Multiple resistance to imazethapyr, atrazine, and glyphosate in a recently introduced Palmer amaranth (Amaranthus palmeri) accession in Wisconsin

Authors: Faleco, Felipe A., Oliveira, Maxwel C., Arneson, Nicholas J., Renz, Mark, Stoltenberg, David E., et al.

Source: Weed Technology, 36(3) : 344-351

Published By: Weed Science Society of America

URL: https://doi.org/10.1017/wet.2022.22

The BioOne Digital Library (https://bioone.org/) provides worldwide distribution for more than 580 journals and eBooks from BioOne's community of over 150 nonprofit societies, research institutions, and university presses in the biological, ecological, and environmental sciences. The BioOne Digital Library encompasses the flagship aggregation BioOne Complete (https://bioone.org/subscribe), the BioOne Complete Archive

Downloaded From: https://staging.bioone.org/journals/Weed-Technology on 02 May 2025
Terms of Use: https://staging.bioone.org/terms-of-use

Weed Technology

www.cambridge.org/wet

Multiple resistance to imazethapyr, atrazine, and glyphosate in a recently introduced Palmer amaranth (*Amaranthus palmeri*) accession in Wisconsin

Felipe A. Faleco[1], Maxwel C. Oliveira[2], Nicholas J. Arneson[3], Mark Renz[4], David E. Stoltenberg[4] and Rodrigo Werle[5]

[1]Graduate Student, Department of Agronomy, University of Wisconsin–Madison, Madison, WI, USA; [2]Postdoctoral Researcher, Department of Agronomy, University of Wisconsin–Madison, Madison, WI, USA; [3]Outreach Program Manager, Department of Agronomy, University of Wisconsin–Madison, Madison, WI, USA; [4]Professor, Department of Agronomy, University of Wisconsin–Madison, Madison, WI, USA and [5]Assistant Professor, Department of Agronomy, University of Wisconsin–Madison, Madison, WI, USA

Research Article

Cite this article: Faleco FA, Oliveira MC, Arneson NJ, Renz M, Stoltenberg DE, Werle R (2022) Multiple resistance to imazethapyr, atrazine, and glyphosate in a recently introduced Palmer amaranth (*Amaranthus palmeri*) accession in Wisconsin. Weed Technol. **36**: 344–351. doi: 10.1017/wet.2022.22

Received: 4 November 2021
Revised: 11 February 2022
Accepted: 3 March 2022
First published online: 18 April 2022

Associate Editor:
Scott McElroy, Auburn University

Nomenclature:
atrazine; dicamba; glufosinate; glyphosate; imazethapyr; lactofen; mesotrione; metribuzin; sulfentrazone; *S*-metolachlor; 2,4-D; Palmer amaranth; *Amaranthus palmeri* S. Watson; corn; *Zea mays* L.; soybean; *Glycine max* (L.) Merr.

Keywords:
ALS inhibitor resistance; PSII inhibitor resistance; auxin mimics resistance; herbicide efficacy

Author for correspondence:
Rodrigo Werle, Department of Agronomy, University of Wisconsin–Madison, 1575 Linden Drive, Madison, WI 53706.
Email: rwerle@wisc.edu

Abstract

The continued dispersal of Palmer amaranth can impose detrimental impacts on cropping systems in Wisconsin. Our objective was to characterize the response of a recently introduced Palmer amaranth accession in southern Wisconsin to postemergence (POST) and preemergence (PRE) herbicides commonly used in corn and soybean. Greenhouse experiments were conducted with the Wisconsin putative herbicide-resistant accession (BRO) and two additional control accessions from Nebraska, a glyphosate-resistant (KEI2) and a glyphosate-susceptible (KEI3) accession. POST treatments were 2,4-D, atrazine, dicamba, glufosinate, glyphosate, imazethapyr, lactofen, and mesotrione at 1X and 3X label rates. PRE treatments were atrazine, mesotrione, metribuzin, *S*-metolachlor, and sulfentrazone at 0.5X, 1X, and 3X label rates. Plant survival of each accession was ≥63% after exposure to imazethapyr POST 3X rate. Survival of BRO and KEI2 was 44% (±13) and 50% (±13), respectively, after exposure to atrazine POST 3X rate. Survival of BRO was 69% (±12) after exposure to glyphosate POST 1X rate, whereas survival of KEI2 was 44% (±13) after exposure to glyphosate POST 3X rate. After exposure to 2,4-D POST 1X rate, KEI2 and KEI3 survival was 38% (±13) and 50% (±13), respectively. Survival of all accessions was ≤31% after exposure to 2,4-D POST 3X rate or dicamba, glufosinate, lactofen, and mesotrione POST at either rate. Plant density reduction of KEI2 was 77% (±13) after exposure to atrazine PRE 1X rate, whereas density reduction of BRO was 56% (±13) after exposure to atrazine PRE 3X rate. Plant density reduction of all accessions was ≥94% after exposure to mesotrione PRE 1X and 3X rates or metribuzin, *S*-metolachlor, and sulfentrazone PRE at either rate. Our results suggest that each accession is resistant (≥50% survival) to imazethapyr POST, that BRO and KEI2 are resistant to atrazine and glyphosate POST, and that KEI2 and KEI3 are resistant to 2,4-D POST. The recently introduced BRO accession exhibited multiple resistance to imazethapyr, atrazine, and glyphosate POST. In addition, atrazine PRE was ineffective for BRO control, suggesting that diversified resistance management strategies will be critical for its effective management.

Introduction

Palmer amaranth is a C_4 annual plant species native to the Sonoran Desert in the southwestern United States and northern Mexico (Ehleringer 1983; Sauer 1957). Currently, in the United States, Palmer amaranth is ranked as one of the most common and most troublesome weed species among several crops, including corn, soybean, cotton (*Gossypium hirsutum* L.), peanuts (*Arachis hypogaea* L.), and sorghum [*Sorghum bicolor* (L.) Moench] (Van Wychen 2019, 2020). Crop–weed competition studies have shown that Palmer amaranth is highly competitive with both corn and soybean (Bensch et al. 2003; Massinga et al. 2001). Its competitive ability is attributed to several biological characteristics, including an extended period of emergence, aggressive growth rate, and high-water use efficiency (Ehleringer 1983; Horak and Loughin 2000; Keeley et al. 1987). Moreover, some reproductive characteristics, such as dioecious, prolific pollen, seed production and dispersal, and low rates of interspecific hybridization (Franssen et al. 2001; Gaines et al. 2012; Jhala et al. 2021; Sosnoskie et al. 2012; Walkington 1960), facilitate the adaptation of Palmer amaranth into new environments and might accelerate herbicide-resistance evolution (Tehranchian et al. 2017; Jhala et al. 2021).

Palmer amaranth dispersal has been attributed to natural and agricultural causes, including seed transport in waterfowl digestive tracts during migration (Farmer et al. 2017), water movement (Norsworthy et al. 2014), hurricanes (Menges 1987), use of weed-contaminated seeds for

© University of Wisconsin-Madison, 2022. This is a work of the US Government and is not subject to copyright protection within the United States. Published by Cambridge University Press on behalf of the Weed Science Society of America. This is an Open Access article, distributed under the terms of the Creative Commons Attribution licence (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted re-use, distribution and reproduction, provided the original article is properly cited.

