1 and SS2, glufosinate-susceptible standards collected in South Carolina in 1986 (SS1) and in Arkansas in 2001 (SS2); WSSA, Weed Science Society of America.

<sup>b</sup>Crop oil concentrate at 1% v/v was added in applications with atrazine, diuron, or mesotrione; Non-ionic surfactant at 0.25% v/v was added to applications with fomesafen or imazethapyr.

Table 2. Herbicides used in the preemergence experiments.<sup>a</sup>

	WSSA/HRAC group			
Herbicide	number	Trade name	Manufacturer	Rate
				g ai ha <sup>-1</sup>
Acetochlor	15	Warrant	Bayer CropScience	1,270
Atrazine	5	Aatrex <sup>®</sup>	Syngenta Crop Protection, LLC	1,680
Diuron	7	Direx <sup>®</sup>	ADAMA	1,120
Flumioxazin	14	Valor <sup>®</sup>	Valent U.S.A. LLC	72
Fluridone	12	Brake®	SePRO Ag, LLC	170
Fomesafen	14	Flexstar®	Syngenta Crop Protection, LLC	280
Imazaquin	2	Scepter®	Amvac Chemical Corporation	130
Isoxaflutole	27	Balance® Flexx	Bayer CropScience	90
Mesotrione	27	Callisto®	Syngenta Crop Protection, LLC	220
Metribuzin	5	TriCor®	UPL NA Inc.	750
Pendimethalin	3	Prowl® H <sub>2</sub> 0	BASF Ag Products	1,120
Pyroxasulfone	15	Zidua®	BASF Ag Products	170
Saflufenacil	14	Sharpen <sup>®</sup>	BASF Ag Products	50
S-metolachlor	15	Dual II Magnum®	Syngenta Crop Protection, LLC	1,400
Trifludimoxazin <sup>b</sup>	14	-	BASF Ag Products	50

<sup>a</sup>Abbreviations: HRAC, Herbicide Resistance Action Committee; WSSA, Weed Science Society of America.

<sup>b</sup>The herbicide trifludimoxazin is not currently commercially labeled for use in the United States.

the dicamba treatment. One day after the postemergence treatments, the entire experimental area was sprayed with pyroxasulfone at 170 g ai  $ha^{-1}$  (Zidua<sup>\*</sup>; BASF) to avoid Palmer amaranth emergence.

Two 0.25-m<sup>-2</sup> quadrats were randomly placed in all plots of the preemergence and postemergence experiments. Palmer amaranth seedlings were counted from each quadrat at 3 and 6 WAT (the end of the preemergence experiment) during the preemergence experiment, and visible control was rated at 3, 4, 5, and 6 WAT. In the postemergence experiment, live Palmer amaranth plants were counted at 4 WAT (the end of the postemergence experiment), and visible control was rated at 1, 2, 3, and 4 WAT. The visible control assessments followed a 0% (no control) to 100% (complete control) rating scale in comparison to the nontreated (Frans et al. 1986). At the end of each experiment, Palmer amaranth biomass was collected from the two quadrats in each plot in both experiments. Biomass reduction was calculated as a percent compared to the nontreated, using Equation 1:

Biomass reduction (%) = 
$$\left[\frac{\text{nontreated-individual plot}}{\text{nontreated}}\right] \times 100$$
 [1]

## Data Analysis

The mortality (%) data obtained in the dose-response experiment were analyzed using the Fit Curve Platform with JMP<sup>®</sup> Pro 18.0.2 software (SAS Institute, Cary, NC). The lowest calculated Akaike information criterion corrected and Bayesian information criterion (Burnham and Anderson 2004), root mean square error, and  $R^2$  were used to evaluate the fit of various nonlinear models. Based on these criteria, the best nonlinear model fit for the dose-response data was the Weibull growth curve, which is defined by Equation 2:

$$Y = a * \left\{ 1 - \exp\left[ -\left(\frac{\text{Herbicide rate}}{b}\right)^c \right] \right\}$$
[2]

where *Y* is the mortality (%), *a* is the asymptote, *b* is the location parameter, and *c* is the growth rate. The mortality data were pooled



Figure 1. Rainfall (cm) events at the experimental location in 2022, 2023, and 2024, from the beginning to the termination of preemergence experiments.

across experimental runs. Individual Weibull growth curves were fit for each herbicide by accession, and the regression parameters are available in Table 4. The predicted rates causing 50% ( $LD_{50}$ ) and 90% ( $LD_{90}$ ) mortality for each herbicide and accession were calculated. The lower and upper 95% estimated confidence intervals were also calculated to determine whether glufosinateresistant accessions (MSR1, MSR2, and CCR) differed from the susceptible standards (SS1 and SS2). The resistant:susceptible (R/S) fold was calculated by dividing the  $LD_{50}$  or  $LD_{90}$  estimated values of each glufosinate-resistant accession by the  $LD_{50}$  or  $LD_{90}$ values of both susceptible standards. If the confidence intervals did not overlap with the ones predicted for SS1 and SS2, the R/S-fold was considered significant (indicated with \* in the tables).

Data collected in the preemergence and postemergence experiments were subjected to ANOVA. To observe the response of MSR2 across different environmental scenarios, year and replications nested within year were considered random effects. All data collected were subjected to Shapiro-Wilk normality and goodness of fit tests. Palmer amaranth control (%), counts (plants per square meter), and biomass reduction (%) were analyzed using the generalized linear mixed model with JMP Pro18 software with a beta, Poisson, and normal distribution, respectively. If significant, means were separated using Fisher's protected LSD ( $\alpha = 0.05$ ).

Graphs were produced with SigmaPlot 15.0 software (Systat Software Inc., San Jose, CA).

