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Confirmation of a four-way herbicide-resistant Palmer amaranth (*Amaranthus palmeri*) population in Iowa

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Abstract

Palmer amaranth (*Amaranthus palmeri* S. Watson) was first reported in Iowa in 2013 and has continued to spread across the state over the last decade. *Amaranthus palmeri* is widely recognized as one of the more economically important weeds in production agriculture. The presence of *A. palmeri* in Iowa is concerning as the species has evolved resistance to ten herbicide sites of action, however, no formal characterization has been conducted on Iowa populations. Therefore, herbicide assays were conducted on an *A. palmeri* population collected in Harrison County, IA, in 2023 (Southwest Palmer Amaranth [SWPA]) and a known herbicide-susceptible population collected from Nebraska in 2001 (Palmer Amaranth Susceptible [PAS]). The two populations were treated with preemergence and postemergence herbicides commonly used in Iowa. The treatments included preemergence applications of atrazine, metribuzin, and mesotrione and postemergence applications of atrazine, imazethapyr, glyphosate, lactofen, mesotrione, glufosinate, 2,4-D, and dicamba at 1× and 4× the labeled rates. Survival frequency of SWPA was >90% when treated postemergence with 1× rates of imazethapyr, atrazine, glyphosate, and mesotrione compared with ≤6% for PAS. Both SWPA and PAS had 0% survival when treated with lactofen, glufosinate, 2,4-D, and dicamba at the 1× or 4× rates. Plant population density reduction for SWPA was 53% and 40% in response to 1× rates of preemergence-applied mesotrione and atrazine, respectively. Metribuzin applied preemergence reduced SWPA plant population density by >90% at both rates. Dose–response experiments revealed the 50% effective doses (ED_{50}) of mesotrione, glyphosate, imazethapyr, and atrazine for SWPA were 9.5-,8.5-, 71-, and 40-fold greater than for PAS, respectively. The results confirm that SWPA is four-way multiple-herbicide resistant. *Amaranthus palmeri* infestations are likely to continue to spread within Iowa; therefore, diversified weed management programs that include early detection, rapid response, and effective multi-tactic management strategies will be required for control.

Introduction

Palmer amaranth (*Amaranthus palmeri* S. Watson) is a summer annual weed native to northern Mexico and the southwestern United States (Sauer 1957). It is currently considered one of the most common and troublesome weeds in U.S. annual row crops, causing significant yield losses in cotton (*Gossypium hirsutum* L.), soybean [*Glycine max* (L.) Merr.], sorghum [*Sorghum bicolor* (L.) Moench], and corn (*Zea mays* L.) (Bensch et al. 2003; Massinga et al. 2001; Moore et al. 2004; Morgan et al. 2001; Van Wychen 2020, 2022). *Amaranthus palmeri* has several biological characteristics that allow it to compete and persist within many crop systems. These characteristics include, but are not limited to, an extended emergence period, a rapid growth rate, and prolific seed production (Horak and Loughin 2000; Keeley et al. 1987; Ward et al. 2013).

Amaranthus palmeri is a dioecious species with obligate outcrossing, increasing its genetic variability and thus its adaptability (Franssen et al. 2001; Ward et al. 2013). *Amaranthus palmeri* readily evolves herbicide resistance. The first confirmed case of herbicide resistance in *A. palmeri* was reported in 1989 to the microtubule-inhibiting herbicides (i.e., trifluralin) (Gossett et al. 1992). Currently, resistance to ten herbicide sites of action (SOA) including photosystem II–serine 264 binders (PS II), PS II–histidine 215 binders, acetolactate synthase (ALS), 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), glutamine synthetase (GS), protoporphyrinogen oxidase (PPO), very long-chain fatty acid synthase (VLCFAS), microtubule-inhibitors, synthetic auxins, and hydroxyphenyl pyruvate dioxygenase (HPPD) inhibitors has been reported in A. palmeri populations however the specific evolved resistances

