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Confirmation of glufosinate-resistant Palmer amaranth and response to other herbicides

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Abstract

The ability of weed populations to evolve resistance to herbicides affects management strategies and the profitability of crop production. The objective of this research was to screen Palmer amaranth accessions from Arkansas for glufosinate resistance. Additional efforts focused on the effectiveness of various herbicides, across multiple sites of action (SOAs), on each putative-resistant accession. The three putative accessions were selected from 60 Palmer amaranth accessions collected in 2019 and 2020 and screened with to 0.5× and 1× rates of glufosinate. A dose-response experiment was conducted for glufosinate on accessions A2019, A2020, and B2020. The effectiveness of various preemergence- and postemergence-applied herbicides were evaluated on each accession. Resistance ratios of A2019, A2020, and B2020 to glufosinate ranged from 5.1 to 27.4 when comparing LD_{50} values to two susceptible accessions, thus all three accessions were resistant to glufosinate. All three accessions (A2019, A2020, and B2020) were found to have a reduction equal to or greater than 20 percentage points in mortality to at least one herbicide from five different SOAs equal to or greater than five sites of action. Herbicides from nine different SOAs controlled A2019 at least 20 percentage points less than the susceptible accessions, which points to a need for additional research to characterize the response of this accession.

Introduction

Herbicides are valuable tools in agricultural production for weed control. In row-crop production systems, herbicides are often the best option for controlling weeds due to their relatively low cost and ease of implementation. However, the widespread use of herbicides since the 1940s has led to herbicide-resistant biotypes.

Herbicide-resistant biotypes have typically been controlled by the use of a herbicide with a different site of action (SOA); however, this approach may aid in selection for multiple herbicide-resistant biotypes. Weed species that harbor multiple resistance mechanisms include but are not limited to black grass (*Alopecurus myosuroides* Huds.), common waterhemp [*Amaranthus tuberculatus* (Moq.) JD Sauer], Palmer amaranth (*Amaranthus palmeri* S. Wats), barnyardgrass (*Echinochloa crus-galli*), Italian ryegrass (*Lolium perenne* L. ssp. *multiflorum*), rigid ryegrass (*Lolium rigidum* Gaudin), and wild radish (*Raphanus raphanistrum*); see Owen et al. (2015); Preston et al. (1996); Schwartz-Lazaro et al. (2017); Shergill et al. (2018); Spaunhorst et al. (2019); Tehranchian et al. (2019); and Yu et al. (2009). Weed species such as rigid ryegrass, Palmer amaranth, and barnyardgrass have been confirmed to be resistant to seven, six, and five different herbicides SOAs in a single biotype, respectively (Heap 2021; Shyam et al. 2020). With an increase in weeds that harbor multiple resistance mechanisms, the number of effective herbicides available in crops such as soybean [*Glycine max* (L.) Merr.] and cotton (*Gossypium hirsutum* L.) has diminished.

Following the evolution of inhibitor resistance to acetolactate synthase, photosystem II, 5-enolpyruvate shikimate 3-phosphate, and protoporphyrinogen oxidase in Palmer amaranth populations, glufosinate-resistant crops and the use of glufosinate became a commonly used option to control emerged weeds in soybean and cotton (Heap 2021; USDA-NASS 2021). Since the commercial launch of glufosinate-resistant soybean and cotton in the United States, in-season annual use of glufosinate has increased from 34,375 kg in 2007 to 4,705,000 kg in 2019, which is a 137-fold increase over a 12-yr period (USDA-NASS 2021).

In the past, overreliance on a single SOA has led to evolution of herbicide resistance in weed populations (Perez-Jones et al. 2005; Powles et al. 1997; Simarmata et al. 2005). Glufosinate resistance has not been reported in broadleaf weed species throughout the world (Heap 2021). The objective of this research was to determine the extent of glufosinate-resistant Palmer amaranth persistence in Arkansas and to identify the sensitivity of troublesome populations to other herbicides.