## **Results and Discussion**

## Whole-Plant Dose-Response Assay with Multiple Postemergence Herbicides

A whole plant dose-response assay was conducted to obtain the resistance profile of glufosinate-resistant Palmer amaranth accessions (MSR1, MSR2, and CCR) to postemergence herbicides. Additionally, the mortality levels at the labeled rate of each herbicide were provided for all accessions evaluated. Based on the R/S-folds (>1.7) and the presence of survivors at the labeled rate (Table 5), we can determine that the previously described Palmer amaranth accessions carrying glufosinate (Group 10) resistance were also confirmed to be resistant to 2,4-D (Group 4), diuron (Group 5), fomesafen (Group 14), glyphosate (Group 9), imazethapyr (Group 2), and mesotrione (Group 27). CCR is also resistant to dicamba. Therefore, accessions MSR1, MSR2, and CCR have evolved resistance to postemergence herbicides pertaining to seven SOAs. This is the first case of a seven-way herbicide resistance evolution in any Palmer amaranth population. It is

Herbicide	WSSA/HRAC group number	Trade name	Manufacturer	Rate
				g ai or ae ha <sup>-1</sup>
2,4-D	4	Enlist One®	Corteva Agriscience USA	1,064
Atrazine	5	Aatrex <sup>®</sup>	Syngenta Crop Protection, LLC	1,680
Carfentrazone	14	Aim®	FMC Corporation	22
Dicamba	4	XtendiMax <sup>®</sup>	Bayer CropScience	560
Diuron	7	Direx®	ADAMA	840
Flumioxazin	14	Valor <sup>®</sup>	Valent U.S.A. LLC	72
Fomesafen	14	Reflex <sup>®</sup>	Syngenta Crop Protection, LLC	280
Glufosinate	10	Liberty®	BASF Ag Products	656
Glyphosate	9	Roundup PowerMAX <sup>®</sup> 3	Bayer CropScience	1,120
Isoxaflutole	27	Balance <sup>®</sup> Flexx	Bayer CropScience	90
Mesotrione	27	Callisto <sup>®</sup>	Syngenta Crop Protection, LLC	220
Paraquat	22	Gramoxone®	Syngenta Crop Protection, LLC	700
Saflufenacil	14	Sharpen®	BASF Ag Products	25
Tembotrione	27	Laudis	Bayer CropScience	50
Trifloxysulfuron	2	Envoke®	Amvac Chemical Corporation	10.5
Trifludimoxazin <sup>c</sup>	14		BASF Ag Products	50

<sup>a</sup>Abbreviations: HRAC, Herbicide Resistance Action Committee; WSSA, Weed Science Society of America.

<sup>b</sup>Crop oil concentrate at 1% v/v was added in applications with atrazine, carfentrazone, diuron, or mesotrione; Nonionic surfactant at 0.25% v/v was added to applications with flumioxazin, fomesafen, paraquat, or trifloxysulfuron; volatility reduction agent at 1.46 L ha<sup>-1</sup> and drift reduction agent at 0.5% v/v were added to applications with dicamba; methylated seed oil at 1% v/v was added to applications with saflufenacil or tembotrione; methylated seed oil at 1% v/v and ammonium sulfate at 1% w/v were added to applications.

<sup>c</sup>The herbicide trifludimoxazin is not currently commercially labeled for use in the United States.

important to also note that control failures were previously observed with preemergence herbicides from Group 3 (pendimethalin) and Group 15 (S-metolachlor) with these accessions, but further soil-applied dose-response studies are needed to confirm the presence or absence of resistance to these herbicides (Priess et al. 2022). Furthermore, Group 3 (pendimethalin and trifluralin) and Group 15 (S-metolachlor) herbicides failed to control other Palmer amaranth accessions from Arkansas (Brabham et al. 2019; González-Torralva and Norsworthy 2021; Kouame et al. 2022; Schwartz-Lazaro et al. 2017).

Besides the accumulation of resistance genes through gene flow, multiple herbicide-resistant weeds can also arise due to subsequent selection. For instance, a field in which the presence of herbicideresistant species is confirmed will receive applications of a herbicide from a different SOA. The continued use of that herbicide might further select individuals that become resistant to multiple herbicides, especially when resistance is metabolic (Beckie et al. 2019; Heap and LeBarron 2001; Zimdahl and Basinger 2024). Resistance to 2,4-D, fomesafen, glufosinate, glyphosate, imazethapyr, or mesotrione has been previously described in different Palmer amaranth accessions from Arkansas (Hwang et al. 2023; Norsworthy et al. 2008; Priess et al. 2022; Salas et al. 2016; Schwartz-Lazaro et al. 2017; Singh et al. 2018; Varanasi et al. 2018). Therefore, the sequential selection of individuals carrying resistance to two or more SOAs is plausible. The presence of multiple herbicide resistance in Palmer amaranth and waterhemp [Amaranthus tuberculatus (Moq.) Sauer] has been confirmed in Kansas and Missouri (Kumar et al. 2019; Shergill et al. 2018; Shyam et al. 2021), which further displays the adaptability of this genus. Chemical control of a biotype carrying seven-way herbicide resistance will be considerably challenging due to the lack of available effective products, especially if the weed emerges, and colossal selection is expected to be exerted on the few remaining effective options.

There have been no previous reports of dicamba resistance in Palmer amaranth populations in Arkansas (Heap 2024). However, dicamba resistance in Palmer amaranth was documented in Tennessee in 2022 (Foster and Steckel 2022), and has been a reason for concern among farmers in Arkansas. For dicamba, the R/S-fold from accessions MSR1 and MSR2 did not differ from the susceptible standards and ranged from 0.94-fold to 1.9-fold with LD<sub>50</sub> and from 1.1-fold to 1.3-fold with LD<sub>90</sub>, respectively. In contrast, the dicamba R/S-fold of accession CCR significantly differed from the susceptible standards, ranging from 1.9-fold to 3.7-fold based on LD<sub>50</sub> values and from 2.4-fold to 2.8-fold based on LD<sub>90</sub> values. The CCR accession was collected in Crittenden County, Arkansas, which borders Tennessee. Additionally, it has been previously shown that selection with sequential sublethal applications of dicamba has the potential to decrease the sensitivity of Palmer amaranth plants to herbicides belonging to Group 4, which eventually culminates in the evolution of resistance (Tehranchian et al. 2017). Therefore, the movement of resistance across state lines or the accumulation of genes involved in dicamba resistance are likely involved in the evolution of resistant accessions in Arkansas. Although no Palmer amaranth accession has been previously confirmed to be resistant to diuron, resistance to this herbicide was reported in Powell amaranth (A. powellii S. Watson) and redroot pigweed (A. retroflexus L.) (Heap 2024).