Downloaded From: https://staging.bioone.org/journals/Weed-Technology on 02 May 2025
Terms of Use: https://staging.bioone.org/terms-of-use

the Conservation Reserve Program (CRP; Hartzler and Anderson 2016), animal feed contaminated with seeds and subsequent manure applications (Hartzler and Anderson 2016; Sprague 2014; Van de Stroet and Clay 2019; Yu et al. 2021), and movement of farm equipment (Hartzler and Anderson 2016; Sauer 1957; Werle et al. 2019). Given its nature, characteristics, and confirmed resistance to many herbicide sites of action (SOA), the continued dispersal of Palmer amaranth could impose detrimental impacts on cropping systems in Wisconsin and neighboring states. Currently, in the United States, Palmer amaranth has evolved resistance to nine herbicide SOAs: acetolactate synthase (ALS), microtubule assembly disruptors, auxin mimics (AM), photosynthesis at photosystem II–serine 264 binders (PSII), enolpyruvyl shikimate phosphate synthase (EPSPS), glutamine synthetase (GS), protoporphyrinogen oxidase (PPO), very long-chain fatty acid synthesis (VLCFA), and hydroxyphenyl pyruvate dioxygenase (HPPD) (Heap 2021). Moreover, a single Palmer amaranth accession has been documented to be resistant to five SOAs (Kumar et al. 2019).

In Wisconsin, Palmer amaranth was first identified in 2011 in Rock County (Zimbric et al. 2018). In the following years, Palmer amaranth presence has increased steadily (Renz 2018; Stoltenberg 2018), although it is not widespread in the state. To date, 12 Palmer amaranth points of infestation have been confirmed in nine counties in Wisconsin (Zimbric et al. 2018). An accession identified in Wisconsin by Davis and Recker (2014) was confirmed glyphosate-resistant (Butts and Davis 2015). Drewitz et al. (2016) then confirmed the first case of multiple herbicide resistance in a Palmer amaranth accession from Iowa County, WI, demonstrating high-level resistance to imazethapyr and low-level resistance to thifensulfuron and tembotrione. Currently Palmer amaranth herbicide resistance in Wisconsin has been confirmed for ALS-, EPSPS-, and HPPD-inhibitor herbicides.

The combination of effective postemergence (POST) and preemergence (PRE) herbicides, as part of integrated weed management (IWM), is important to delay herbicide-resistance evolution, to preserve the usefulness of newly developed herbicide-resistant crops, and for the long-term economic success and sustainability of agricultural production (Norsworthy et al. 2012). In 2018, the Wisconsin Cropping Systems Weed Science Program was contacted by agronomists expressing concern about a soybean field near Broadhead, WI, recently infested with an unknown *Amaranthus* weed species in that region. The agronomists suspected that this species may have been introduced from outside Wisconsin, as the field is located adjacent to a facility that processes food-grade soybean from different regions of the United States. After visiting the area, Palmer amaranth was identified, and seed samples were collected to conduct our investigation. Therefore our objective was to characterize the response of this recently introduced Palmer amaranth accession in southern Wisconsin to POST and PRE herbicides commonly used in corn and soybean. We hypothesized that ALS, EPSPS, and HPPD would be ineffective on this accession, whereas AM and inhibitors of PSII, GS, PPO, and VLCFA would be effective.

Materials and Methods

Seed Sources and Research Site

Three Palmer amaranth accessions were included in the experiments: a putative herbicide-resistant accession (BRO) identified near Broadhead, WI (42.6183°N, 89.3762°W) in 2018 and two control accessions from Nebraska, a glyphosate-resistant accession (KEI2) and a glyphosate-susceptible accession (KEI3), both from Keith County, NE (for complete information regarding the control accessions, see Oliveira et al. 2020). Seeds from the BRO accession were collected from a field cultivated with soybean, whereas the KEI2 and KEI3 accession was from a field cultivated with soybean and corn, respectively (Oliveira et al. 2020); herbicide use records of all accessions were not available. After collection from the field, seeds were threshed, cleaned, and stored at 5 C until the onset of the experiments, which were conducted at the University of Wisconsin–Madison Walnut Street Greenhouses (43.076194°N, 89.423611°W), Madison, WI.

Palmer Amaranth Response to POST Herbicides

The experiment was organized in a randomized complete block design (RCBD) with eight replications per treatment and repeated over time (two experimental runs). Treatments were arranged as 3 × 8 × 2 factorial consisting of three accessions (BRO, KEI2, and KEI3), eight herbicides (Table 1), and two herbicide rates (1X and 3X the recommended label rates). A nontreated control (NTC) of each accession was included.

Palmer amaranth seeds were planted at 1.5-cm depth in potting mix (PRO-MIX® HP MYCORRHIZAE™, Premier Tech Horticulture, Rivière-du-Loup, QC, Canada) in 23-cm-diameter disposable aluminum pans. Seedlings at the 2-true-leaf stage were transplanted into potting mix as described, contained in 656-mL pots (D40H Deepot™, Stuewe and Sons Inc., Tangent, OR, USA). The experimental unit was one seedling per pot. Postemergence herbicide treatments were applied when plants reached 5 to 10 cm in height (4- to 6-true-leaf stage) using a single-nozzle research track spray chamber (DeVries Manufacturing, Hollandale, MN, USA) equipped with a TP8002EVS nozzle (TeeJet® Technologies, Wheaton, IL, USA). Owing to vapor drift concerns within an enclosed environment (greenhouse) with the presence of several sensitive broadleaf species, the dicamba and 2,4-D herbicide treatments were applied at the University of Wisconsin–Madison Arlington Agricultural Research Station (43.302631°N, 89.345367°W). Palmer amaranth plants were transported to this field location on the morning of the application and returned to the greenhouse at the end of the day to allow for better herbicide absorption while minimizing potential unintended vapor drift issues. A CO_2-pressurized backpack spray boom with four TTI110015 nozzles (TeeJet® Technologies) was used for the application. A carrier volume of 140 L ha^{-1} was used in all applications (spray chamber and backpack). Plants were maintained in the greenhouse at 20 to 35 C with a natural ventilation system. Natural lighting was supplemented with 400 W high-pressure sodium lightbulbs simulating a 16-h photoperiod. The soil was watered daily and fertigated weekly with 20-10-20 water-soluble fertilizer (Peters® Professional, ICL Fertilizers, Dublin, OH, USA) delivering 500 ppm of both N and K and 250 ppm of P.