Materials and Methods

Dose Response

A preliminary study was conducted by collecting 30 Palmer amaranth accessions from soybean and cotton fields in the state of Arkansas in 2019 and 2020 (60 total accessions). Accessions were collected from fields where a synthetic auxin or glufosinate had been sprayed during the growing season and seed-producing Palmer amaranth plants persisted. Accessions were collected and brought back to the Altheimer Laboratory at the Milo J. Shult Agricultural Research and Extension Center in Fayetteville, AR. The accessions were planted and grown to the 5- to 6-leaf stage in a greenhouse and then treated with glufosinate at 297 (0.5×) and 595 g ai ha^{-1} (1×).

Three accessions that were not effectively (less than 70%) controlled by a 0.5× or 1× rate of glufosinate were selected for use in the dose-response experiment. Two additional susceptible accessions collected from Arkansas in 2001 were also included in the experiment for comparison. For the two susceptible and three putative-resistant accessions, two experimental runs were completed. Each experimental run was conducted as a completely randomized design with three spatial replications, with each spatial replication containing 15 to 20 Palmer amaranth plants. A minimum of 100 plants per herbicide dose was treated.

Palmer amaranth plants were grown in trays containing mediated potting soil (Sungro® Horticulture, Agawam, MA) until the cotyledon to 1-leaf stage. A single plant cell was transplanted into mediated potting soil in a 20-cell trays (Greenhouse Megastore, Danville, IL). Potting mix was maintained moist throughout the experiment through daily irrigation. Plants were grown in a greenhouse at 25 ± 8 C, and light was supplemented to provide 1,000 ± 320 µmol m^{-2} s^{-1} in a 16-h day.

The three putative-resistant accessions (A2019, A2020, B2020) and two susceptible accessions (S1 and S2) were grown to the 5- to 6-leaf stage. When plants reached the 5- to 6-leaf stage herbicide treatments were applied. Treatments applied to susceptible accessions included glufosinate at 0, 37.2, 74.3, 148.8, 297.5, 595, and 1,190 g ai ha^{-1}. Putative-resistant accessions were subjected to a log scale of six herbicide rates based on their previous response to glufosinate, a 1× field rate of each herbicide was 595 g ai ha $^{-1}$. Differing rate structures were used to account for the variability in herbicide sensitivity among biotypes.

Applications were made using a two-nozzle track sprayer equipped with TeeJet 1100067 nozzles (TeeJet Technologies, Spraying Systems Co., Glendale Heights, IL). The track sprayer was calibrated to deliver 187 L ha^{-1} at 1.61 km h^{-1}. Prior to application the number of live plants were counted, and the remaining live plants were counted again 28 d after application (DAA). These values were used to calculate percent mortality of Palmer amaranth 28 DAA. Putative-resistant plants that survived greater than a 1× rate were kept to increase seed production for additional experiments; therefore, biomass was not assessed.

Response to Labeled Herbicide Rates

In addition to the dose-response study, sensitivity of the three putative-resistant accessions and S1 was evaluated to herbicides from 11 distinct SOAs. The study was set up similar to the dose-response experiment, with two experimental runs completed. A minimum of 100 plants per postemergence herbicide and a total of 300 seeds per preemergence herbicide were subjected to treatments. This sample size has been shown to be sufficient to assess for herbicide resistance (Burgos et al. 2013), albeit confirmation of resistance was not the intent of this experiment. Plants were grown in similar manner and under the same greenhouse conditions as the dose-response experiment.

Postemergence applications were made to 6- to 8-leaf Palmer amaranth plants and included the following herbicides: 2,4-D, atrazine, dicamba, diuron, fomesafen, glyphosate, imazethapyr, mesotrione, paraquat, and tembotrione. Respective herbicide group numbers as classified by the Weed Science Society of America (WSSA), common names, family names, adjuvants, and use rates are included in Table 1. Use rates of herbicides are representative of 1× rates applied in corn (*Zea mays* L.), cotton, and soybean.