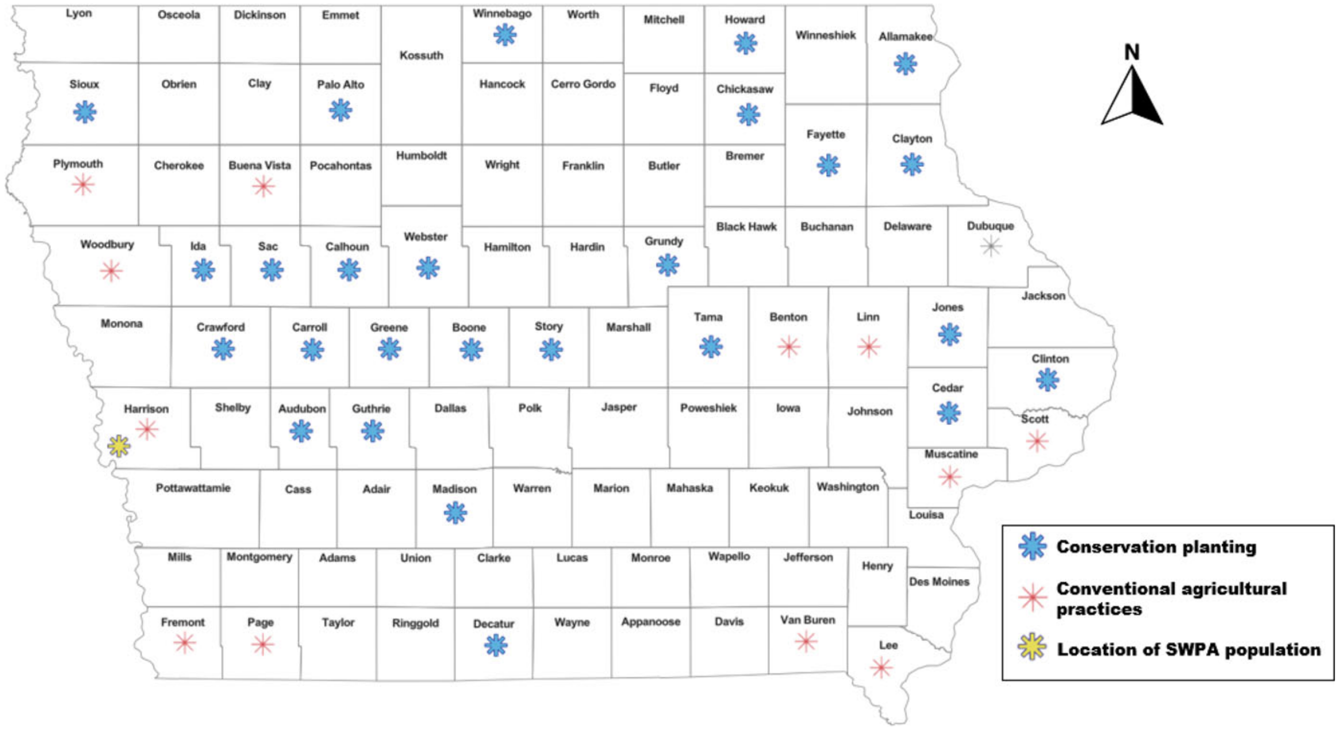

Figure 1. Iowa counties with confirmed *Amaranthus palmeri* infestations found in conservation plantings or conventional agricultural fields and the location of the Southwest Palmer Amaranth (SWPA) study population.

vary within populations (Heap 2023). Moreover, *A. palmeri* populations have evolved resistance to multiple herbicide SOAs, one example being a six-way multiple herbicide–resistant (MHR) population in Kansas (Shyam et al. 2021).

Amaranthus palmeri populations have expanded well beyond their original native range in the southwestern United States and northern Mexico (Roberts and Florentine 2022; Ward et al. 2013). This expansion was facilitated by natural and human mechanisms, including migratory waterfowl, native seed mixes for conservation plantings, and other agricultural practices (Bagavathiannan and Norsworthy 2016; Farmer et al. 2017; Hartzler and Anderson 2016). The expansion of *A. palmeri* is also global, with populations in Africa, Asia, Europe, Oceania, and South America (Kistner and Hatfield 2018; Küpper et al. 2017; Mennan et al. 2021; Milani et al. 2021; Sukhorukov et al. 2021). A recent study using climate models predicted that as global average temperatures increase, the range suitable for *A. palmeri* will continue to expand northward into the U.S. Midwest, Canada, and Europe (Kistner and Hatfield 2018).

In Iowa, the first reported sighting of *A. palmeri* occurred in 2013 in Harrison County, located less than 10 km from the Missouri River and Nebraska (Hartzler and Anderson 2016). The field had been fallow for the spreading of waste from a fermentation plant in southern Nebraska, a region where *A. palmeri* was present. The *A. palmeri* was likely present for several years before discovery, based on the observed population density when discovered in August 2013 (Hartzler and Anderson 2016). Seeds were believed to have traveled via vehicles carrying waste from a nearby processing plant. Subsequent reports of *A. palmeri* infestations were confirmed in five additional counties across southern Iowa between 2013 and 2015, all where fields used inputs or machines originating from outside Iowa (Hartzler and Anderson 2016). Increased planting of native seed mixes occurred in Iowa in 2016, due in part to incentives via the Conservation Reserve Program. However, Iowa seed producers could not meet the demand for the native seed mixtures requested by growers and thus imported seed mixtures from other states. These imported seed mixtures were contaminated with *A. palmeri* seed. Subsequently, *A. palmeri* infestations were confirmed in 49 counties in Iowa at the end of 2016 (Hartzler and Anderson 2016) (Figure 1).

Previous research reported that newly introduced *A. palmeri* populations in the United States, Brazil, Turkey, and South Africa were confirmed to be MHR (Faleco et al. 2022; Küpper et al. 2017; Mennan et al. 2021; Reinhardt et al. 2022). Wisconsin researchers reported a recently discovered *A. palmeri* population was three-way MHR and survived labeled rates of imazethapyr, glyphosate, and atrazine (Faleco et al. 2022). Similarly, a recently discovered *A. palmeri* population in South Africa was confirmed resistant to glyphosate and chlorimuron-ethyl (Reinhardt et al. 2022). Herbicide-resistant *A. palmeri* may be a significant threat to Iowa agriculture; however, to our knowledge, no Iowa *A. palmeri* populations have been evaluated for herbicide resistance. Therefore, the objective of this research was to evaluate the responses of an Iowa *A. palmeri* population to commonly used preemergence and postemergence herbicides.

Materials and Methods

Sample Collection and Processing

Seed samples of an *A. palmeri* population collected in fall 2022 near Modale, IA (designated Southwest Palmer Amaranth [SWPA]) were sent to the Iowa State University Department of Agronomy for identification and evaluation for herbicide resistance. The SWPA population was discovered just south of Modale, IA (41.6190°N, 96.0115°W) (Figure 1). The population sample was

Table 1. Herbicide treatments used to evaluate the response of the *Amaranthus palmeri* populations.[a]

Active ingredient	Application timing	Trade name	Formulation	SOA (HG)	Label rate (1×)	Adjuvant[b]	Herbicide manufacturer
					g ai/ae ha^{-1}		
Imazethapyr	POST	Pursuit®	2 L	ALS (HG2)	70	AMS + COC	BASF Corp., Research Triangle Park, NC
Atrazine	POST	Aatrex®	4 L	PSII (HG5)	1,121	COC	Syngenta Crop Protection, Greensboro, NC
Glyphosate	POST	Roundup PowerMax®	4.5 L	EPSPS (HG9)	857	AMS	Bayer Crop Science, St Louis, MO
Lactofen	POST	Cobra®	2 L	PPO (HG14)	208	COC	Valent U.S.A Corp., Walnut Creek, CA
Mesotrione	POST	Callisto®	4 SC	HPPD (HG27)	105	COC + UAN	Syngenta Crop Protection, Greensboro, NC
Glufosinate	POST	Liberty®	280 SL	GS (HG 10)	660	AMS	BASF Corp., Research Triangle Park, NC
2,4-D	POST	Enlist One™	3.8 L	AM (HG 4)	795	—	Corteva Agriscience LLC, Indianapolis, IN
Dicamba	POST	XtendiMax®	2.9 L	AM (HG 4)	552	—	Bayer Crop Science, St. Louis, MO
Atrazine	PRE	Aatrex®	4 L	PSII (HG5)	2,240	—	Syngenta Crop Protection, Greensboro, NC
Metribuzin	PRE	Tricor®	75 DF	PSII (HG5)	515	—	United Phosphorus Inc., King of Prussia, PA
Mesotrione	PRE	Callisto®	4 SC	HPPD (HG27)	240	—	Syngenta Crop Protection, Greensboro, NC

