Weed Technology

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Susceptibility of Arkansas Palmer amaranth accessions to common herbicide sites of action

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Note

Cite this article: González-Torralva F, Norsworthy JK, Piveta LB, Varanasi VK, Barber T, Brabham C (2020) Susceptibility of Arkansas Palmer amaranth accessions to common herbicide sites of action. Weed Technol. **34**: 770–775. doi: 10.1017/wet.2020.56

Received: 13 February 2020
Revised: 20 April 2020
Accepted: 15 May 2020
First published online: 26 May 2020

Associate Editor:
Amit Jhala, University of Nebraska, Lincoln

Keywords:
herbicide screening; herbicide resistance; multiple herbicide resistance

Nomenclature:
atrazine; dicamba; fomesafen; glufosinate; glyphosate; imazethapyr; *S*-metolachlor; tembotrione; trifluralin; Palmer amaranth; *Amaranthus palmeri* (S.) Watson

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Abstract

Palmer amaranth is one of the most difficult-to-control weeds in row crop systems and has evolved resistance to several herbicide sites of action (SOAs). A late-season weed-escape survey had been conducted earlier to determine the distribution of protoporphyrinogen oxidase–inhibitor resistant Palmer Amaranth in Arkansas. The objective of this study was to evaluate the susceptibility of Arkansas Palmer amaranth accessions to commonly used herbicide SOAs. The SOAs evaluated were group 2 + 9, 3, 4, 5, 10, 14, 15, and 27, and the representative herbicide from each group was imazethapyr + glyphosate (79 + 860 g ha^{-1}), trifluralin (1,120 g ha^{-1}), dicamba (280 and 560 g ha^{-1}), atrazine (560 g ha^{-1}), glufosinate (594 g ha^{-1}), fomesafen (395 g ha^{-1}), *S*-metolachlor (1,064 g ha^{-1}), and tembotrione (92 g ha^{-1}), respectively. Palmer amaranth mortality varied among accessions across SOAs. Averaged across accessions, the mortality rates, by treatment in order from lowest to highest, were as follows: glyphosate + imazethapyr (16%), tembotrione (51%), dicamba at 280 g ha^{-1} (51%), fomesafen (76%), dicamba at 560 g ha^{-1} (82%), atrazine (85%), trifluralin (87%), *S*-metolachlor (96%), and glufosinate (99.5%). This study provides evidence that Palmer amaranth accessions with low susceptibility to glyphosate + imazethapyr, fomesafen, and tembotrione are widespread throughout Arkansas. Of the remaining SOAs, most Palmer amaranth accessions were sensitive; however, within each herbicide SOA, except glufosinate, control of some accessions was less than expected and resistance is suspected.

Introduction

The evolution and spread of herbicide-resistant weeds are partially driven by herbicide use pattern (Kniss 2018). This can be exemplified by the change in soybean [*Glycine max* (L.) Merr.] herbicide use patterns in the southern United States for control of herbicide-resistant Palmer amaranth. Before the development of glyphosate-resistant crops, acetolactate synthase (ALS; Group 2) and microtubule polymerization-inhibiting (MT; Group 3) herbicides were the primary site of action (SOA) used to control Palmer amaranth (Gossett et al. 1992; Kniss 2018; Webster and Coble 1997). By 2000, glyphosate (Group 9) became the dominant herbicide used, mainly because of its simplicity and effectiveness in glyphosate-resistant crops and the prevalence of ALS- and MT-resistant Palmer amaranth (Dill et al. 2008). Glyphosate-resistant Palmer amaranth was initially confirmed in 2005, and by 2009, nearly all soybean-producing states in the southern United States were infested with glyphosate-resistant accessions (Heap 2020). Subsequently, protoporphyrinogen oxidase (PPO)-inhibiting herbicides (Group 14) became a common PRE and POST option for Palmer amaranth control (Riar et al. 2013), which eventually lead to PPO resistance in Palmer amaranth being confirmed in the mid-southern United States (Copeland et al. 2018; Varanasi et al. 2018).