Field soil characterized as a Leaf silt loam (fine, mixed, active, thermic Typic, Albaqualts) with 34% sand, 53% silt, 13% clay, and 1.5% organic matter, pH 5.9, was sieved and used to test sensitivity of accessions to preemergence-applied herbicides, specifically pendimethalin and *S*-metolachlor. Field soil was placed in 30-cm by 17-cm flats and wetted. After wetting, 50 Palmer amaranth seeds were spread and lightly covered with 0.25 to 0.5 cm of field soil. A total of three replications per herbicide were included in each run, thus a total 300 seeds were treated per herbicide. All herbicides were applied using the same methodology as the dose-response experiment, and herbicides were incorporated through overhead irrigation to simulate approximately 1.5 cm of rainfall.

For the postemergence herbicides, the number of total plants sprayed at the time of application was recorded, and live plants that persisted 28 DAA were counted to capture mortality percentages. For the assessment of preemergence herbicide efficacy, the number of Palmer amaranth plants with one true leaf were counted at 14 DAA, and the number of emerged plants was reported as a percentage relative to the nontreated to account for variability in germination and emergence among accessions.

Data Analysis

Dose Response

In the dose-response experiment, the percent mortality of Palmer amaranth was analyzed in the Fit Curve Platform of JMP Pro 16.2 software (SAS Institute Inc., Cary, NC). A Weibull growth curve ($y = a * \{1 - \mathrm{Exp}[-(\mathrm{rate}/b)^c]\}$, where a = asymptote, b = inflection point, and c = growth rate) was found to be the best fit compared to other models, including but not limited to Exponential 3P, Mechanistic growth, Gompertz, Logistic 3P, etc., when corrected Akaike information criterion, Bayesian information criterion, sum of squares error, mean square error, and R^2 values were used to model the percent mortality of Palmer amaranth. The Weibull growth curve has been used to fit dose-response data in ecotoxicology, weed science, and other types of research (Christensen et al. 1984; Knezevic et al. 2007; Ritz 2010). Data were pooled over experimental runs and individual nonlinear Weibull growth models

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Table 1. Herbicides used with accessions S1, A2019, A2020, and B2020.[e]

Timing of application	WSSA group number	Herbicide	Herbicide family	Product	Use rate
					g ai ha^{-1} or g ae ha^{-1} [d]
PRE					
	3	Pendimethalin	Dinitroaniline	Prowl H_2O® 3.8 L	970
	15	*S*-metolachlor	Chloroacetamide	Dual II Magnum® 7.34 EC	1,067
POST					
	2	Imazethapyr[a]	Imidazolinone	Pursuit® 2 L	72
	4	2,4-D[a]	Phenoxy	Enlist One® 3.8 L	1,064*
	4	Dicamba[a]	Benzoic acid	XtendiMax® plus VaporGrip® 2.9 L	560*
	5	Atrazine[c]	Triazine	Aatrex 4 L	1,120
	7	Diuron[a]	Ureas	Direx 4 L	894
	9	Glyphosate	Glycine	Roundup Powermax II® 4.5 L	866*
	10	Glufosinate	Phosphinic acid	Liberty® 2.34 L	595
	14	Fomesafen[a]	Diphenyl ethers	Reflex® 2 SL	395
	22	Paraquat[a]	Bipyridylium	Gramoxone® 3 SL	709
	27	Mesotrione[b]	Triketone	Callisto® 4 SC	105
	27	Tembotrione[c]	Triketone	Laudis® 3.5 L	92

[a]Nonionic surfactant at 0.25% (vol/vol) was included.
[b]Crop oil concentrate at 1% (vol/vol) was included.
[c]Methylated seed oil at 1% (vol/vol) was included.
[d]Rates displayed with an asterisk (*) are ae, those without an asterisk are ai.
[e]Abbreviations: ae, acid equivalent; ai, active ingredient; POST, postemergence; PRE, preemergence; WSSA, Weed Science Society of America.