At 21 days after treatment (DAT), plant survival was assessed visually as dead (no green tissue; assessed value of 0) or alive (green tissue and evidence of regrowth; assessed value of 1; Figure 1). Accessions with ≥50% (± standard error) plant survival were classified as resistant to each herbicide × rate treatment (adapted from Schultz et al. 2015; Vennapusa et al. 2018). Aboveground biomass was harvested and force air-dried at 52 C to constant mass. The biomass data were converted into percent biomass reduction compared to the NTC using Equation 1 (adapted from Wortman 2014):

Downloaded From: https://staging.bioone.org/journals/Weed-Technology on 02 May 2025
Terms of Use: https://staging.bioone.org/terms-of-use

Table 1. Postemergence herbicide treatments used to evaluate the response of three Palmer amaranth accessions.[a]

Active ingredient	Trade name	Formulation	WSSA SOA[d]	Rate[b] Herbicide 1X	Rate[b] Herbicide 3X	Rate[b] Adjuvant[c] HSOC	Rate[b] Adjuvant[c] AMS	Herbicide manufacturer
				— g ai or ae ha^{-1} —		v/v %	g ha^{-1}	
Imazethapyr	Pursuit®	2 L	ALS (2)	72	216	0.63	2,352	BASF Corporation, Research Triangle Park, NC, USA
Dicamba	XtendiMax®	2.9 L	AM (4)	565	1,695	—	—	Monsanto Company, St. Louis, MO, USA
2,4-D	Enlist One™	3.8 L	AM (4)	800	2,400	—	—	Dow AgroSciences LLC, Indianapolis, IN, USA
Atrazine	Aatrex®	4 L	PSII (5)	2,242	6,726	0.83	—	Syngenta Crop Protection LLC, Greensboro, NC, USA
Glyphosate	Roundup PowerMAX®	4.5 L	EPSPS (9)	864	2,592	—	2,184	Monsanto Company, St. Louis, MO, USA
Glufosinate	Liberty®	280 SL	GS (10)	654	1,962	—	2,242	Bayer CropScience LP, Research Triangle Park, NC, USA
Lactofen	Cobra®	2 EC	PPO (14)	218	654	0.42	504	Valent USA Corporation, Walnut Creek, CA, USA
Mesotrione	Callisto®	4 SC	HPPD (27)	106	318	0.5	1,428	Syngenta Crop Protection LLC

[a]Abbreviations: L, liquid; SL, soluble liquid; EC, emulsifiable concentrate; SC, soluble concentrate; HSOC, high surfactant oil concentrate; AMS, ammonium sulfate.
[b]The 1X herbicide rate and adjuvant rate were based on the respective herbicide label crop use directions for POST application in corn or soybean and recommendations for controlling Palmer amaranth when specified.
[c]A dash indicates that adjuvant was not included.
[d]Weed Science Society of America (WSSA) herbicide site of action (SOA): ALS, acetolactate synthase (Group 2); AM, auxin mimics (Group 4); PSII, photosynthesis at photosystem II–serine 264 binders (Group 5); EPSPS, enolpyruvyl shikimate phosphate synthase (Group 9); GS, glutamine synthetase (Group 10); PPO, protoporphyrinogen oxidase (Group 14); HPPD, hydroxyphenyl pyruvate dioxygenase (Group 27).

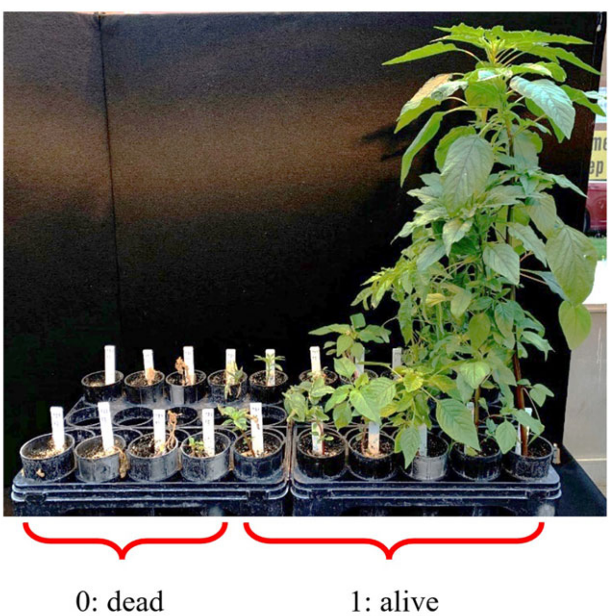

Figure 1. Plant survival rating used for herbicide resistance classification for Palmer amaranth response to POST herbicides.

$$\text{Biomass reduction}(\%) = \left(1 - \frac{\text{BEU}}{\overline{\text{BNTC}}}\right) \times 100 \qquad [1]$$

where BEU represents the biomass of the experimental unit and $\overline{\text{BNTC}}$ represents the biomass mean of the NTC for the respective accession. Seed production of survivor plants was not determined.

Palmer Amaranth Response to PRE Herbicides

The experiment was organized in a RCBD with four replications per treatment and repeated over time (two experimental runs). Treatments were arranged as $3 \times 5 \times 3$ factorial consisting of three accessions (BRO, KEI2, and KEI3), five herbicides (Table 2), and three herbicide rates (0.5X, 1X, and 3X the recommended label rate). A NTC of each accession was included.

Experimental units were approximately 130 seeds (measured by volume) planted 1.5 cm deep in 360-mL pots (8.9 cm Kord Traditional Square Pot, HC Companies, Twinsburg, OH, USA) filled with nonsterilized field soil (silt loam; 7.0 pH; 2.8% organic matter; 21% sand, 57% silt, 22% clay by weight). The soil was watered immediately after planting and before herbicide application to facilitate seed germination and herbicide activation. Preemergence herbicide treatments were applied using the spray chamber and carrier volume described earlier, equipped with a AI9502EVS nozzle (TeeJet® Technologies). Plants were maintained in a greenhouse under the same conditions described previously.

At 25 DAT, emerged plants per experimental unit were counted. The count data were converted into percent plant density reduction compared with the NTC using Equation 2 (adapted from Wortman 2014):

$$\text{Plant density reduction}(\%) = \left(1 - \frac{\overline{\text{PCEU}}}{\overline{\text{PCNTC}}}\right) \times 100 \qquad [2]$$

where $\overline{\text{PCEU}}$ represents the plant counts of the experimental unit and $\overline{\text{PCNTC}}$ represents the plant counts mean of the NTC for the respective accession.

Herbicide × rate treatments that provided <90% (± standard error) plant density reduction were classified as ineffective for each accession (adapted from Vennapusa et al. 2018).