[a] Abbreviations: ALS, acetolactate synthase; AM, auxin mimics; EPSPS, 5-enolpyruvylshikimate-3-phosphate synthase; GS, glutamine synthetase; HG, herbicide group; HPPD, 4-hydroxyphenylpyruvate dioxygenase; POST, postemergence; PPO, protoporphyrinogen oxidase; PRE, preemergence; PSII, photosystem II; SOA, site of action,

[b] Ammonium sulfate (AMS) at 2g 100 ml^{-1}, crop oil concentrate (COC) at 1% v/v, and urea ammonium nitrate (UAN) at 2.5% v/v.

approximately 5 to 10 *A. palmeri* seed heads collected from 20 plants near or on the borders of a field site that had been in corn/soybean production for many years. How the SWPA was introduced to Iowa, as well as its herbicide exposure history, is unknown. The SWPA seed sample was air-dried at room temperature for 72 h, and then processed by hand to remove the seeds from the inflorescences. The seeds were then processed through multiple sieves and, finally, an air column separator to remove any remaining plant material from the sample. The seeds were then stored at 0 C in the dark until herbicide response experiments were initiated. A known susceptible *A. palmeri* population (designated Palmer Amaranth Susceptible [PAS]) was used for comparison and was originally collected in 2001 in a Nebraska agricultural field. The PAS population was confirmed susceptible to all herbicides tested through preliminary experiments conducted shortly before the postemergence herbicide-resistance assays. Preliminary germination tests revealed high germination percentages within SWPA; therefore, dormancy-breaking procedures were not needed. All preemergence, postemergence, and dose–response experiments were conducted at the Iowa State University Department of Agronomy Greenhouse in Ames, IA, between December 2022 and July 2023.

Postemergence Herbicide-Resistance Assays

Amaranthus palmeri seeds from the SWPA and PAS populations were grown in 28 cm by 56 cm plastic seed trays (Jiffy Products of America, Lorain, OH) filled with commercial potting mixture (Metro-Mix® 820, Sun Gro®, Agawam, MA). Individual seedlings that reached the 2-leaf stage were transplanted into 2.5-cm-diameter by 16-cm-deep cones (Cone-tainer™, Stewe and Sons, Tangent, OR) that contained potting mixture fertilized with Osmocote® Smart-Release® fertilizer (Scotts, Marysville, OH) (methods adapted from Hamberg et al. [2023]). The transplanted seedlings were watered once daily. Greenhouse conditions were maintained at 30/25 C day/night temperatures with supplemental artificial light from metal-halide lamps (600 µmol m^{-2}s^{-1}) providing a 14-h photoperiod. All plants were kept under the abovementioned conditions both before and after herbicide applications.

The SWPA and PAS populations were treated with eight herbicides applied at 1× and 4× labeled rates (Table 1). The experimental design was a completely randomized design with eight replications of one plant per replication, and the experiments were repeated once. Eight nontreated control plants from each population were used for comparison. All postemergence herbicide treatments were applied using an enclosed laboratory spray chamber equipped with a single 0015EVS nozzle (TeeJet® Spraying Systems, Wheaton, IL) calibrated to deliver 140 L ha^{-1} at 276 kPa at 4.7 km h^{-1} when all *A. palmeri* plants were 5- to 7-cm tall. When auxinic herbicides were sprayed, treated plants were moved to a separate greenhouse room and isolated to avoid any unwanted injury to other treatments due to possible volatilization. Visual injury observations were made 28 d after treatment (DAT) using a scale of 0% to 100%, where 0% was no injury and 100% was plant death compared with nontreated control plants. At 28 DAT, the survival of each plant was evaluated individually; plants with ≤65% visual injury were considered to have survived the herbicide. Herbicide survival frequency was calculated by dividing the number of surviving plants by the total number of treated plants and multiplying by 100. The *A. palmeri* population was considered resistant if the survival frequency was >50% of the 1× labeled rate. Aboveground biomass for each plant was harvested at 28 DAT and oven-dried at 60 C for 72 h, and dry weights were recorded. Dry plant biomass reduction relative to a nontreated control was calculated using the following formula:

$$\text{Biomass reduction } (\%) = \frac{\overline{\text{C}} - \text{B}}{\overline{\text{C}}} \times 100 \qquad [1]$$

where $\overline{\text{C}}$ is the mean biomass of eight nontreated control plants, and B is the biomass of an individual treated experimental unit.