Table 2. Weibull growth curve fit to data by herbicide and Palmer amaranth accession.[a]

Herbicide	Accession[b]	Asymptote	Inflection point	Growth rate	R^{2c}
Glufosinate	S1	100.00	0.08	2.50	0.99
	S2	98.53	0.08	1.56	0.98
	A2019	91.99	0.41	2.09	0.97
	A2020	99.22	1.50	1.53	0.98
	B2020	92.23	1.74	4.74	0.99

[a]The Weibull growth curve is $y = a * \{1 - \text{Exp}[-(\text{rate}/b)^c]\}$, where a = asymptote, b = inflection point, and c = growth rate.
[b]S1 and S2 are susceptible standards, and A2019, A2020, and B2020 are putative-resistant accessions.
[c]R^2 values display the percentage of the response variability explained by the model.

were fit to each accession by herbicide. Parameter estimates and R^2 values for models fit are displayed in Table 2. Predictions of the herbicide rate needed to kill 50% of the population (e.g., LD_{50}) and 80% of the population (e.g., LD_{80}) were made along with the lower and upper estimates of the 95% confidence interval. Confidence intervals were used to determine whether the LD_{50} and LD_{80} predictions were different from other accessions sprayed with the same herbicide. If confidence intervals of prediction estimates did not overlap, the predications were considered different, and resistant-fold values were calculated by dividing the LD_{50} or LD_{80} estimate of the resistant biotype by the respective LD_{50} or LD_{80} estimate of the susceptible biotypes.

Response to Labeled Herbicide Rates

Analysis of variance confirmed that there were no differences between experimental runs ($P = 0.6857$); therefore, data were pooled over runs. Moss et al. (1999) and Walsh et al. (2004) used 20% survival as a threshold for classifying a weed as resistant to a labeled rate of various herbicides when screening for multiple resistance, but as methodologies have improved to classify weed species as herbicide-resistant over the last 20 yr, this experiment will be used only to assess effectiveness of alternative control options relative to a standard accession.

Results and Discussion

Dose Response

Glufosinate

The two susceptible accessions were proven to be sensitive to glufosinate. When the LD_{50} values of accessions A2019, A2020, and B2020 were compared with the susceptible accessions there was a 5- to 6-, 17- to 19-, and 24- to 27-fold increase in the glufosinate rate needed to achieve comparable mortality of the putative-resistant accessions, respectively (Table 3). The glufosinate dose required to kill 80% of the three putative-resistant accessions was 5.4 to 21.0 times greater than the susceptible accessions (Table 3). As of 2021, glufosinate resistance has not been documented in any broadleaf weed (Heap 2021). The rate of glufosinate needed to kill 50% of the resistant Palmer amaranth accessions (A2019, A2020, B2020) was 0.46 to 2.5 kg ai ha^{-1}. Based on the LD_{50} and LD_{80} values; all three accessions that were suspected of having resistance to glufosinate can be deemed "resistant". All three fields where accession A2019, A2020, and B2020 originated had at least one glufosinate application fail to control Palmer amaranth plants in 2019 or 2020, and some plants in the 2019 field survived as many as five applications of glufosinate.

Effectiveness of Labeled Herbicides on Glufosinate-Resistant Palmer Amaranth

The same S1 standard accession collected in 2001 and used in the previous dose-response experiments was used to confirm sensitivity of Palmer amaranth to the tested herbicides. Unfortunately, imazethapyr resulted in 0% mortality of the standard in both experimental runs (Table 4). This finding is not surprising because Palmer amaranth populations with resistance to acetolactate synthase-inhibiting herbicides, including imazethapyr, were first documented in 1994 in Arkansas (Heap 2021). The standard accession used in the experiment appeared to be effectively controlled by all other herbicides tested, with mortality ranging from 77% to 100%. In contrast, accessions A2019, A2020, and B2020 were not effectively controlled (20 percentage points less than the susceptible standard) by several herbicides (Table 4).