The R/S-fold values derived from the  $LD_{50}$  and  $LD_{90}$  of susceptible and resistant accessions were significant for atrazine (Table 5). However, the labeled rate of atrazine completely controlled the Palmer amaranth accessions under greenhouse conditions, indicating that these accessions should not be classified as being resistant to this herbicide. Nonetheless, it seems that a shift toward increased tolerance to Group 5 herbicides is occurring in the glufosinate-resistant accessions compared to the susceptible ones. Resistance to atrazine has been reported in Palmer amaranth accessions from other states, but not Arkansas (Heap 2024).

Except for imazethapyr, the mortality of susceptible standards (SS1 and SS2) was >99% with the labeled rate of all herbicides tested (Table 5). When applied with the recommended crop rate of imazethapyr ( $1 \times = 70.6$  g ai ha<sup>-1</sup>), the accessions SS1 and SS2 had mortality values of 20% and 79%, respectively. The SS2 accession was completely controlled with a rate equivalent to  $2 \times$  of the

Table 4. Weibull growth curve regression parameters by herbicide and Palmer amaranth accession.<sup>a</sup>

			Re	egression parameters (±SE	) <sup>b</sup>		
Herbicide	WSSA/HRAC group number	Accession <sup>c</sup>	Asymptote	Location	Growth rate	RMSE <sup>d</sup>	R <sup>2e</sup>
2,4-D	4	SS1	100.11 (4.49)	225.75 (34.38)	1.08 (0.17)	9.7	0.95
		SS2	102.13 (7.80)	343.63 (73.94)	1.11 (0.24)	13.7	0.91
		MSR2	98.47 (4.56)	785.31 (81.04)	2.32 (0.53)	11.2	0.95
		MSR1	99.46 (5.18)	1,084.83 (150)	$\begin{array}{c c} \underline{+SE})^{b} \\ \hline \\ $	10.7	0.95
		CCR	99.73 (2.87)	797.56 (56.12)	1.89 (0.28)	8.0	0.97
Atrazine	5	SS1	99.91 (2.84)	236.35 (16.45)	1.71 (0.20)	5.7	0.98
		SS2	99.53 (2.09)	173.20 (8.26)	2.03 (0.21)	5.0	0.99
		Accession <sup>c</sup> Asymptote  Location  Growth rate  RMSE <sup>c</sup> SS1  100.11 (4.49)  225.75 (34.38)  1.08 (0.17)  9.7    SS2  102.13 (7.80)  343.63 (73.94)  1.11 (0.24)  13.7    MSR2  98.47 (4.56)  785.31 (81.04)  2.32 (0.53)  11.2    MSR1  99.46 (5.18)  1.084.83 (150)  1.47 (0.30)  10.7    CCR  99.73 (2.87)  797.56 (56.12)  1.89 (0.28)  8.0    SS1  99.91 (2.84)  236.35 (16.45)  1.71 (0.20)  5.7    SS2  99.53 (2.09)  173.20 (8.26)  2.03 (0.21)  5.0    MSR1  98.88 (1.81)  427.47 (72.89)  1.85 (0.19)  5.0    CCR  90.02 (2.95)  345.75 (25.85)  1.75 (0.24)  6.7    SS1  97.87 (2.42)  100.16 (8.07)  1.41 (0.19)  6.5    SS2  97.04 (7.73)  58.66 (18.62)  0.30 (0.20)  8.1    MSR1  99.61 (3.52)  1.00.10 (0.27)  1.74  MSR2    SS1  97.52 (3.32)	9.1	0.96			
		MSR1	98.88 (1.81)	427.47 (22.89)	n parameters $(\pm SE)^b$ RN    Location  Growth rate  RN    125.75 (34.38)  1.08 (0.17)  1    128.31 (81.04)  2.32 (0.53)  1    188.33 (150)  1.47 (0.30)  1    197.56 (56.12)  1.89 (0.28)  1    123.32 (16.45)  1.71 (0.20)  1    197.56 (56.12)  1.89 (0.28)  1    124.89 (25.08)  2.51 (0.45)  1    127.47 (22.89)  1.85 (0.19)  1    124.75 (25.85)  1.75 (0.24)  1    0.016 (8.07)  1.41 (0.19)  5    58.66 (18.62)  0.91 (0.27)  1    99.18 (10.52)  1.30 (0.20)  1    0.040 (12.89)  1.20 (0.19)  1    1.44.0 (20.31)  1.08 (0.12)  1    75.70 (7.76)  1.62 (0.29)  1    30.56 (3.52)  2.21 (0.66)  1    1.74.30 (19.82)  1.55 (0.29)  1    15.06 (1.32)  1.71 (0.27)  1    25.67 (2.28)  1.52 (0.22)  1	5.0	0.99
		CCR	99.02 (2.95)	345.75 (25.85)	1.75 (0.24)	6.7	0.98
Dicamba	4	SS1	97.87 (2.42)	100.16 (8.07)	1.41 (0.19)	6.5	0.98
		SS2	97.04 (7.73)	58.66 (18.62)	0.91 (0.27)	17.4	0.83