Downloaded From: https://staging.bioone.org/journals/Weed-Technology on 02 May 2025
Terms of Use: https://staging.bioone.org/terms-of-use

Table 2. Preemergence herbicide treatments used to evaluate the response of three Palmer amaranth accessions.[a]

Active ingredient	Trade name	Formulation	WSSA SOA[c]	Herbicide rate[b] 0.5X	1X	3X	Herbicide manufacturer
				g ai ha^{-1}			
Atrazine	Aatrex®	4 L	PSII (5)	1,121	2,242	6,726	Syngenta Crop Protection LLC, Greensboro, NC, USA
Metribuzin	Tricor®	75 DF	PSII (5)	262	525	1,575	United Phosphorus Inc., King of Prussia, PA, USA
Sulfentrazone	Spartan®	4 F	PPO (14)	140	280	840	FMC Corporation, Philadelphia, PA, USA
S-metolachlor	Dual II Magnum®	7.64 EC	VLCFA (15)	892	1,785	5,355	Syngenta Crop Protection LLC
Mesotrione	Callisto®	4 SC	HPPD (27)	135	270	810	Syngenta Crop Protection LLC

[a]Abbreviations: L, liquid; DF, dry flowable; F, flowable; EC, emulsifiable concentrate; SC, soluble concentrate.
[b]The 1X herbicide rate was based on the respective herbicide label crop use directions for PRE application in corn or soybean on medium not highly erodible soils with 2.8% organic matter and on recommendations for controlling Palmer amaranth when specified.
[c]Weed Science Society of America (WSSA) herbicide site of action (SOA): PSII, photosynthesis at photosystem II–serine 264 binders (Group 5); PPO, protoporphyrinogen oxidase (Group 14); VLCFA, very long-chain fatty acid synthesis (Group 15); HPPD, hydroxyphenyl pyruvate dioxygenase (Group 27).

Statistical Analyses

A generalized linear mixed model with Gaussian distribution was fitted to the biomass reduction data (POST) and plant density reduction data (PRE) using the GLMMTMB package version 1.0.2.1 (Brooks et al. 2017). Analysis of variance (ANOVA) type II Wald chi-square was performed followed by Tukey's honestly significant difference ($\alpha = 0.05$) pairwise comparisons using the EMMEANS package version 1.5.4 (Lenth 2020). Both response variables were logit-transformed to improve normality assumptions (Barnes et al. 2020; Davies et al. 2019, 2020; Striegel et al. 2020; Warton and Hui 2011). Back-transformed means are presented. Accession, herbicide, and rate were considered as fixed effects, whereas experimental run was considered as a random effect. Statistical analyses were performed using R version 4.0.3 (R Core Team 2020).

Results and Discussion

Palmer Amaranth Response to POST Herbicides

Plant survival of each accession was ≥63% after exposure to imazethapyr POST 3X rate (Figure 2). Survival of BRO and KEI2 was 44% (±13) and 50% (±13), respectively, after exposure to atrazine POST 3X rate. Survival of BRO was 69% (±12) after exposure to glyphosate POST 1X rate, whereas survival of KEI2 was 44% (±13) after exposure to glyphosate POST 3X rate. After exposure to 2,4-D POST 1X rate, KEI2 and KEI3 survival was 38% (±13) and 50% (±13), respectively. Survival of all accessions was ≤31% after exposure to 2,4-D POST 3X rate or dicamba, glufosinate, lactofen, and mesotrione POST at either rate evaluated in this study. No plants of any accession survived exposure to glufosinate at either rate. Our glyphosate results for KEI2 and KEI3 accessions corroborate the findings of Oliveira et al. (2020), who reported these accessions as glyphosate-resistant and glyphosate-susceptible, respectively.

The ANOVA exhibited a significant three-way interaction among accession, herbicide, and rate for biomass reduction (P value ≤ 0.0001). For imazethapyr 1X rate, the biomass reduction did not differ between KEI3 (67%) and KEI2 (50%) nor between KEI2 and BRO (30%; Figure 3). For imazethapyr 3X rate, the biomass reduction did not differ between KEI2 (88%) and KEI3 (81%), which was greater than for BRO (43%). For glyphosate 1X rate, the biomass reduction was greater for KEI3 (98%) than for BRO (65%), which was greater than it was for KEI2 (33%). The biomass reduction did not differ among accessions for glyphosate 3X rate (each ≥ 96%). For atrazine 1X rate, biomass reduction was greater for KEI3 (97%) than for BRO (89%) and KEI2 (80%), which did not differ. For atrazine 3X rate, biomass reduction did not differ between KEI3 (97%) and BRO (95%) but was greater than it was for KEI2 (88%). The biomass reduction did not differ among accessions for 2,4-D, dicamba, glufosinate, lactofen, and mesotrione at either rate (≥91%).

The reduced performance of imazethapyr and glyphosate POST on the three Palmer amaranth accessions evaluated in our study is consistent with previous findings (Chahal et al. 2017; Drewitz et al. 2016; Kumar et al. 2020; Norsworthy et al. 2008; Oliveira et al. 2020; Schwartz-Lazaro et al. 2017). The adoption of genetically modified herbicide-resistant crops substantially reduced herbicide SOA diversity in cotton and soybean cropping systems in past decades (Kniss 2018), and the overreliance on a single herbicide, such as glyphosate, contributed to rapid resistance evolution (Culpepper et al. 2006; Legleiter and Bradley 2008; Norsworthy et al. 2012; VanGessel 2001). Recently, field escapes and greenhouse screenings have identified Palmer amaranth accessions resistant to dicamba and glufosinate in TN and AR, respectively (Barber et al. 2021; Steckel 2020), threatening the sustainability of recently introduced herbicide-tolerant soybean traits in the market. Additionally, several Palmer amaranth accessions have been confirmed resistant to multiple SOAs (Kohrt et al. 2017; Kumar et al. 2018; Schwartz-Lazaro et al. 2017), with one known accession confirmed resistant to five SOAs: ALS, PSII, AM, EPSPS, and HPPD (Kumar et al. 2019).

Palmer Amaranth Response to PRE Herbicides

Plant density reduction of KEI2 was 77% (±13) after exposure to atrazine PRE 1X rate, whereas density reduction of BRO was 56% (±13) after exposure to atrazine PRE 3X rate (Figure 4). After exposure to mesotrione PRE 0.5X rate, BRO and KEI plant density reduction was 83% (±8) and 83% (±12), respectively. Plant density reduction of all accessions was ≥94% after exposure to mesotrione PRE 1X and 3X rates or metribuzin, *S*-metolachlor, and sulfentrazone PRE at either rate evaluated in this study.

The three-way interaction among accession, herbicide, and rate was not significant for plant density reduction (P value = 0.75). The ANOVA exhibited a significant two-way interaction between accession and herbicide for plant density reduction (P value < 0.0001). For atrazine, plant density reduction was greater for KEI3 (95%) than it was for KEI 2 (83%), which were greater than it was for BRO (34%; Figure 5). Plant density reduction did not differ among accessions for mesotrione, metribuzin, *S*-metolachlor, or sulfentrazone (≥95%). Comparing atrazine and metribuzin, both PSII inhibitors but from different chemical families

Downloaded From: https://staging.bioone.org/journals/Weed-Technology on 02 May 2025
Terms of Use: https://staging.bioone.org/terms-of-use