Herbicide resistance is often a chronic trait, even in the absence of selection; thus, it is not surprising that reports of Palmer amaranth resistant to multiple SOAs are frequent. In response, industry has developed new herbicide-resistant traits to enable the use of alternative SOAs for control of herbicide-resistant Palmer amaranth and other troublesome weeds. Enlist™ (Pioneer, Johnson, IA) and Xtend® (Asgrow, Monmouth, IL) soybean and cotton (*Gossypium hirsutum* L.) cultivars are now commercially available and are resistant to auxinic herbicides (Group 4) such as 2,4-D or dicamba (Behrens et al. 2007; Wright et al. 2010). Unique traits that confer resistance to 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides (Group 27) such as isoxaflutole are now available in soybean and will soon be available in cotton (Dreesen et al. 2018; Hawkes et al. 2010). In addition, with the evolution and widespread occurrence of

Table 1. Common name, trade name, rate, application timing, and manufacturer information of herbicides used to determine the susceptibility of Palmer amaranth accessions.

Common name	Trade name	Rate	Application timing	Manufacturer	Location
		g ai or ae ha^{-1}			
Atrazine	AAtrex	560	POST	Syngenta Crop Protection, LLC	Greensboro, NC
Dicamba[a]	Clarity	560 and 280	POST	BASF Corporation	Research Triangle Park, NC
Fomesafen[a]	Flexstar	395	POST	Syngenta Crop Protection, LLC	Greensboro, NC
Glufosinate	Liberty	594	POST	Bayer CropScience	Research Triangle Park, NC
Glyphosate	Roundup Powermax	860	POST	Bayer CropScience	Research Triangle Park, NC
+	+	+		BASF Corporation	
imazethapyr[a]	Newpath	79			
S-metolachlor	Dual II Magnum	1,064	PRE	Syngenta Crop Protection, LLC	Greensboro, NC
Tembotrione[b]	Laudis	92	POST	Bayer CropScience	Research Triangle Park, NC
Trifluralin	Treflan	1,120	PPI	Loveland Products, INC	Loveland, CO

[a] A nonionic surfactant at 0.25% vol/vol was included with dicamba, fomesafen, and glyphosate + imazethapyr.

[b] A methylated seed oil at 1% vol/vol was used with tembotrione.

glyphosate-resistant weeds in soybean-producing regions of the United States, the utility of glyphosate on weeds like Palmer amaranth is becoming limited. Thus, it would be expected that crop traits that allow for in-crop use of glufosinate (Group 10) will increase in coming years. In the coming decade, a combination of herbicide-resistant traits in cotton and soybean, all of which enable the use of glufosinate, will likely be commercialized to improve control of herbicide-resistant weed populations (Gage et al. 2019; Nandula 2019), albeit multiple herbicide resistance could jeopardize the utility of technologies involving multiple traits.

Herbicide resistance in Palmer amaranth is a significant issue and resistance surveys are commonly used to determine the geographic magnitude of resistance (Bagavathiannan and Norsworthy 2016; Bond et al. 2006; Copeland et al. 2018; Garetson et al. 2019; Kumar et al. 2020; Singh et al. 2018; Varanasi et al. 2018; Wise et al. 2009). Alternatively, the efficacy of SOAs can be determined through resistance surveys, which can aid the development and evaluation of current weed management programs (Beckie et al. 2000; Burgos et al. 2013). Previous Palmer amaranth resistance surveys in Arkansas revealed widespread resistance to glyphosate, ALS-, and PPO-inhibiting herbicides (Bagavathiannan and Norsworthy 2016; Singh et al. 2018; Varanasi et al. 2018). The objective of the current study was to evaluate the susceptibility of different Palmer amaranth accessions to available SOAs in Arkansas row crops using many of the same accessions previously screened for fomesafen resistance (Varanasi et al. 2018).

Materials and Methods

Plant Materials

Palmer amaranth accessions from corn (*Zea mays* L.), cotton, soybean, and rice (*Oryza sativa* L.) fields in Arkansas were collected in the fall 2016. As stated in Varanasi et al. (2018), growers, crop consultants, extension agents, and graduate students collected the majority of accessions from soybean fields. At least 10 inflorescences were collected from each field (considered one unique accession) and threshed to make a composite seed sample.