		MSR2	95.20 (2.71)	Regression parameters (±SE) <sup>o</sup> RMSE <sup>d</sup> Location  Growth rate  RMSE <sup>d</sup> 225.75 (34.38)  1.08 (0.17)  9.7    343.63 (73.94)  1.11 (0.24)  13.7    785.31 (81.04)  2.32 (0.53)  11.2    1,084.83 (150)  1.47 (0.30)  10.7    797.56 (56.12)  1.89 (0.28)  8.0    236.35 (16.45)  1.71 (0.20)  5.7    173.20 (8.26)  2.03 (0.21)  5.0    348.99 (25.08)  2.51 (0.45)  9.1    427.47 (22.89)  1.85 (0.19)  5.0    345.75 (25.85)  1.75 (0.24)  6.7    100.16 (8.07)  1.41 (0.19)  6.5    58.66 (18.62)  0.91 (0.27)  17.4    99.18 (10.52)  1.30 (0.20)  8.1    100.40 (12.89)  1.20 (0.19)  9.4    214.40 (20.31)  1.08 (0.12)  7.3    75.70 (7.76)  1.62 (0.29)  11.2    30.56 (3.52)  2.21 (0.66)  12.5    174.30 (19.82)  1.55 (0.29)  11.7    366 (3.52)	0.96		
		MSR1	99.61 (3.62)	100.40 (12.89)	1.20 (0.19)	9.4	0.96
		CCR	100.01 (2.91)	214.40 (20.31)	1.08 (0.12)	7.3	0.97
Diuron	5	SS1	97.52 (3.32)	75.70 (7.76)	1.62 (0.29)	11.2	0.93
		SS2	92.54 (3.27)	30.56 (3.52)	2.21 (0.66)	12.5	0.91
		MSR2	99.13 (4.00)	174.30 (19.82)	1.55 (0.29)	11.7	0.94
		MSR1	100.49 (3.50)	367.58 (33.71)	1.41 (0.19)	9.4	0.96
		CCR	97.43 (3.57)	237.86 (38.39)	0.86 (0.12)	11.2	0.93
Fomesafen	14	SS1	96.79 (2.72)	15.06 (1.32)	1.71 (0.27)	8.2	0.97
		SS2	100.08 (2.87)	25.67 (2.28)	1.52 (0.22)	7.7	0.97
		MSR2	103.21 (8.41)	ptoteLocationGrowth rateRMS $(4.49)$ 225.75 (34.38)1.08 (0.17)9 $(7.80)$ 343.63 (73.94)1.11 (0.24)13 $(4.56)$ 785.31 (81.04)2.32 (0.53)11 $(5.18)$ 1,084.83 (150)1.47 (0.30)10 $(2.87)$ 797.56 (56.12)1.89 (0.28)88 $(2.84)$ 236.35 (16.45)1.71 (0.20)55 $(2.09)$ 173.20 (8.26)2.03 (0.21)55 $(3.24)$ 348.99 (25.08)2.51 (0.45)99 $(1.81)$ 427.47 (22.89)1.85 (0.19)55 $(2.95)$ 345.75 (25.85)1.75 (0.24)66 $(2.42)$ 100.16 (8.07)1.41 (0.19)66 $(7.73)$ 58.66 (18.62)0.91 (0.27)17 $(2.71)$ 99.18 (10.52)1.30 (0.20)8 $(3.62)$ 100.40 (12.89)1.20 (0.19)99 $(2.91)$ 214.40 (20.31)1.08 (0.12)7 $(3.22)$ 75.70 (7.76)1.62 (0.29)11 $(3.50)$ 367.58 (33.71)1.41 (0.19)9 $(3.57)$ 237.86 (38.39)0.86 (0.12)11 $(2.72)$ 15.06 (1.32)1.71 (0.27)8 $(2.87)$ 25.67 (2.28)1.52 (0.22)7 $(3.64)$ 273.62 (79.39)0.94 (0.23)14 $(2.72)$ 25.67 (2.28)1.52 (0.22)7 $(3.64)$ 273.62 (79.39)0.94 (0.23)14 $(2.72)$ 25.65 (3.01)1.37 (0.11)4 $(4.66)$ 6,064.92 (855)0.99 (0.12) <td< td=""><td>10.2</td><td>0.94</td></td<>	10.2	0.94	
		MSR1	97.87 (4.27)	285.54 (41.37)	1.18 (0.20)	9.9	0.95
		CCR	99.19 (9.19)	401.53 (103.60)	1.08 (0.26)	13.7	0.90
Glyphosate	9	SS1	98.28 (8.64)	273.62 (79.39)	0.94 (0.23)	14.7	0.88
		SS2	92.45 (2.84)	43.63 (4.67)	1.67 (0.33)	9.4	0.95
		MSR2	101.78 (4.66)	6,064.92 (855)	0.99 (0.12)	7.1	0.97
		MSR1	93.62 (3.11)	1,945.54 (186)	1.84 (0.37)	9.0	0.96
		CCR	94.23 (4.00)	2,341.39 (378)	1.12 (0.21)	10.9	0.94
Imazethapyr	2	SS1	88.10 (4.42)	333.64 (72.90)	0.64 (0.07)	7.0	0.96
		SS2	97.84 (2.50)	50.85 (3.01)	1.37 (0.11)	4.4	0.99
		MSR2	70.17 (4.69)	838.36 (180.03)	0.80 (0.09)	5.1	0.96
		MSR1	78.33 (5.60)	670.21 (163.81)	0.74 (0.09)	6.7	0.95
		CCR	63.01 (11.43)	1,208.63 (829)	0.53 (0.08)	4.5	0.94
Mesotrione	27	SS1	99.27 (3.57)	51.06 (4.21)	1.88 (0.32)	9.1	0.96
		SS2	96.95 (5.50)	39.87 (6.38)	1.12 (0.17)	10.2	0.94
		MSR2	99.69 (9.16)	157.94 (46.01)	0.81 (0.15)	11.6	0.91
		MSR1	95.15 (2.94)	198.06 (10.75)	1.82 (0.21)	6.3	0.98
		CCR	93.78 (3.45)	204.83 (21.88)	1.18 (0.14)	8.0	0.96

<sup>a</sup>Abbreviations: HRAC, Herbicide Resistance Action Committee; RMSE, Root mean square error; WSSA, Weed Science Society of America;.

<sup>b</sup>The regression parameters were estimated by a Weibull growth curve,  $Y = a * \{1 - \text{Exp}[-\frac{(\text{Rate})}{b}c]\}$ , where a = asymptote, b = location parameter, and <math>c = growth rate.<sup>c</sup>Accessions SS1 and SS2 are the susceptible standards; Accessions MSR1, MSR2, and CCR are confirmed glufosinate-resistant accessions (Priess et al. 2022).