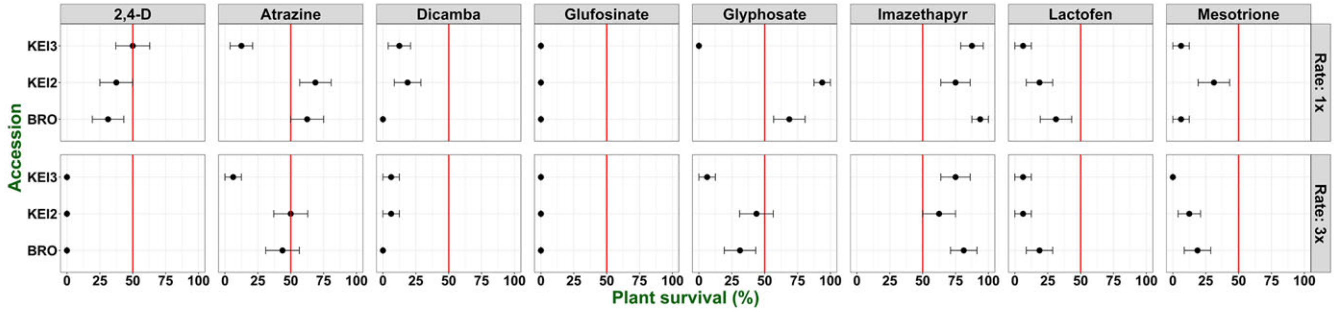

Figure 2. Palmer amaranth plant survival (± standard error) of accessions from Wisconsin (BRO) and Nebraska (KEI2 and KEI 3) in response to POST herbicides. Accessions with survival ≥50% (represented by the red line) were classified as ineffectively controlled by each herbicide × rate treatment.

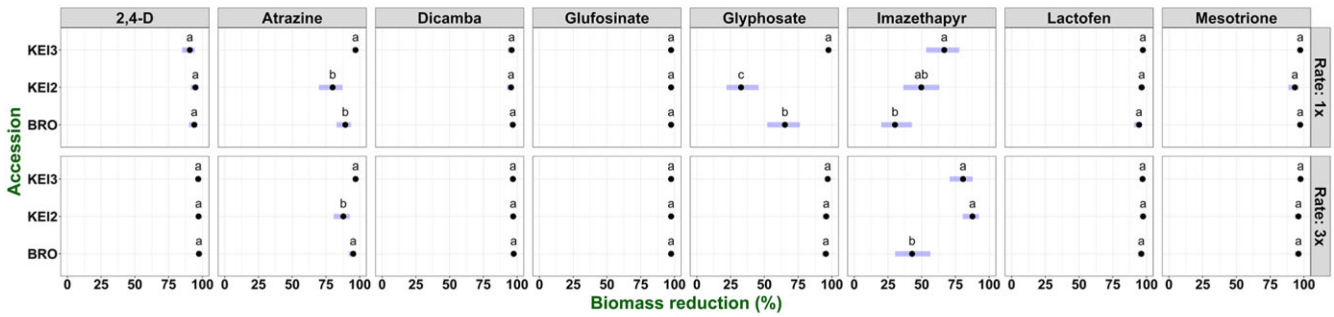

Figure 3. Palmer amaranth biomass reduction of accessions from Wisconsin (BRO) and Nebraska (KEI2 and KEI 3) represented by the three-way interaction among accession, POST herbicide, and rate. The blue boxes represent the 95% confidence intervals. Treatments with the same letters did not differ according to Tukey's honestly significant difference, α = 0.05.

Figure 4. Palmer amaranth plant density reduction (± standard error) of accessions from Wisconsin (BRO) and Nebraska (KEI2 and KEI 3) in response to PRE herbicides. Treatments with plant density reduction <90% (represented by the red line) were classified as ineffective.

(triazine and triazinone, respectively), we observed different responses when applied PRE. Similarly, Vennapusa et al. (2018) reported higher efficacy of metribuzin than atrazine for control of waterhemp [*Amaranthus tuberculatus* (Moq.) J. D. Sauer] accessions from Nebraska, both applied PRE. In contrast, Schwartz-Lazaro et al. (2017) reported higher mortality of Palmer amaranth accessions from Arizona with atrazine compared to metribuzin, both applied PRE. Additionally, Fuerst et al. (1986) observed cross-resistance between atrazine and metribuzin applied PRE to smooth pigweed (*Amaranthus hybridus* L.).

The ANOVA also exhibited a significant two-way interaction between herbicide and rate for plant density reduction (P value = 0.0001). At the 0.5X rate, plant density reduction for sulfentrazone did not differ compared to *S*-metolachlor and metribuzin (each ≥95%) and was greater than for mesotrione (92%) and atrazine (52%; Figure 6). At the 1X and 3X rates, plant density reductions for sulfentrazone, *S*-metolachlor, metribuzin, and mesotrione (each ≥97%) were greater than it was for atrazine (≤90%). The use of reduced PRE herbicide rates as an attempt to reduce costs, herbicide carryover, and/or environmental impacts may increase the selection pressure and lead to rapid herbicide-resistance evolution (Belz 2020; Manalil et al. 2011; Maxwell and Mortimer 1994; Norsworthy 2012; Tehranchian et al. 2017; Vieira et al. 2020). Our results suggest

Downloaded From: https://staging.bioone.org/journals/Weed-Technology on 02 May 2025
Terms of Use: https://staging.bioone.org/terms-of-use

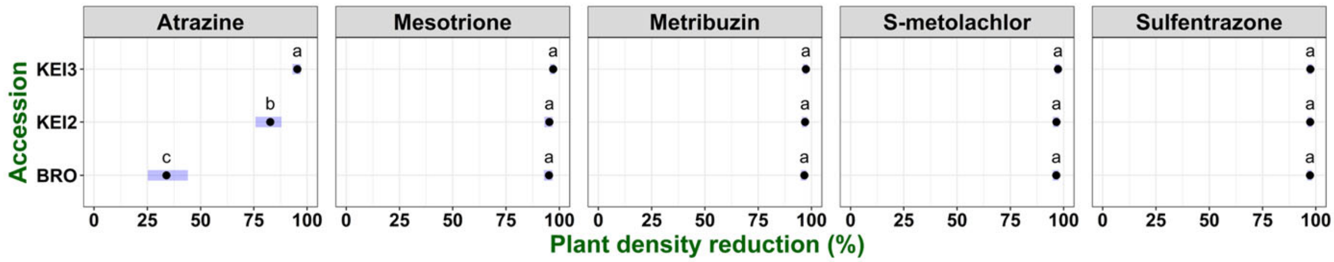

Figure 5. Palmer amaranth plant density reduction of accessions from Wisconsin (BRO) and Nebraska (KEI2 and KEI 3) represented by the two-way interaction between accession and PRE herbicide. The blue boxes represent the 95% confidence intervals. Treatments with the same letters did not differ according to Tukey's honestly significant difference, $\alpha = 0.05$.