Soil-applied pendimethalin and *S*-metolachlor resulted in only 77% and 48% mortality, respectively, of the A2019 accession,

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Table 3. LD_{50} predictions from glufosinate dose-response experiment conducted on accessions S1, S2, A2019, A2020, and B2020.[a]

Herbicide		Accession	Predicted rate	Confidence interval (95%) Lower	Upper	Level of resistance to S1	Level of resistance to S2
			g ai ha^{-1}			resistance ratio[b,c]	
Glufosinate	LD_{50}	S1	42	36	48		
		S2	36	30	42		
		A2019	214	184	244	5.1*	5.9*
		A2020	708	583	833	16.9*	19.7*
		B2020	988	898	1,071	23.5*	27.4*
	LD_{80}	S1	60	54	65		
		S2	65	60	71		
		A2019	339	309	369	5.7*	5.4*
		A2020	1,232	1,107	1,357	21.0*	19.6*
		B2020	1,202	1,119	1,291	20.5*	19.1*

[a]Resistance ratio was determined by dividing the predicted value of the putative resistant (R) accession by the predicted value of the susceptible (S) accession.
[b]Predicted glufosinate rates are shown in g ai ha^{-1}.
[c]Significant R/S ratios based on 95% confidence intervals are indicated with an asterisk (*).

Table 4. Percent mortality of Palmer amaranth accessions A2019, A2020, and B2020 following applications of various preemergence and postemergence herbicides.[e]

	WSSA group number	Herbicide	Herbicide family	Palmer amaranth mortality 28 DAA A2019	A2020	B2020
				%, percentage point difference from standard accession[d]		
PRE	3	Pendimethalin	Dinitroaniline	77 (20)*	86 (11)	87(10)
	15	*S*-metolachlor	Chloroacetamide	48 (52)*	88 (12)	98 (2)
POST	2	Imazethapyr[a]	Imidazolinone	0 (0)	4 (-4)	0 (0)
	4	2,4-D[a]	Phenoxy	47 (39)*	43 (43)*	77 (9)
	4	Dicamba[a]	Benzoic acid	72 (18)	74 (16)	87 (3)
	5	Atrazine[c]	Triazine	86 (14)	100 (0)	97 (3)
	7	Diuron[a]	Ureas	58 (42)*	100 (0)	100 (0)
	9	Glyphosate	Glycine	0 (84)*	4 (80)*	2 (82)*
	10	Glufosinate	Phosphinic acid	80 (20)*	46 (54)*	6 (94)*
	14	Fomesafen[a]	Diphenyl ethers	4 (83)*	82 (5)	62 (25)*
	22	Paraquat[a]	Bipyridylium	100 (0)	100 (0)	100 (0)
	27	Mesotrione[b]	Triketone	2 (76)*	9 (69)*	45 (33)*
	27	Tembotrione[c]	Triketone	7 (70)*	73 (4)	73 (4)

[a]Nonionic surfactant at 0.25% (vol/vol) was included.
[b]Crop oil concentrate at 1% (vol/vol) was included.
[c]Methylated seed oil at 1% (vol/vol) was included.
[d]Asterisk (*) indicates at least 20 percentage point reduced mortality compared with standard accession.
[e]Abbreviations: DAA, days after application; POST, postemergence; PRE, preemergence; WSSA, Weed Science Society of America.

which was more than 20 percentage points less effective than the susceptible standard (Table 4). Mortality of the A2019 accession following a postemergence application of 2,4-D, diuron, fomesafen, glyphosate, glufosinate, mesotrione, and tembotrione was 20 percentage points less than the susceptible standard, and imazethapyr resulted in 0% mortality (Table 4). Additionally, mortality percentages declined by 18 and 14 percentage points when dicamba and atrazine, respectively, were applied postemergence to A2019. Atrazine and paraquat were the only herbicide options tested that resulted in greater than 85% mortality of A2019 (Table 4). Again, A2019 is suspected to harbor resistance to at least one herbicide from at least nine SOAs, with these including WSSA Groups 2, 3, 4, 7, 9, 10, 14, 15, and 27. To date, no population of Palmer amaranth with resistance to herbicides from more than six SOAs has been found (Shyam et al. 2020). Likewise, there has been no documented resistance to a Group 7 herbicide in this weed species. The failure of diuron on this accession is not surprising because Group 7 herbicides have been used repeatedly for control of Palmer amaranth in this field in years when cotton was grown.