<sup>d</sup>RMSE values show the average distance between observed and predicted data points by the model.

 $eR^2$  values show the variability proportion in the observed data explained by the model.

labeled rate (data not shown), whereas the SS1 accession was classified as being resistant to this chemical. The R/S fold based on LD<sub>50</sub> values ranged from 6.4 to 71 in the three resistant and SS1 accessions. No imazethapyr rate produced mortality levels above 90% in this study for the accessions SS1, MSR1, MSR2, and CCR. Therefore, LD<sub>90</sub> values were assumed to be above the highest rate sprayed (4,518 g ha<sup>-1</sup>). Although the first case of Palmer amaranth resistance to imazethapyr was reported in 1993, previous studies showed that control of this species with imazethapyr has been difficult since the early 1990s (Heap 2024; Horak and Peterson 1995; Mayo et al. 1995).

## Preemergence Experiment

Control of MSR2 with preemergence treatments significantly differed in all weeks evaluated (Table 6). At 3 WAT, 11 out of the 16 preemergence herbicides tested showed MSR2 control levels >90%, and this number decreased to eight at 4 WAT, three at 5

WAT, and only two at 6 WAT. For all weeks evaluated, the herbicides atrazine, pyroxasulfone, trifludimoxazin, and metribuzin produced the highest preemergence control levels. High residual control with at least one of these herbicides was also observed on other Palmer amaranth accessions in other research (Hay et al. 2018; Houston et al. 2019; Kohrt and Sprague 2017; Meyers et al. 2017; Witschel et al. 2021). The herbicide trifludimoxazin is currently under development and is expected to be registered for preplant burndown applications targeting major weeds in corn, soybean, and other production systems (Findley et al. 2020). In the present study, trifludimoxazin provided prolonged residual control of MSR2, with average control above 85% up to 6 WAT, which makes this herbicide a desired addition to the row crops portfolio. Previous research has also observed the prolonged residual activity of trifludimoxazin when evaluating Palmer amaranth and other dicotyledon species such as sicklepod [Senna obtusifolia (L.) Irwin & Barneby] and common lambsquarters (Chenopodium album L.) (Rapado et al. 2024).

## Table 5. Predicted rates to obtain mortality levels of 50% and 90% by Palmer amaranth accession and herbicide.<sup>a,b</sup>

			Confidence interva	l (95%)		Resistance degree to	Resistance degree to	Mortality at labeled rate
Herbicide		Accession	Predicted rate	Lower	Upper	SS1	SS2	(1×)
			g ai or	ae ha <sup>-1</sup> —		——— R/S f	old <sup>c</sup> , <sup>d</sup> ———	%
2,4-D	$LD_{50}$	SS1	160	94	227			
		SS2	240	97	383			
		MSR2	676	520	834	4.2*	2.8*	
		MSR1	851	561	1,141	5.3*	3.5*	
		CCR	658	550	/6/	4.1"	2.1*	00
	LD <sub>90</sub>	551	488	420	555 925			99
		MSR2	1 157	998	025	2.4*	1 7*	88
		MSR2 MSR1	1 937	1 644	2 231	2. <del>4</del> 4 0*	2.8*	68
		CCR	1,247	1.137	1.357	2.6*	1.8*	82
Atrazine	$LD_{50}$	SS1	191	159	223			
	50	SS2	145	129	161			
		MSR2	307	258	356	1.6*	2.1*	
		MSR1	354	309	398	1.8*	2.5*	
		CCR	283	233	333	1.5*	2.0*	
	LD <sub>90</sub>	SS1	386	354	418			100
		SS2	264	247	280			100
		MSR2	513	464	562	1.3*	1.9*	100
		MSRI	687	643 F10	(33	1.8"	2.6"	100
Dicamba			209	519	620	1.5	2.1	100
Dicamba	LD <sub>50</sub>	551	79 41	5	95 77			
		MSR2	79	59	99	1†	1 9†	
		MSR2 MSR1	74	49	99	0.94†	1.8†	
		CCR	153	113	192	1.9*	3.7*	
	LDao	SS1	193	177	209	210		100
	50	SS2	169	132	205			100
		MSR2	226	205	247	1.2†	1.3†	91
		MSR1	204	179	229	1.1†	1.2†	98
		CCR	464	424	504	2.4*	2.8*	96
Diuron	LD <sub>50</sub>	SS1	62	47	77			
		SS2	27	20	34			
		MSR2	139	100	177	2.2*	5.1*	
		MSR1	282	217	347	4.5*	10*	
		CCR	162	88	236	2.6^	6^	100
	LD <sub>90</sub>	551	135	120	151			100
		332 MSD2	24 205	48	244	2.2*	5.6*	100
		MSR2 MSR1	505 656	590	722	2.3 4 9*	J.0 12*	97
		CCR	716	640	791	5.3*	13*	89
Fomesafen	LDEO	SS1	12	10	15	5.5	15	65
	30	SS2	20	16	24			
		MSR2	235	47	423	20*	12*	
		MSR1	215	135	295	18*	11*	
		CCR	289	89	489	24*	14*	
	LD <sub>90</sub>	SS1	27	24	29			100
		SS2	44	40	49			100
		MSR2	882	691	1,073	33*	20*	51
		MSR1	625	544	706	23^	14^	62
Churchasata		CCR SC1	894	691	1,096	33	20	62
Glyphosate	LD <sub>50</sub>	331	190	20	344 17			
		MSR2	4 083	2 4 3 0	5 736	21*	110*	
		MSR2 MSR1	1 681	1 319	2 043	8.8*	45*	
		CCR	1.824	1.089	2.559	9.6*	49*	
	LDao	SS1	716	561	872			100
	50	SS2	94	85	104			100
		MSR2	13,178	11,502	14,854	18*	140*	13
		MSR1	3,690	3,324	4,055	5.2*	39*	29
		CCR	6,440	5,696	7,183	9.0*	68*	35
Imazethapyr	$LD_{50}$	SS1	254	112	395		6.4*	
		SS2	40	34	46			
		MSR2	1,102	752	1,453	-	27*	
		MSR1	685	367	1,004	-	17*	
		CCR	2,846	1,227	4,465	-	(1^	20
	LD <sub>90</sub>	221	>4,518 100	-	-		>45.Z	20
		JSZ MCDJ	100 \_1 51Q	54	100	-	<u>∖45</u> 2*	19
		MUNZ	∕¬,5±0				/ TJ.L	v
								(Continued