Table 2. Survival frequency and biomass reduction of two *Amaranthus palmeri* populations (± SE) 28 d after treatment to herbicides applied postemergence at two herbicide rates.[a,b]

	Plant survival[c]		Biomass reduction[d]		Plant survival[c]		Biomass reduction[d]	
Herbicide	PAS	SWPA	PAS	SWPA	PAS	SWPA	PAS	SWPA
	1×[e]				4×[f]			
	%							
Imazethapyr	0 a (0)	100 b (0)	91 a (0.9)	12 b (2)	0 a (0)	100 b (0)	96 a (1)	11 b (2)
Atrazine	19 a (0.1)	100 b (0)	89 a (2)	45 b (5)	0 a (0)	69 b (0.1)	96 a (0.4)	35 b (9)
Glyphosate	0 a (0)	100 b (0)	87 a (1)	13 b (3)	0 a (0)	88 b (0.1)	92 a (1)	28 b (6)
Lactofen	0 a (0)	0 a (0)	96 a (0.5)	94 a (2)	0 a (0)	0 a (0)	97 a (0.5)	97 a (0.4)
Mesotrione	6 a (0.1)	91 b (0.1)	94 a (1)	48 b (7)	0 a (0)	69 b (0.1)	96 a (0.5)	66 b (5)
Glufosinate	0 a (0)	0 a (0)	97 a (0.4)	97 a (0.4)	0 a (0)	0 a (0)	97 a (0.3)	96 a (0.5)
2,4-D	0 a (0)	0 a (0)	93 a (5)	91 a (4)	0 a (0)	0 a (0)	98 a (0.7)	96 a (0.4)
Dicamba	0 a (0)	0 a (0)	95 a (2)	92 a (2)	0 a (0)	0 a (0)	98 a (0.5)	99 a (0.2)

[a] Abbreviations: SWPA, Southwest Palmer Amaranth is an *A. palmeri* population collected southwest of Modale, IA, in 2022; PAS, Palmer Amaranth Susceptible is a known susceptible *A. palmeri* population collected from a field in Nebraska in 2001.

[b] Means for each response variable across the rows with no common letters are significantly different according to Welch's two-sample *t*-test, where $P \leq 0.05$.

[c] Plants were considered to have survived if visual injury was ≤65%.

[d] Biomass reduction was calculated as the percent biomass reduction of one experimental unit compared with the mean biomass of eight nontreated control plants.

[e] Herbicide label use rate.

[f] Four times the herbicide label use rate.

Preemergence Herbicide-Resistance Assays

The experimental design was a completely randomized design with four replications, and the experiment was repeated once. Treatments consisted of two *A. palmeri* populations (SWPA and PAS) treated with three herbicides at 1× and 4× labeled rates (Table 1). Each experimental unit consisted of an 8.9 cm by 8.9 cm by 6.4-cm deep square nursery pot (Kord Square Pot, HC Companies, Twinsburg, OH) filled with herbicide-free field soil (Canisteo silty clay loam (fine-loamy, mixed, superactive, calcareous, mesic Typic Endoaquolls), 4.0% organic matter and pH 6.9) with 50 *A. palmeri* seeds (counted individually) planted at 1-cm depth. The soil was collected from a field southeast of Boone, IA, with a known history of no herbicide applications for at least 1.5 yr and no *A. palmeri* infestation. The *A. palmeri* seeds were placed evenly in the square nursery pot, avoiding placement near the pot edges. Nontreated controls for both populations were included for each replication. Herbicide applications were applied with an enclosed laboratory spray chamber with the same specifications mentioned previously. The pots were watered after treatments to moisten the soil and to allow herbicide activation. Pots were watered uniformly every 1 to 2 d throughout the experimental period. Greenhouse conditions were kept consistent with the specifications mentioned previously.

The total number of emerged plants was counted for each experimental unit at 28 DAT. The percent reduction in plant population density was compared with the nontreated control using the following equation:

$$\text{Population density reduction (\%)} = \frac{\text{ECEU}}{\text{ECNTC}} \times 100 \qquad [2]$$

where ECEU is the plant emergence count of each individual experimental unit, and ECNTC is the mean plant emergence counts of the nontreated control units. Herbicide treatments that provided <90% plant population density reduction were classified as resistant (adapted from Faleco et al. [2022]).

Dose–Response Assays

Herbicide dose–response experiments were conducted to assess the resistance levels in the SWPA population and repeated once. The herbicides selected for dose–response assays were based on the results of the postemergence herbicide-resistance assays. Herbicides were selected if survival frequencies were >50% to 4× the labeled rate (Table 2). The herbicide treatments used for the experiments were ⅛×, ¼×, ½×, 1×, 2×, 4×, and 6× the recommended herbicide labeled rates of atrazine, imazethapyr, glyphosate, and mesotrione (Table 1). Nontreated controls for each population were included for comparison. Each treatment consisted of eight replications with one plant per replication, and the experiment was repeated once. *Amaranthus palmeri* plants were seeded and grown in the greenhouse following the same methods mentioned previously. Treatments were applied when plants reached 7-cm tall, as previously described.

No auxin mimic herbicides were tested in the dose–response experiments, so visual injury and plant survival observations were conducted at 21 DAT instead of 28 DAT. Visual injury was assessed on a scale of 0% to 100%, where 0% was no visual injury, and 100% was plant death compared with nontreated control plants. Individual plant survival was based upon the visual injury observations, in which plants with ≤65% visual injury were considered to have survived the herbicide application. Finally, at 21 DAT, individual aboveground plant biomass was harvested and dried at 60 C for 72 h, and dry weights were recorded.

Statistical Analysis

Plant biomass, survival frequency, and plant population density reduction for each herbicide and rate combination were analyzed with a Welch's two-sample *t*-test in R v. 4.3.1 (R Core Team 2023) using the *t-test* function. *Amaranthus palmeri* dry biomass and survival percentage were analyzed using nonlinear regression models in R v. 4.3.1 (R Core Team 2023) using the drc package v. 3.0-1 (Knezevic et al. 2007; Ritz et al. 2015). A three-parameter log-logistic model was fit to the dry plant biomass data using the following equation:

$$y = \frac{d}{1 + \exp[b(\log x - \log e)]} \qquad [3]$$

where y is the dry biomass of *A. palmeri*, b is the slope at the inflection point, d is the upper limit, and e is the dose required to achieve a 50% reduction in dry biomass (ED_{50}). The ED_{50} was calculated for both populations with all herbicides tested. The R:S ratios were calculated by dividing the ED_{50} of SWPA by the ED_{50} of PAS. Parameter estimates were generated using raw dry plant biomass data; however, for easier interpretation, figures show dry plant biomass reduction relative to a nontreated control (Equation 1).