Accession A2020 displayed at least a 20 percentage point reduction in mortality compared with the susceptible standard following an application of 2,4-D, glyphosate, glufosinate, and mesotrione (Table 4). Greater than 46% mortality was not observed when A2020 was treated with labeled rates of 2,4-D, glyphosate, glufosinate, imazethapyr, or mesotrione, thus, these herbicides would be considered ineffective control options. A2020 is suspected to harbor multiple resistance to 2,4-D, glyphosate, glufosinate, imazethapyr, and mesotrione, but further experiments would be needed to confirm this resistance. Pendimethalin and *S*-metolachlor, both preemergence-applied herbicides, resulted in more than 85% mortality of A2020. Postemergence application of atrazine, diuron, and paraquat also resulted in greater than 85% mortality of A2020, whereas dicamba and fomesafen resulted in 74% and 82% mortality, respectively (Table 4).

When labeled rates (shown in Table 1) of glyphosate, glufosinate, imazethapyr, and mesotrione were applied to accession B2020, no more than 9% mortality was observed. Additionally, only 62% morality was observed when B2020 was treated with fomesafen, which was a 25 percentage point reduction compared with the susceptible standard (Table 4). Labeled rates of *S*-metolachlor, pendimethalin, atrazine, dicamba, diuron, and paraquat resulted in greater than 85% mortality of B2020, thus potential options for chemical control of this accession exist.

Practical Implications and Conclusions

All three accessions of Palmer amaranth for which glufosinate failed to provide control in the field in 2019 or 2020 likely harbors multiple herbicide resistance. Resistance to glufosinate was confirmed in A2020 and B2020 with resistance ratios of 16.9 to 27.4. Further efforts should focus on determining which other herbicide SOAs to which this accession is resistant. The number of useful herbicide options to control Palmer amaranth in cotton and soybean in the southern United Stated is diminishing. With few herbicide options left in soybean and cotton, additional nonchemical control strategies will be needed to combat these Palmer amaranth populations. In the future, any novel herbicide that is brought to market is likely to undergo increased selection due to the lack of alternative in-crop herbicide options for Palmer amaranth control in cotton and soybean (Culpepper et al. 2006; Perez-Jones et al. 2005; Powles et al. 1997; Simarmata et al. 2005). Furthermore, the selection for resistance to an auxin herbicide without any recently known use of such herbicide is a concern for the long-term sustainability of effective herbicide-based weed control programs.

Glufosinate resistance in Palmer amaranth further limits control options for corn, cotton, and soybean growers. Rotation to a crop such as rice (*Oryza sativa* L.) for which the field can be flooded as a nonchemical means of control was used in 2020 to control glufosinate-resistant accessions. Other strategies such as drill-seeded or narrow-row crops, cover crops, deep tillage, and harvest weed seed control techniques are additional options that may aid long-term management of this weed (Norsworthy et al. 2012).

In the future, accessions A2019, A2020, and B2020 will undergo additional testing to confirm their resistance to other SOAs and elucidate the mechanisms responsible for herbicide failure. Additional research should also assess whether any fitness penalty is associated with the resistant mechanisms, especially considering that A2019 did not appear to exhibit growth that was as vigorous as the other accessions we tested. Field research should also aim at identifying herbicide combinations and programs that effectively control these accessions. Mixtures of herbicides may also increase control and should be evaluated on these populations as potential chemical options.

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