#### Table 5. (Continued)

			Confidence interval	l (95%)		Resistance degree to	Resistance degree to	Mortality at labeled rate
Herbicide		Accession	Predicted rate	Lower	Upper	SS1	SS2	(1×)
		MSR1	>4,518	-	-	-	>45.2*	8
		CCR	>4,518	-	-	-	>45.2*	13
Mesotrione	LD <sub>50</sub>	SS1	42	34	50			
		SS2	30	18	42			
		MSR2	101	12	190	2.4†	3.4†	
		MSR1	169	148	189	4.0*	5.6*	
		CCR	163	120	205	3.9*	5.4*	
	LD <sub>90</sub>	SS1	81	73	89			100
	50	SS2	94	82	107			100
		MSR2	450	360	540	5.6*	4.8*	59
		MSR1	357	336	378	4.4*	3.8*	64
		CCR	550	508	593	6.8*	5.8*	74

<sup>a</sup>Accessions SS1 and SS2 are the susceptible standards; accessions MSR1, MSR2, and CCR are confirmed glufosinate-resistant accessions (Priess et al. 2022).

 $^{b}\text{LD}_{50}$  and  $\text{LD}_{90}$  are the estimated lethal doses to control each population by 50% and 90%, respectively.

<sup>c</sup>The resistant:susceptible (R/S) fold was calculated by dividing the  $LD_{50}$  or  $LD_{90}$  of each glufosinate-resistant population by the  $LD_{50}$  or  $LD_{90}$  of the susceptible standards (SS1 and SS2). <sup>d</sup>Resistant:susceptible (R/S) fold based on confidence intervals (95%) are indicated with an asterisk (\*) if significant or  $\dagger$  if not significant.

Table 6. Preemergence control of glufosinate-resistant Palmer amaranth accession MSR2 at 3, 4, 5, and 6 wk after treatment.<sup>a,b,c</sup>

			PRE Palmer an	naranth control	
Treatment Atrazine Pyroxasulfone Trifludimoxazin Metribuzin Mesotrione Isoxaflutole Flumioxazin Diuron S-metolachlor	WSSA/HRAC group number	3 WAT	PRE Palmer amaranth control    3 WAT  4 WAT  5 WAT		6 WAT
			q	%	
Atrazine	5	100 a	97 a	94 a	92 a
Pyroxasulfone	15	99 ab	97 a	93 a	91 a
Trifludimoxazin	14	97 abc	96 ab	91 ab	86 ab
Metribuzin	5	96 abcd	94 abc	88 abc	82 abc
Mesotrione	27	96 abcd	93 abc	87 abcd	72 cde
Isoxaflutole	27	95 bcd	91 bcd	83 bcd	74 bcde
Flumioxazin	14	95 bcd	89 cd	84 bcd	75 bc
Diuron	5	94 cde	91 bcd	85 bcd	79 bc
S-metolachlor	15	92 de	88 cd	78 de	68 cde
Fomesafen	14	91 de	86 de	80 cd	74 bcd
Fluridone	12	88 ef	86 de	79 cde	70 cde
Acetochlor	15	80 fg	78 ef	67 ef	58 e
Saflufenacil	14	74 g	69 fg	59 fg	59 de
Pendimethalin	3	69 g	61 g	47 gh	40 f
Imazaquin	2	57 g	59 g	37 ĥ	29 f
P-value		<.0001	<.0001	<.0001	<.0001

<sup>a</sup>Abbreviations: HRAC, Herbicide Resistance Action Committee; PRE, preemergence; WAT, weeks after treatment; WSSA, Weed Science Society of America.

<sup>b</sup>Means within the same column followed by the same letter are not statistically different according to Fisher's protected LSD ( $\alpha = 0.05$ ).

Data were averaged across years in experiments conducted at the Milo J. Shult Agricultural Research and Extension Center, Fayetteville, AR, in 2022, 2023, and 2024.

The treatments with imazaquin and pendimethalin consistently obtained the lowest control levels across all weeks evaluated (Table 6). Control with imazaquin was 57% at 3 WAT and dropped to 29% by 6 WAT, while control with pendimethalin was 69% at 3 WAT, dropping to 40% by 6 WAT. Resistance to herbicides from Groups 2 and 3 has been confirmed since the 1990s and is widespread (Gossett et al. 1992; Horak and Peterson 1995). The detection of resistance to additional sites of action will impact control responses. For instance, atrazine provided little preemergence control of a Palmer amaranth accession in Nebraska, whereas saflufenacil provided >80% control up to 90 d after application (Kaur et al. 2024). Atrazine-resistant Palmer amaranth is widespread in Nebraska, whereas resistance to Group 14 herbicides has not been reported (Heap 2024).

The number of seedlings and biomass reduction followed a similar pattern as the visual control assessments. Like Palmer amaranth control, the lowest numbers of MSR2 seedlings at 3 WAT were encountered in treatments with atrazine, pyroxasulfone, trifludimoxazin, and metribuzin, with an average of 0.2, 0.6, 1.7, and 2.1 seedlings m<sup>-2</sup>, respectively (Figure 2). At 6 WAT, the best emergence suppression was with pyroxasulfone, trifludimoxazin, and atrazine, with an average of 3, 3.4, and 6.3 seedlings m<sup>-2</sup>, respectively. For comparison, an average of 93 and 139 seedlings m<sup>-2</sup> were present on nontreated plots across the site-years at 3 and 6 WAT, respectively. In addition to low emergence, the highest biomass reduction relative to the nontreated was with atrazine (73%; Supplementary Figure 1). Similarly, in a study assessing residual control options for a Palmer amaranth accession resistant to Group 14 herbicides, the most efficacious residual herbicides tested were atrazine and pyroxasulfone (Houston et al. 2019).