A two-parameter log-logistic model was fit to the plant survival percentages using the following equation:

$$y = \frac{1}{1 + \{b[\log(x) - \log(e)]\}} \qquad [4]$$

where y is the survival percentage of the *A. palmeri* population, b denotes the slope at the inflection point, and e denotes the lethal dose required to kill 50% of the *A. palmeri* population (LD_{50}). The R:S ratios were calculated by dividing the LD_{50} of SWPA by the LD_{50} of PAS.

Table 3. Population density reduction of two *Amaranthus palmeri* populations (± SE) 28 d after treatment with herbicides applied preemergence.[a]

	Plant population density reduction[b,c]							
Herbicide	PAS		SWPA		PAS		SWPA	
	1×[d]				4×[e]			
				%				
Atrazine	91 a	(3)	53 b	(1)	98 a	(7)	63 b	(5)
Metribuzin	97 a	(2)	93 a	(2)	100 a	(0)	99 a	(1)
Mesotrione	97 a	(1)	40 b	(8)	100 a	(0)	70 b	(5)

[a] Abbreviations: SWPA, Southwest Palmer Amaranth is an *A. palmeri* population collected southwest of Modale, IA, in 2022; PAS< Palmer Amaranth Susceptible is a known susceptible *A. palmeri* population collected from a field in Nebraska in 2001.

[b] Density reduction was calculated by comparing the number of plants emerged in herbicide treatment pots 28 DAT to the number emerged in the nontreated control pots.

[c] Means across rows with no common letters are significantly different according to Welch's two sample *t*-test, where $P \leq 0.05$.

[d] Herbicide label use rate.

[e] Four times the herbicide label use rate.

Results and Discussion

Postemergence Herbicide-Resistance Assays

A paired *t*-test determined no differences between the two experimental runs; thus, data were combined for analysis. Neither SWPA or PAS survived applications of lactofen, glufosinate, 2,4-D or dicamba (Table 2). These herbicides may be effective for the postemergence management of *A. palmeri* in Iowa, thus far (Heap 2023).

A high level of resistance to imazethapyr was detected in the SWPA *A. palmeri* population. Plant biomass reduction was significantly different for SWPA and PAS and was 12% and 91%, respectively, to the 1× rate of imazethapyr (Table 2). A similar response was observed to the 4× imazethapyr rate (Table 2). SWPA had 100% survival to imazethapyr. The ALS resistance in the SWPA population was not surprising, given that high levels of ALS resistance have been reported in *A. palmeri* populations across the United States (Chahal et al. 2017; Faleco et al. 2022; Garetson et al. 2019). Cross-resistance to ALS inhibitors among the imidazolinone, pyrimidinyl thiobenzoic acid, triazolopyrimidine, and sulfonylurea chemical families is common in *A. palmeri* and is caused by a less-sensitive ALS enzyme (Burgos et al. 2001; Ward et al. 2013). Although additional ALS-inhibitor herbicides were not tested, it is likely that SWPA may exhibit cross-resistance to multiple ALS families.

Plant biomass reduction was significantly different between populations for atrazine regardless of rate (Table 2). Plant biomass reductions in response to the 1× atrazine rate were 45% and 89% for SWPA and PAS, respectively, and 35% and 96% at the 4× rate, respectively (Table 2). The survival frequency of SWPA to atrazine exceeded 50% (Table 2). Survival of the SWPA population to atrazine was similar to the reported survival frequency in a Wisconsin *A. palmeri* population in which >50% and 44% survival to 1× and 3x atrazine rates, respectively, were observed (Faleco et al. 2022).

Plant biomass reduction for glyphosate was significantly different between SWPA and PAS (Table 2). Plant biomass reductions for glyphosate treatments never exceeded 28% for SWPA; however, they were 87% and 92% 1× and 4× rates, respectively, for PAS (Table 2). Survival frequency of SWPA in response to 1× and 4× glyphosate rates was 100% and 88%, respectively, compared with 0% for the PAS population, suggesting that the SWPA population is highly resistant to glyphosate. Glyphosate-resistant *A. palmeri* populations are prevalent across the southern United States and have been reported farther north in newly introduced *A. palmeri* populations in Illinois, Michigan, and Wisconsin, so the data supporting the evolved glyphosate resistance in SWPA is not unexpected (Butts 2015; Davis et al. 2015; Chahal et al. 2017; Culpepper et al. 2008; Keating 2019; Norsworthy et al. 2008; Sprague 2012).

Plant biomass reduction for mesotrione was significantly different when comparing SWPA and PAS. Plant biomass reduction in response to the 1× rate of mesotrione was 48% and 94% for SWPA and PAS, respectively. Survival to the 1× mesotrione rate was >90% for SWPA and 6% for PAS (Table 2). Plant biomass reduction of SWPA was higher at the 4× mesotrione rate; however, survival percentage was 66% (Table 2). *Amaranthus palmeri* resistant to HPPD inhibitors was first reported in Kansas in 2009 and has been discovered in Nebraska and Wisconsin in subsequent years (Drewitz et al. 2016; Heap 2023; Jhala et al. 2014).

Data provide evidence that SWPA is still susceptible to several herbicides commonly used in Iowa corn and soybean production. However, *A. palmeri* populations resistant to lactofen, glufosinate, and 2,4-D have been reported in the United States (Priess et al. 2022; Shyam et al. 2021). The SWPA population resistance to imazethapyr, atrazine, glyphosate, and mesotrione suggests that future reliance on dicamba, glufosinate or 2,4-D for *A. palmeri* control will result in evolved resistance to these herbicides.