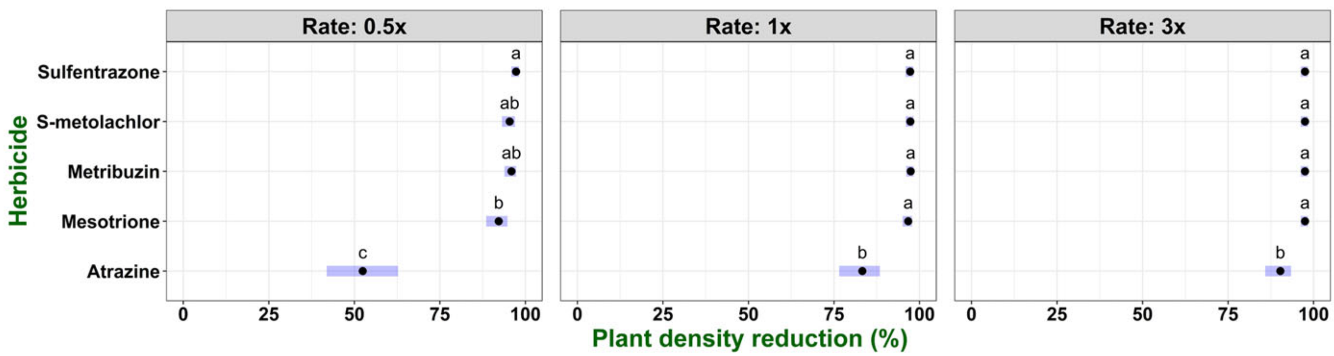

Figure 6. Palmer amaranth plant density reduction represented by the two-way interaction between PRE herbicide and rate. The blue boxes represent the 95% confidence intervals. Treatments with the same letters did not differ according to Tukey's honestly significant difference, $\alpha = 0.05$.

that herbicides applied PRE at the 0.5X label rate may provide reduced Palmer amaranth control, particularly for atrazine and mesotrione. Consequently, the reliance on herbicides applied POST may increase, and in the end, the short-term economic benefits associated with using reduced herbicide rates are quickly outweighed by the future costs related to herbicide-resistance evolution and spread (Gressel 1997).

The Concerns of Palmer Amaranth Introduction in Wisconsin

The indication that this recently introduced Palmer amaranth accession (BRO) in Wisconsin is likely to carry multiple herbicide-resistance traits is cause for great concern. The most notable source of new Palmer amaranth infestations in Iowa, Ohio, Illinois, and Minnesota was credited to the use of Palmer amaranth–contaminated seeds for CRP (Hartzler and Anderson 2016; Yu et al. 2021). The 2021 State-Noxious-Weed Seed Requirements Recognized in the Administration of the Federal Seed Act (USDA 2021) designates Palmer amaranth as a prohibited noxious weed seed in Wisconsin, prohibiting the sale of agricultural seeds contaminated with Palmer amaranth seed. Similarly, Iowa and Minnesota designate Palmer amaranth as a noxious weed, whereas Illinois and Michigan do not. Minnesota went beyond and now requires a genetic test of any *Amaranthus* contaminant to determine if Palmer amaranth is present in agricultural seeds (USDA 2021; Yu et al. 2021).

Animal feed contaminated with Palmer amaranth seeds and subsequent manure applications have been reported as a possible cause of Palmer amaranth spread. In 2018, the Minnesota Department of Agriculture identified animal feed and manure as pathways for the introduction of Palmer amaranth in the state, after contaminated sunflower feed was used for cattle (Yu et al. 2021). Whole cottonseed is another example of a low-cost by-product with good nutritional value commonly used in dairy diets (Warner et al. 2020). If not properly monitored, it may become a pathway for Palmer amaranth introduction in new areas, particularly because the Cotton Belt is one of the areas in the United States most harshly affected by Palmer amaranth (Norsworthy et al. 2014; Ward et al. 2013; Webster and Nichols 2012). Kellog et al. (2001) reported that from 133 dairy farms surveyed across the United States, 71% used whole cottonseed as a feed source, with the greatest use in the western United States. The 2017 to 2018 Wisconsin Statutes and Annotations, in chapter 94.72, "Commercial Feed" (Wisconsin Statutes 2020), do not list Palmer amaranth as a noxious weed seed in commercial feed, which is cause for concern. More research is needed to evaluate the impact of animal feed sources on dispersal of noxious weed seeds in Wisconsin, the second-largest dairy state in the United States, with a production of 13.88 million tons of milk in 2018 and a herd size of 1.28 million cows distributed among approximately 9,037 farms (USDA 2020).

In conclusion, our results suggest that each accession is resistant (≥50% survival) to imazethapyr POST, that BRO and KEI2 accessions are resistant to atrazine and glyphosate POST, and that KEI2 and KEI3 are resistant to 2,4-D POST. In contrast, each accession was susceptible (<50% survival) to dicamba, glufosinate, lactofen, and mesotrione POST. The recently introduced BRO accession exhibited multiple resistance to imazethapyr, atrazine, and glyphosate POST. In addition, atrazine PRE was ineffective (<90% plant density reduction) for BRO control. Metribuzin, sulfentrazone, *S*-metolachlor, and mesotrione PRE effectively controlled (≥90% plant density reduction) each accession at 1X and 3X rates. Atrazine and mesotrione PRE at 0.5X rate provided reduced Palmer amaranth control and may impose selection pressure on POST herbicides. Community efforts, training, economic

Downloaded From: https://staging.bioone.org/journals/Weed-Technology on 02 May 2025
Terms of Use: https://staging.bioone.org/terms-of-use

incentives, policies, and proactive scouting to prevent new Palmer amaranth infestations, which, according to our findings, are likely to carry herbicide resistance, and the use of effective PRE and POST herbicides as part of an IWM are vital for Palmer amaranth management in Wisconsin.

Acknowledgments. The authors thank the Wisconsin Soybean Marketing Board for funding FF's graduate research assistantship and the University of Wisconsin–Madison Cropping Systems Weed Science Program for its technical assistance with the greenhouse experiments. No conflicts of interest have been declared.

References

Barber T, Norsworthy J, Butts T (2021) Arkansas Palmer amaranth found resistant to field rates of glufosinate. Row Crops Blog, University of Arkansas. https://arkansascrops.uaex.edu/posts/weeds/palmer-amaranth.aspx. Accessed: February 19, 2021

Barnes ER, Knezevic SZ, Lawrence NC, Irmak S, Rodriguez O, Jhala AJ (2020) Control of velvetleaf (*Abutilon theophrasti*) at two heights with POST herbicides in Nebraska popcorn. Weed Technol 34:560–567

Belz RG (2020) Low herbicide doses can change the responses of weeds to subsequent treatments in the next generation: metamitron exposed PSII-target-site resistant *Chenopodium album* as a case study. Pest Manag Sci 76:3056–3065

Bensch CN, Horak MJ, Peterson D (2003) Interference of redroot pigweed (*Amaranthus retroflexus*), Palmer amaranth (*A. palmeri*), and common waterhemp (*A. rudis*) in soybean. Weed Sci 51:37–43

Brooks ME, Kristensen K, Van Bethem KJ, Magnusson A, Berg CW, Nielsen A, Skaug HJ, Marchler M, Bolker BM (2017) glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. R J 9:378–400

Butts TR, Davis VM (2015) Palmer amaranth (*Amaranthus palmeri*) confirmed glyphosate-resistant in Dane County, Wisconsin. University of Wisconsin–Madison Crop Weed Science Blog. https://wcws.cals.wisc.edu/documents/. Accessed: April 17, 2020

Chahal PS, Varanasi VK, Jugulam M, Jhala AJ (2017) Glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) in Nebraska: confirmation, EPSPS gene amplification, and response to POST corn and soybean herbicides. Weed Technol 31:80–93