## Postemergence Experiment

There were stark differences in postemergence control of MSR2 among the herbicides tested (Table 7), which is further evidence of resistance to many of the herbicides evaluated here. Options to



**Figure 2.** Number of glufosinate-resistant Palmer amaranth (MSR2) seedlings (plants per square meter) emerged following preemergence herbicide applications at 3 and 6 wk after treatment (WAT). The data were averaged across years for 2022, 2023, and 2024. Standard errors of the means are represented by error bars. Means followed by the same uppercase or lowercase letters are not statistically different according to Fisher's protected LSD ( $\alpha = 0.05$ ) at 3 and 6 WAT, respectively.

control glufosinate-resistant Palmer amaranth accession MSR2 with postemergence herbicides were limited, and paraquat was the only herbicide that provided >90% control at all evaluations. Similarly, previous studies have shown high control levels of Palmer amaranth accessions with paraquat at burndown applications (Crow et al. 2015; Hay et al. 2019; Houston et al. 2019). Acceptable control levels were observed with trifludimoxazin, saflufenacil, and atrazine at 1 WAT, ranging from 83% to 88%. Palmer amaranth control ranged from 79% to 80% at 2 WAT, and 76% to 77% at 3 WAT with atrazine and trifludimoxazin, respectively. Except for paraquat, Palmer amaranth control was <72% at 4 WAT for all treatments.

The Palmer amaranth control results obtained in this study are based on a single application of each herbicide (Table 7), and sequential applications or mixtures are often advised by product labels for better performance. For instance, trifludimoxazin is likely to be recommended in a mixture with saflufenacil or in sequential applications to delay resistance evolution (Witschel et al. 2021). Previously, optimal control (above 85%) has been observed with trifludimoxazin applications in different species, including Palmer amaranth and waterhemp (Rapado et al. 2024; Steppig et al. 2024). Even though the evolution of PPO target-site mutations has been extensively documented (Salas et al. 2016; Varanasi et al. 2018), trifludimoxazin was shown to fully inhibit PPO2 enzymes carrying target site resistance (TSR) mutations in vitro, and to suppress the growth of Arabidopsis plants ectopically expressing the PPO2 TSR mutation (Porri et al. 2023). Although trifludimoxazin exhibited greater inhibitory potency against PPO2 enzymes carrying TSR mutations, nontarget site resistance to Group 14 herbicides has been detected in Palmer amaranth in Arkansas (Porri et al. 2023; Varanasi et al. 2019). The potential effect of nontarget site resistance mechanisms toward trifludimoxazin has still to be evaluated. A PPO-resistant Palmer amaranth accession from Georgia showed resistance to

trifludimoxazin in greenhouse assays with a resistance factor >10 (Randell-Singleton et al. 2024). However, in the same assay, trifludimoxazin at 25 g ha<sup>-1</sup> gave more than 90% control of such biotype. Therefore, the test of this PPO-resistant Palmer amaranth accession with trifludimoxazin should be further assessed in more natural conditions, such as in the field, to enable a more conclusive assessment.

In addition to glufosinate resistance, the dose-response assay results (Table 5) showed that the Palmer amaranth accession MSR2 was also resistant to 2,4-D, diuron, fomesafen, glyphosate, imazethapyr, and mesotrione. Except for imazethapyr, all aforementioned herbicides were included in the postemergence field experiment. Due to the presence of resistance, none of the herbicides achieved control above 77% at any evaluation timing (Table 7). Interestingly, while MSR2 was classified as susceptible to dicamba under greenhouse conditions, this sensitivity level was not observed when plants were sprayed under field conditions. Field control of MSR2 with dicamba ranged from 67% to 75% averaged across years. One possible explanation for this contrast in results is the nozzle type used in each application. The spray chamber used for the dose-response experiments was equipped with 1100067 nozzles applying 187 L ha<sup>-1</sup>, and the field applications were made at 140 L ha<sup>-1</sup> using 110015 TTI nozzles. The smaller orifice nozzle produces fine droplets with excellent coverage while the TTI nozzle has medium to coarse droplets, which limits the coverage (Creech et al. 2015). Additionally, plants growing under controlled conditions are likely submitted to less stress compared with those in the field, which might affect herbicide response.

The lowest number of Palmer amaranth plants present at 4 WAT was in treatments with paraquat or trifludimoxazin (Figure 3). Averaged across years, 0.4 and 8 Palmer amaranth plants  $m^{-2}$  were encountered in treatments with paraquat and trifludimoxazin, respectively. For comparison, nontreated plots had an average of 85 plants  $m^{-2}$ . Paraquat reduced biomass relative

			POST Palmer a	maranth control	
Treatment	WSSA/HRAC group number	1 WAT	2 WAT	3 WAT	4 WAT
				- %	
Paraquat	22	99 a	97 a	97 a	95 a
Trifludimoxazin	14	88 b	79 b	76 b	62 bc
Saflufenacil	14	87 bc	76 bc	66 bc	56 bc
Atrazine	5	83 bc	80 b	77 b	72 b
Diuron	5	77 cd	72 bcd	69 bc	56 bc
Fomesafen	14	73 de	57 efg	38 defg	35 de
Dicamba	4	71 de	68 cde	75 b	67 b
Tembotrione	27	68 def	59 efg	46 de	37 de
2,4-D	4	67 def	60 def	55 cd	48 cd
Glufosinate	10	62 ef	47 gh	27 fgh	20 ef
Mesotrione	27	58 fg	48 fgh	38 defg	35 de
Carfentrazone	14	56 fg	44 h	32 efgh	30 ef
Flumioxazin	14	45 gh	39 hi	42 def	31 ef
Isoxaflutole	27	45 gh	41 hi	35 efgh	30 ef
Glyphosate	9	42 h	30 i	26 gh	23 efg
Trifloxysulfuron	2	28 i	18 j	20 ĥ	15 g
P-value		<.0001	<.0001	<.0001	<.0001

Table 7. Postemergence control of glufosinate-resistant Palmer amaranth accession MSR2 at 3, 4, 5, and 6 wk after treatment.<sup>a-b,c,d</sup>

<sup>a</sup>Abbreviations: HRAC, Herbicide Resistance Action Committee; POST, postemergence; WAT, weeks after treatment; WSSA, Weed Science Society of America.