Preemergence Herbicide-Resistance Assays

A paired *t*-test determined no differences between the two experimental runs; thus, data were combined for analysis. Plant population density was reduced by 91% and 98% for PAS when treated with 1× and 4× atrazine applied preemergence, respectively (Table 3). However, the population density of SWPA was only reduced 53% and 63% when treated with 1× and 4× atrazine, respectively. Plant population density reductions were significantly different for atrazine rates (Table 3). The SWPA population was not controlled (>90% population density reduction) with atrazine applied preemergence (Table 3). *Amaranthus palmeri* populations that are resistant to atrazine applied postemergence were also poorly controlled (<60%) when atrazine is applied preemergence (Hay et al. 2019). The findings of this study indicate that SWPA is

Table 4. Regression parameter estimates for the dry biomass of two *Amaranthus palmeri* populations 21 d after treatment with atrazine, imazethapyr, mesotrione, and glyphosate in whole-plant dose–response experiments.[a]

	Parameter estimates[b] (± SE)			
	b	*d*	ED_{50}	R/S[c]
			g ai/ae ha^{-1}	
Atrazine				
SWPA	1.28 (0.59)	1.54 (0.05)	12,224 (3,802)	
PAS	1.27 (0.16)	1.89 (0.07)	172 (18.6)	71×
Imazethapyr				
SWPA	3.24 (3.77)	1.58 (0.02)	258 (155)	
PAS	1.76 (0.40)	1.88 (0.06)	6.40 (0.79)	40×
Mesotrione				
SWPA	0.96 (0.08)	1.59 (0.05)	153 (16.7)	
PAS	1.40 (0.15)	1.60 (0.06)	17 (1.61)	9.5×
Glyphosate				
SWPA	1.68 (0.23)	1.59 (0.04)	2,281 (200)	
PAS	3.70 (0.61)	1.91 (0.06)	268 (13.0)	8.5×

[a] Abbreviations: SWPA, Southwest Palmer Amaranth is an A. palmeri population collected southwest of Modale, IA, in 2022; PAS< Palmer Amaranth Susceptible is a known susceptible A. palmeri population collected from a field in Nebraska in 2001.

[b] *b* is the slope at the inflection point, *d* is the upper limit, and ED_{50} is the dose required to reduce biomass by 50%.

[c] R/S (resistance ratio) is calculated by dividing the ED_{50} of SWPA by the ED_{50} of PAS.

Table 5. Regression parameter estimates for the survival frequency of two *Amaranthus palmeri* populations 21 days after treatment with atrazine, mesotrione and glyphosate in whole-plant dose response studies.[a]

	Parameter estimates (± SE)		
	b[b]	ED_{50}	R/S[c]
		g ai/ae ha^{-1}	
Atrazine			
SWPA	1.60 (0.67)	18,731 (8,669)	
PAS	11.6 (1.91)	537 (50.3)	35×
Mesotrione			
SWPA	1.28 (0.39)	256 (67.7)	
PAS	2.04 (0.59)	29.9 (6.56)	8.5×
Glyphosate			
SWPA	1.55 (0.55)	4,460 (1,436)	
PAS	5.81 (2.03)	303 (39.9)	14.7×

[a] Abbreviations: SWPA, Southwest Palmer Amaranth is an A. palmeri population collected southwest of Modale, IA, in 2022; PAS< Palmer Amaranth Susceptible is a known susceptible A. palmeri population collected from a field in Nebraska in 2001.

[b] *b* is the slope at the inflection point, and ED_{50} is the dose required to population survival by 50%.

[c] R/S (resistance ratio) is calculated by dividing the ED_{50} of SWPA by the ED_{50} of PAS.

resistant to atrazine applied preemergence and postemergence and represents a future management problem in Iowa.

Plant population density reduction for SWPA and PAS was 99% with a 1× metribuzin rate and >90% with 4× the labeled rate (Table 3). Interestingly, although atrazine and metribuzin are PSII inhibitors with the same target site, the response observed in this study differed with metribuzin providing higher control than atrazine. Faleco et al. (2022) reported a similar difference in plant population density reduction when atrazine and metribuzin were applied preemergence. Previous research with the closely related species waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer] has shown that non–target site (NTS) based atrazine resistance confers a narrower spectrum of cross-resistance to other PSII–Ser-264 binders (Patzoldt et al. 2003). Atrazine resistance in *A. palmeri* is also reported to be NTS mediated (Nakka et al. 2017). Therefore, the difference in response between both PSII inhibitors observed in this study may suggest the mechanism of atrazine resistance within the SWPA population is NTS mediated and does not confer cross-resistance to metribuzin, but further research is needed.

Plant population density reduction was significantly different between PAS and SWPA for mesotrione (Table 3). Reduction in plant population density was 97% and 40% in response to a 1× mesotrione rate for PAS and SWPA, respectively. Schwartz-Lazarro et al. (2017) reported two MHR *A. palmeri* populations from Arkansas had low (<55%) mortality to 1× (213 g ai ha^{-1}) mesotrione applied preemergence compared with 100% mortality to a known susceptible population. At the 4× rate of mesotrione, SWPA population density was reduced only 70%, which suggested the population was resistant (Table 3). These data provide evidence that SWPA is resistant to mesotrione applied preemergence and potentially represents a future major control issue in Iowa, given that 47% of corn acres are treated with mesotrione annually (USDA-NASS 2021).

Dose–Response Assays

A paired *t*-test determined no differences between the two experimental runs; thus, data were combined for analysis. The ED_{50} values for SWPA and PAS for atrazine were 12,224 and 172 g ae ha^{-1}, respectively, indicating a 71-fold resistance ratio (Table 4). Moreover, atrazine at the highest rate (7,326 g ae ha^{-1}) never reduced SWPA biomass by >40% compared with the nontreated control plants (Figure 2D). Plant biomass reduction for the PAS population reached >90% at 1,121 g ae ha^{-1}, which is the atrazine field labeled rate (Figure 2D). Survival frequency of SWPA was never below 75% regardless of atrazine rate, while the PAS population did not survive the atrazine field rate (Figure 3C). The LD_{50} values for SWPA and PAS were 18,731 and 537 g ae ha^{-1}, respectively, providing strong evidence that the SWPA population is highly resistant to atrazine (Table 5).