Culpepper AS, Grey TL, Vencill WK, Kichler JM, Webster TM, Brown SM, York AC, Davis JW, Hanna WW (2006) Glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) confirmed in Georgia. Weed Sci 54:620–626

Davies LR, Hull R, Moss S, Neve P (2019) The first cases of evolving glyphosate resistance in UK poverty brome (*Bromus sterilis*) populations. Weed Sci 67:41–47

Davies LR, Onkokesung N, Brazier-Hicks M, Edwards R, Moss S (2020) Detection and characterization of resistance to acetolactate synthase inhibiting herbicides in *Anisantha* and *Bromus* species in the United Kingdom. Pest Manag Sci 76:2473–2482

Davis VM, Recker RA (2014) Palmer amaranth identified through the late-season weed scape survey. University of Wisconsin–Madison Crop Weed Science Blog. https://wcws.cals.wisc.edu/2014/01/15/palmer-amaranth-identified-through-the-late-season-weed-escape-survey/. Accessed: March 29, 2021

Drewitz N, Hammer D, Conley S, Stoltenberg D (2016) Multiple resistance to ALS- and HPPD-inhibiting herbicides in Palmer amaranth from Iowa County, Wisconsin. University of Wisconsin–Madison Integrated Pest and Crop Management Blog. https://ipcm.wisc.edu/blog/2016/10/multiple-resistance-to-als-and-hppd-inhibiting-herbicides-in-palmer-amaranth-from-iowa-county-wisconsin/. Accessed: April 17, 2020

Ehleringer J (1983) Ecophysiology of *Amaranthus palmeri*, a Sonoran Desert summer annual. Oecologia 57:10–112

Farmer JA, Webb EB, Pierce RA II, Bradley KW (2017) Evaluating the potential for weed seed dispersal based on waterfowl consumption and seed viability. Pest Manag Sci 73:2592–2603

Franssen AS, Skinner DZ, Al-Khatib K, Horak MJ, Kulakow PA (2001) Interspecific hybridization and gene flow of ALS resistance in *Amaranthus* species. Weed Sci 49:598–606

Fuerst EP, Arntzen CJ, Pfister K, Penner D (1986) Herbicide cross-resistance in triazine-resistant biotypes of four species. Weed Sci 34:344–353

Gaines TA, Ward SM, Bekun B, Preston C, Leach JE, Westra P (2012) Interspecific hybridization transfers a previously unknown glyphosate resistance mechanism in *Amaranthus* species. Evol Appl 5:29–38

Gressel J (1997) Burgeoning resistance requires new strategies. Pages 3–14 *in* De Prado R, Jorrín J, García-Torres L, eds. Weed and Crop Resistance to Herbicides. Dordrecht, Netherlands: Springer

Hartzler B, Anderson M (2016) Palmer amaranth: it's here, now what? Proceedings of the Integrated Crop Management Conference. Ames, IA, December 1, 2016

Heap I (2021) The International Herbicide-Resistant Weed Database. http://www.weedscience.org/. Accessed: October 23, 2021

Horak MJ, Loughin TM (2000) Growth analysis of four *Amaranthus* species. Weed Sci 48:347–355

Jhala AJ, Norsworthy JK, Ganie ZA, Sosnoskie LM, Beckie HJ, Mallory-Smith CA, Liu J, Wei W, Wang J, Stoltenberg DE (2021) Pollen-mediated gene flow and transfer of resistance alleles from herbicide-resistant broadleaf weeds. Weed Technol 35:173–187

Keeley PE, Carter CH, Thullen RJ (1987) Influence of planting date on growth of Palmer amaranth (*Amaranthus palmeri*). Weed Sci 35:199–204

Kellog DW, Pennington JA, Johnson ZB, Panivivat R (2001) Survey of management practices used for the highest producing DHI herds in the United States. J Dairy Sci 84:E120–E127

Kniss AR (2018) Genetically engineered herbicide-resistant crops and herbicide-resistant weed evolution in the United States. Weed Sci 66:260–273

Kohrt JR, Sprague CL, Nadakuduti SS, Douches D (2017) Confirmation of a three-way (glyphosate, ALS, and atrazine) herbicide-resistant population of Palmer amaranth (*Amaranthus palmeri*) in Michigan. Weed Sci 65:327–338

Kumar V, Liu R, Boyer G, Stahlman PW (2019) Confirmation of 2,4-D resistance and identification of multiple resistance in Kansas Palmer amaranth (*Amaranthus palmeri*) population. Pest Manag Sci 75:2925–2933

Kumar V, Liu R, Stahlman PW (2020) Differential sensitivity of Kansas Palmer amaranth populations to multiple herbicides. Agron J 112:2152–2163

Kumar V, Stahlman PW, Boyer G (2018) Palmer amaranth populations from Kansas with multiple resistance to glyphosate, chlorsulfuron, mesotrione, and atrazine. Kansas Agric Exp Station Res Rep 4(7)

Legleiter TR, Bradley KW (2008) Glyphosate and multiple herbicide resistance in common waterhemp (*Amaranthus rudis*) populations from Missouri. Weed Sci 56:582–587

Lenth R (2020) emmeans: estimated marginal means, aka least-square means. R package version 1.4.5. https://CRAN.R-project.org/package=emmeans. Accessed: May 10, 2022

Manalil S, Busi R, Renton M, Powles SB (2011) Rapid evolution of herbicide resistance by low herbicide dosages. Weed Sci 59:210–217

Massinga RA, Currie RS, Horak MJ, Boyer J (2001) Interference of Palmer amaranth in corn. Weed Sci 49:202–208

Maxwell BD, Mortimer AM (1994) Selection for herbicide resistance. Pages 1–25 *in* Powles SB, Holtum JAM, eds. Herbicide Resistance in Plants: Biology and Biochemistry. 2nd ed. Boca Raton, FL: CRC Press

Menges RM (1987) Weed seed population dynamics during six years of weed management systems in crop rotations on irrigated soil. Weed Sci 35:328–332

Norsworthy JK (2012) Repeated sublethal rates of glyphosate lead to decreased sensitivity in Palmer amaranth. Crop Manag 11:1–6

Norsworthy JK, Griffith G, Griffin T, Bagavathiannan M, Gbur EE (2014) In-field movement of glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) and its impact on cotton lint yield: evidence supporting a zero-threshold strategy. Weed Sci 62:237–249

Norsworthy JK, Griffith GM, Scott RC, Smith KL, Oliver LR (2008) Confirmation and control of glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) in Arkansas. Weed Technol 22:108–113

Norsworthy JK, Ward SM, Shaw DR, Llewellyn RS, Nichols RL, Webster TM, Bradley KW, Frisvold G, Powles SB, Burgos NR, Witt WW, Barret M (2012)

Downloaded From: https://staging.bioone.org/journals/Weed-Technology on 02 May 2025
Terms of Use: https://staging.bioone.org/terms-of-use

Reducing the risks of herbicide resistance: best management practices and recommendations. Weed Sci 60:31–62