<sup>b</sup>Means within the same column followed by the same letter are not statistically different according to Fisher's protected LSD ( $\alpha = 0.05$ ).

<sup>c</sup>Crop oil concentrate at 1% v/v was added in applications with atrazine, carfentrazone, diuron, or mesotrione; a nonionic surfactant at 0.25% v/v was added to applications with flumioxazin, fomesafen, paraquat, or trifloxysulfuron; a volatility reduction agent at 1.46 L ha<sup>-1</sup> and drift reduction agent at 0.5% v/v were added to applications with dicamba; methylated seed oil at 1% v/v was added to applications with saflufenacil or tembotrione; methylated seed oil at 1% v/v and ammonium sulfate at 1% w/v were added to applications with trifludimoxazin. <sup>d</sup>Data were averaged across years in experiments conducted at the Milo J. Shult Agricultural Research and Extension Center, Fayetteville, AR, in 2022, 2023, and 2024.



**Figure 3.** Number of glufosinate-resistant Palmer amaranth (MSR2) plants (plants per square meter) remaining in plots following postemergence herbicide applications at 4 wk after treatment. Data were averaged across years in experiments conducted in 2023 and 2024. Standard errors of the means are represented by error bars. Means followed by the same uppercase letters are not statistically different according to Fisher's protected LSD ( $\alpha = 0.05$ ).

to nontreated by 99% (Supplementary Figure 2). Biomass reduction with the other herbicides was <70%, with a high variability within each treatment. In this study, Palmer amaranth plants ranged from 5 to 12 cm in 2023 and 2.5 to 15 cm in 2024 at application, and previous research has shown that regrowth may occur when a herbicide is applied to plants taller than 10 cm since plants do not completely die (Morichetti et al. 2012; Steckel et al.

**1997**). This might provide an explanation for the lack of control observed at 4 WAT for some herbicides, including trifludimoxazin.

Besides weed control efficacy, crop safety is also a highly desirable characteristic in postemergence treatments. Although atrazine is an option for postemergence applications to corn (*Zea mays* L.) and grain sorghum [*Sorghum bicolor* (L.) Moench.], the herbicides saflufenacil, trifludimoxazin, or paraquat are unsafe for

over-the-top use. Although modified crops carrying herbicideresistance traits to several Group 14 herbicides, including trifludimoxazin and saflufenacil, are under development (Witschel et al. 2021), trifludimoxazin or its mixture with saflufenacil likely will be initially used for preplant/burndown applications due to the time necessary to obtain regulatory consent.

## **Practical Implications**

Seven-way postemergence herbicide resistance was confirmed in three Palmer amaranth accessions from Arkansas. The accessions MSR1, MSR2, and CCR, previously confirmed to be resistant to glufosinate, were also not controlled by 2,4-D (Group 4), diuron (Group 5) fomesafen (Group 14), glyphosate (Group 9), imazethapyr (Group 2), and mesotrione (Group 27) herbicides. Furthermore, based on the field data, control of highly glufosinateresistant Palmer amaranth accession (MSR2) with postemergence herbicides will be challenging and needs to be partnered earlier in the season with effective residuals such as atrazine, pyroxasulfone, or trifludimoxazin. Multiple resistance within a weed population imposes major selection for further loss of herbicides since active ingredient rotation will be limited due to the lack of effective options (Moss 2017; Shergill et al. 2018; Shyam et al. 2021). It is unlikely that the three Palmer amaranth accessions investigated in this study are the only accessions exhibiting seven-way resistance. Therefore, fields adjacent to the locations where glufosinate resistance has been confirmed in Palmer amaranth will demand a more diverse and proactive management strategy that combines chemical, cultural, and mechanical control tactics (Vulchi et al. 2023). The continued sole reliance on chemical control is not sustainable in the presence of species carrying resistance to several herbicide groups. A single female Palmer amaranth survivor has the potential to produce hundreds of thousands of seeds, leading to severe infestations within a few years and potentially carrying herbicide resistance genes through future generations (Keeley et al. 1987; Norsworthy et al. 2014; Sellers et al. 2003). Therefore, diverse approaches are strongly recommended to avoid seed production and replenishment of soil seedbank or to limit the movement of the six-way resistant weed within and outside fields (Norsworthy et al. 2012, 2014).

Previous research has shown that the amplification of the chloroplastic glutamine synthetase (glufosinate target enzyme) and the 5-enolpyruvylshikimate-3-phosphate synthase (glyphosate target enzyme) are among the mechanisms conferring resistance to glufosinate and glyphosate, respectively, in the accessions MSR1 and MSR2 (Carvalho-Moore et al. 2022, 2024). However, the resistance mechanism to glufosinate in the CCR accession is unknown as are those other herbicides identified here. Future efforts will focus on investigating the mechanisms conferring herbicide resistance in all three accessions. Additionally, studies evaluating the impact of residual herbicides in whole-season control programs are ongoing for the accession MSR2, and documentation of possible resistance to soil-applied dinitroaniline and chloroacetamide herbicides will be conducted.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/wet.2025.8

**Acknowledgments.** We appreciate the support provided by the University of Arkansas Division of Agriculture. We also acknowledge the immense contributions provided by fellow graduate students and research personnel to conduct this research.

Funding. BASF Corporation funded this project.

**Competing Interests.** The authors Ingo Meiners and Aimone Porri are affiliated with BASF Corporation. The other authors declare no conflict of interest.

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