The ED_{50} for SWPA in response to imazethapyr was 258 g ai ha^{-1}, compared with 6.4 g ai ha^{-1} for the PAS population, resulting in an R/S ratio of 40× (Table 4). Plant biomass reduction in response to imazethapyr was never less than 20% for the SWPA population, whereas PAS was >89% at 35 g ai ha^{-1} (Figure 2B). The model was unable to fit the survival frequency data due to lack of mortality to any rate of imazethapyr for SWPA; however, survival frequency for PAS was 0% at 1× the labeled rate (data not shown).

The ED_{50} for SWPA in response to glyphosate was 2,281 g ai ha^{-1}, with plant biomass reduction averaging 85% at the highest rate (5,142 g ai ha^{-1}) (Table 4; Figure 2A). The PAS population ED_{50} was 268 g ai ha^{-1}, and plant biomass reduction reached >89% at the glyphosate labeled rate (Table 4; Figure 2A). The R/S ratio when comparing the plant biomass reduction of SWPA and PAS with glyphosate was 8.5 (Table 4). The LD_{50} values for SWPA and PAS were 4,460 g ai ha^{-1} and 303 g ai ha^{-1}, respectively, resulting in an R/S of 14.7 (Table 5). The modeled survival frequency for SWPA was 88% at the 1× glyphosate rate and 38% at the 6× rate (Figure 3A).

The ED_{50} values for plant biomass in response to mesotrione were 153 and 17 g ai ha^{-1} for SWPA and PAS, respectively, resulting in an R/S ratio of 9.5 (Table 4). The highest mesotrione rate (630 g ai ha^{-1}) caused an 84% plant biomass reduction for the SWPA population; however, the labeled rate (105 g ai ha^{-1}) only reduced SWPA biomass by 48% (Figure 2C). The LD_{50} of SWPA to postemergence mesotrione was 256 g ai ha^{-1} compared with 29.9 g ai ha^{-1} for PAS (Table 5). The highest rate of mesotrione resulted in 25% survival for the SWPA population (Figure 3C). The differences in R/S values between SWPA and PAS confirm that SWPA is resistant to mesotrione (Table 5).

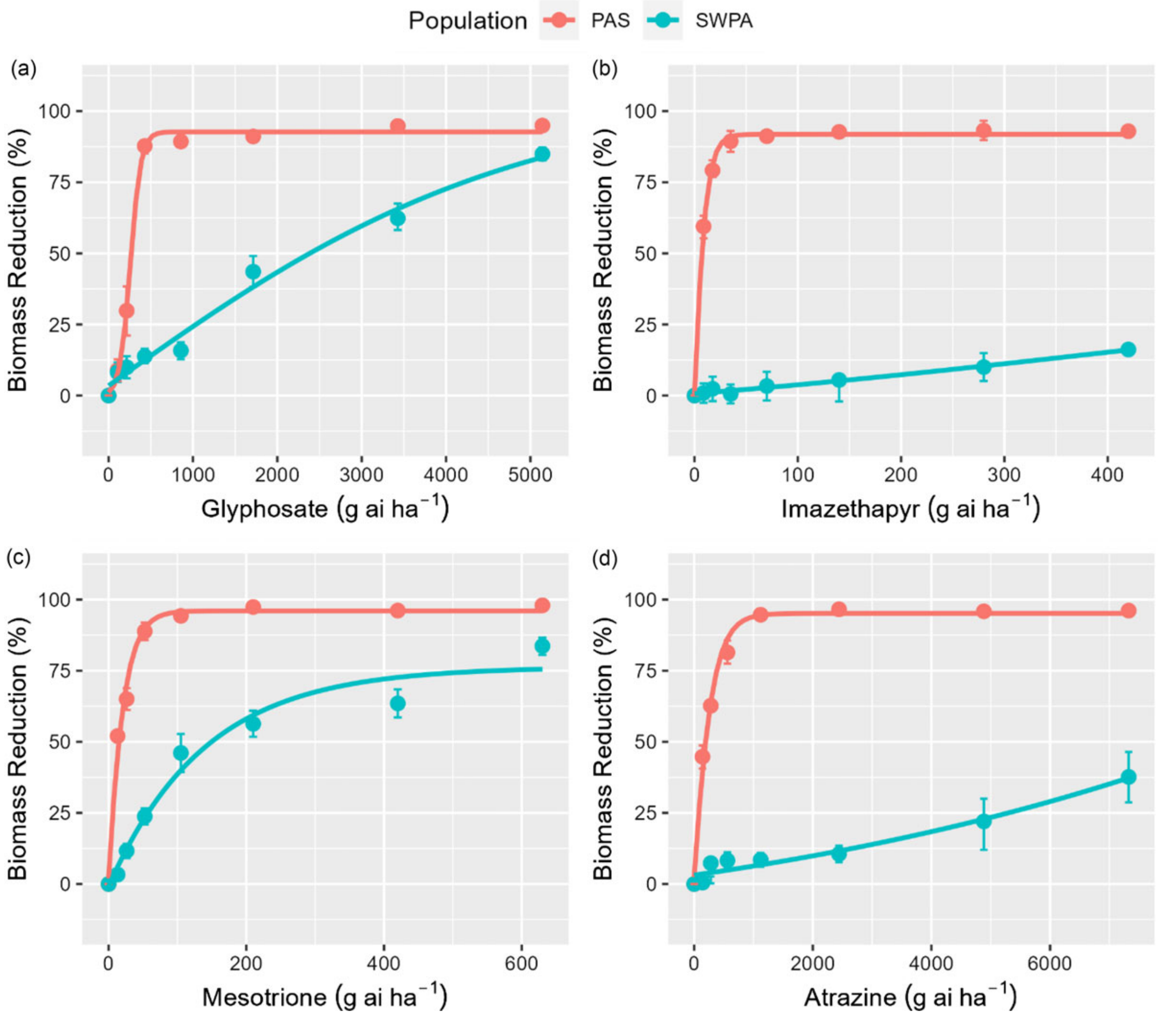

Figure 2. Biomass reduction of *Amaranthus palmeri* populations (SWPA, Southwest Palmer Amaranth; PAS, Palmer Amaranth Susceptible) treated with (a) glyphosate, (b) imazethapyr, (c) mesotrione, and (d) atrazine at 21 d after treatment. Points (±SE) represent actual values, whereas lines represent predicted values from a three-parameter log-logistic model.