Oliveira MC, Giacomini DA, Arsenijevic N, Vieira G, Tranel PJ, Werle R (2020) Distribution and validation of genotypic and phenotypic glyphosate and PPO-inhibitor resistance in Palmer amaranth (*Amaranthus palmeri*) from southwestern Nebraska. Weed Technol 35:65–76

R Core Team (2020) R: a language and environment for statistical computing. R Foundation for Statistical Computing. https://www.R-project.org/. Accessed: May 10, 2022

Renz M (2018) Update on waterhemp and Palmer amaranth in Wisconsin. University of Wisconsin–Madison Integrated Pest and Crop Management Blog. https://ipcm.wisc.edu/blog/2018/08/update-on-waterhemp-and-palmer-amaranth-in-wisconsin/. Accessed: July 27, 2020

Sauer J (1957) Recent migration and evolution of the dioecious amaranths. Evolution 11:11–31

Schultz JL, Chatham LA, Riggins CW, Tranel PJ, Bradley KW (2015) Distribution of herbicide resistances and molecular mechanisms conferring resistance in Missouri waterhemp (*Amaranthus rudis* Sauer) populations. Weed Sci 63:336–345

Schwartz-Lazaro LM, Norsworthy JK, Scott RC, Barber LT (2017) Resistance of two Arkansas Palmer amaranth populations to multiple herbicide sites of action. Crop Prot 96:158–163

Sosnoskie LM, Webster TM, MacRae AW, Grey TL, Culpepper AS (2012) Pollen-mediated dispersal of glyphosate-resistance in Palmer amaranth under field conditions. Weed Sci 60:366–373

Sprague C (2014) Palmer amaranth: managing this new weed problem. https://www.progressiveforage.com/forage-production/management/palmer-amaranth-managing-this-new-weed-problem. Accessed: April 17, 2020

Steckel LE (2020) Dicamba-resistant Palmer amaranth in Tennessee: stewardship even more important. University of Tennessee Crops New Blog. https://news.utcrops.com/2020/07/dicamba-resistant-palmer-amaranth-in-tennessee-stewardship-even-more-important/. Accessed: February 19, 2021

Stoltenberg DE (2018) Current state of herbicide resistance in Wisconsin. Proceedings of the 2018 Wisconsin Agribusiness Classic. Madison, WI, January 9–11, 2018

Striegel A, Eskridge KM, Lawrence NC, Knezevic SZ, Kruger GR, Proctor CA, Hein GL, Jhala AJ (2020) Economics of herbicide programs for weed control in conventional, glufosinate-, and dicamba/glyphosate-resistant soybean across Nebraska. Agron J 112:5158–5179

Tehranchian P, Norsworthy JK, Powles S, Bararpour MT, Bagavathiannan MV, Barber T, Scott RC (2017) Recurrent sublethal-dose selection for reduced susceptibility of Palmer amaranth (*Amaranthus palmeri*) to dicamba. Weed Sci 65:206–212

[USDA] U.S. Department of Agriculture (2020) Consolidation in U.S. Dairy Farming. USDA ERR-274. Washington, DC: USDA. https://www.ers.usda.gov/webdocs/publications/98901/err-274.pdf. Accessed: May 10, 2022

[USDA] U.S. Department of Agriculture (2021) State-Noxious-Weed Seed Requirements Recognized in the Administration of the Federal Seed Act. Washington, DC: USDA. https://www.ams.usda.gov/sites/default/files/media/StateNoxiousWeedsSeedList.pdf. Accessed: May 10, 2022

Van De Stroet B, Clay SA (2019) Management considerations for Palmer amaranth in a northern great plains soybean production system. Agrosyst Geosci Environ 2:1–9

VanGessel MJ (2001) Glyphosate-resistant horseweed in Delaware. Weed Sci 49:703705

Van Wychen L (2019) Survey of the most common and troublesome weeds in broadleaf crops, fruits and vegetables in the United States and Canada. Weed Science Society of America National Weed Survey Dataset. https://wssa.net/wp-content/uploads/2019-Weed-Survey_broadleaf-crops.xlsx. Accessed: May 10, 2022

Van Wychen L (2020) Survey of the most common and troublesome weeds in grass crops, pasture, and turf in the United States and Canada. Weed Science Society of America National Weed Survey Dataset. https://wssa.net/wp-content/uploads/2020-Weed-Survey_grass-crops.xlsx. Accessed: May 10, 2022

Vennapusa AR, Faleco F, Vieira B, Samuelson S, Kruger GR, Werle R, Jugulam M (2018) Prevalence and mechanism of atrazine resistance in waterhemp (*Amaranthus tuberculatus*) from Nebraska. Weed Sci 66:595–602

Vieira BC, Luck JD, Amundsen KL, Werle R, Gaines TA, Kruger GR (2020) Herbicide drift exposure leads to reduced herbicide sensitivity in *Amaranthus* spp. Sci Rep 10:2146

Walkington DL (1960) A survey of the hay fever plants and important atmospheric allergens in the Phoenix, Arizona, metropolitan area. J Allergy 31:25–41

Ward SM, Webster TM, Steckel LE (2013) Palmer amaranth (*Amaranthus palmeri*): a review. Weed Technol 27:12–27

Warner AL, Beck PA, Foote AP, Pierce KN, Robison CA, Hubbell DS, Wilson BK (2020) Effects of utilizing cotton byproducts in a finishing diet on beef cattle performance, carcass traits, fecal characteristics, and plasma metabolites. J Anim Sci 98:1–9

Warton DI, Hui FKC (2011) The arcsine is asinine: the analysis of proportions in ecology. Ecology 92:3–10

Webster TM, Nichols RL (2012) Changes in the prevalence of weed species in the major agronomic crops of the southern United States: 1994/1995 to 2008/2009. Weed Sci 60:145–157

Werle R, Arneson N, Smith D (2019) WiscWeeds research coalition combine weed seed collection project. University of Wisconsin–Madison Weed Science Blog. http://www.wiscweeds.info/post/2019-wiscweeds-research-coalition-combine-weed-seed-collection-project/. Accessed: April 17, 2020

Wisconsin Statutes (2020) Commerical feed. Chapter 94.72 *in* The 2017–18 Wisconsin Statutes and Annotations. https://docs.legis.wisconsin.gov/statutes/statutes/94.pdf#page=28. Accessed: May 10, 2022

Wortman SE (2014) Integrating weed and vegetable crop management with multifunctional air-propelled abrasive grits. Weed Technol 28:243–252

Yu E, Blair S, Hardel M, Chandler M, Thiede D, Cortilet A, Gonsulus J, Becker R (2021) Timeline of Palmer amaranth (*Amaranthus palmeri*) invasion and eradication in Minnesota. Weed Technol, June 21

Zimbric JW, Stoltenberg DE, Renz M, Werle R (2018) Herbicide resistance in Wisconsin: an overview. Proceedings of the 73rd Annual Meeting of the North Central Weed Science Society. Milwaukee, WI, December 3–6, 2018

Downloaded From: https://staging.bioone.org/journals/Weed-Technology on 02 May 2025
Terms of Use: https://staging.bioone.org/terms-of-use