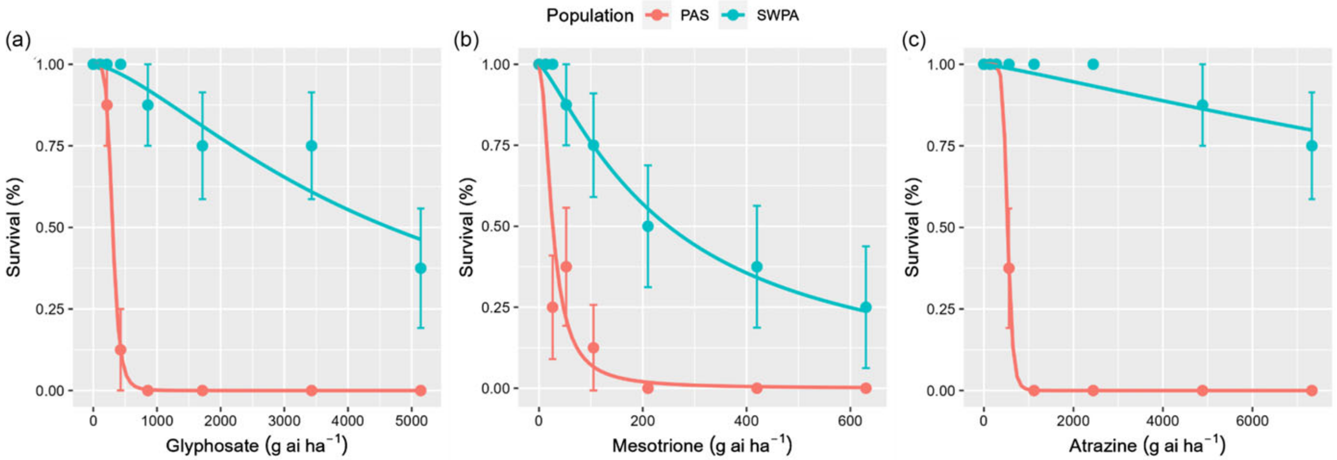

Figure 3. Survival frequency (%) of *Amaranthus palmeri* populations (SWPA, Southwest Palmer Amaranth; PAS, Palmer Amaranth Susceptible) treated with (a) glyphosate, (b) mesotrione, and (c) atrazine at 21 d after treatment. Points (±SE) represent actual values, whereas lines represent predicted values from a two-parameter log-logistic model.

The dose–response experiments show that the SWPA population had higher ED_{50} and LD_{50} values compared with the PAS population and large R/S values (Tables 4 and 5). These data confirm that SWPA is four-way MHR and presents a major future management problem in Iowa crop production. These results align with those of studies that reported four- and six-way MHR *A. palmeri* populations across U.S. states (Faleco et al. 2022; Shyam et al. 2021).

Practical Implications

Amaranthus palmeri has been present in Iowa for at least a decade and is likely to continue to spread in coming years by a variety of mechanisms, such as moving contaminated harvest and tillage equipment from field to field, which are common practices in production agriculture. Our study suggests that other *A. palmeri* populations in Iowa may be resistant to multiple herbicides. Regardless of the evolutionary history of herbicide resistance in *A. palmeri*, the northern spread of *A. palmeri* potentially poses a significant threat to Iowa crop systems. *Amaranthus tuberculatus*, a closely related species to *A. palmeri*, is a major weed in Iowa crop systems, where populations resistant to ALS, PSII inhibitors, and glyphosate are already widespread (Hamberg et al. 2023). Overreliance on herbicides such as 2,4-D, dicamba, glufosinate, and lactofen to control MHR *A. tuberculatus* populations will concurrently select for resistance to these herbicides in sensitive *A. tuberculatus* populations. Given the anticipated increases in *A. palmeri* populations, increased use of the aforementioned herbicides will also increase the prevalence of MHR populations of *A. palmeri*. The discovery of a dicamba-resistant *A. tuberculatus* population in Iowa supports this assumption (Anderson et al. 2023).

Amaranthus palmeri has higher relative growth rate and is more damaging to crop yields than *A. tuberculatus* (Bensch et al. 2003; Horak and Loughin 2000). At optimum soil temperatures, emergence of *A. palmeri* was more rapid than that of *A. tuberculatus* (Steckel et al. 2004). Lillie et al. (2020) reported that *A. palmeri* is more tolerant than *A. tuberculatus* to PPO inhibitors. The rapid growth rate of *A. palmeri* also creates a narrow window for postemergence herbicide applications, where timely application will be challenged by frequent rains in early summer.

Climate predictions estimate that weather in much of the U.S. soybean-growing region, including Iowa, is going to be warmer and drier in future years (Landau et al. 2022). The warmer conditions will likely be more favorable for *A. palmeri* growth and competitiveness in row crops. Using species distribution models, Briscoe Runquist et al. (2019) theorized that historic *A. palmeri* range expansion was facilitated by stochastic, long-distance dispersal events. However, future northward range expansion of *A. palmeri* will likely be facilitated by the projected future increases in temperatures (Briscoe Runquist et al. 2019; Kistner and Hatfield 2018). Furthermore, Davis et al. (2015) suggested the *A. palmeri* damage niche in the Midwest is not limited by weed genotype or maternal environment, and therefore increases in seed abundance will help the widespread invasion into crop systems.

In conclusion, *A. palmeri* has persisted and will likely continue to spread across Iowa. A diversified weed management program, including early detection, rapid response, and multi-tactic management strategies, is required to control *A. palmeri*. Future research should sample *A. palmeri* populations across Iowa and investigate their sensitivity to herbicides to improve herbicide recommendations for growers. Comparative studies such as those conducted by Baker (2021) are needed to accurately predict how the population dynamics of *A. palmeri* and *A. tuberculatus* may change in Iowa crop systems.

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