Herbicide Susceptibility Screening

Herbicide screenings were conducted under greenhouse conditions at the Altheimer Laboratory, University of Arkansas, Fayetteville, AR. The greenhouse was maintained at 35/25 C day/night temperature and a 16-h photoperiod supplemented with light-emitting diodes (a semiconductor light source). The herbicides used in this study are described in Table 1. Total number of Palmer amaranth accessions screened to a particular herbicide depended on seed availability; therefore, not every accession was evaluated for response to all tested herbicides. In all experiments, a susceptible accession collected in 2001 was included (Bond et al. 2006). Experiments were conducted from spring 2017 to fall 2019. For POST herbicide screening experiments, seeds from each accession were germinated in 50-cell plastic trays filled with potting mix (Sunshine Premix No. 1; Sun Gro Horticulture, Bellevue, WA), and seedlings were thinned to one plant in each cell. Once plants reached the 4- to 6-leaf stage (7- to 13-cm tall), they were sprayed with the respective POST herbicide (Table 1). Plant mortality rates were recorded at 14 d after treatment (DAT) for contact herbicides and 21 DAT for systemic herbicides. A plant was considered alive if a meristem was green. Each herbicide screen was repeated in time. Fomesafen-induced mortality rates and target-site resistance mechanisms of the accessions used in this study were previously determined by Varanasi et al. (2018) and are included here for complementary reasons.

For *S*-metolachlor (PRE) and trifluralin (PPI) herbicides (Table 1), screens were conducted using 12.2- × 9.5- × 5.7-cm flats (Insert TO standard; Hummert International, Earth City, MO) filled with a sieved silt loam soil (pH of 6.6 and 2.4% organic matter). The soil was collected from the Milo J. Shult Agricultural Research and Extension Center in Fayetteville, AR. *S*-metolachlor screens were conducted following the methodology described by Brabham et al. (2019). For trifluralin screens, soil-containing flats were initially sprayed, and soil was subsequently emptied into a plastic container, capped, shaken to simulate herbicide incorporation, and poured back into flats. Afterward, 100 seeds were scattered over the soil surface, lightly covered with soil, and watered over the top until soil saturation. For each PRE and PPI herbicide treatment and the nontreated control, there were three replications of each accession, and the experiment was repeated in time. At 21 DAT, the total number of plants with at least one true leaf was recorded, and mortality percentage values were calculated relative to emerged plants in the nontreated control.

Herbicides were applied using a research-chamber track sprayer equipped with 1100067 nozzles calibrated to deliver 187 L ha^{-1} at 1.6 km h^{-1}. Appropriate adjuvants were included with POST herbicides (Table 1). Percent mortality of all treatments in each accession was used to obtain descriptive statistics, using Statistix software (Analytical Software, Tallahassee, FL).

Table 2. Susceptibility of Palmer amaranth accessions collected across Arkansas to different herbicide sites of action.[a]

Herbicide[b]	Rate	Accessions screened	25% Quartile	Median	Mean	75% Quartile
	g ai or ae ha^{-1}		Mortality (%)			
Atrazine	560	144	77	87	85	94
Dicamba	560	127	77	83	82	90
Dicamba	280	134	41	50	51	62
Fomesafen[c]	395	227	62	87	76	98
Glufosinate	594	185	100	100	100	100
Glyphosate + Imazethapyr	860 + 79	140	6	10	16	22
S-metolachlor	1,064	121	94	98	96	100
Tembotrione	92	154	36	51	51	64
Trifluralin	1,120	119	82	92	87	98

[a] Descriptive statistics were generated from mortality rates.

[b] *S*-metolachlor and trifluralin are the only herbicides applied PRE and PPI, respectively.

[c] An in-depth analysis of fomesafen data is presented in Varanasi et al. (2018).

Results and Discussion

Response to Atrazine

The average mortality rate of accessions in response to atrazine applied POST at 560 g ha^{-1} was 85% at 14 DAT (Table 2). Of the 144 accessions screened, 89%, 69%, and 42% of the accessions had a mortality rate of at least 70%, 80%, and 90%, respectively. Although most accessions were sensitive to the atrazine rate tested, a few accessions had less than acceptable mortality values and require additional study. On the basis of these results, atrazine remains an effective herbicide on most Palmer amaranth accessions in Arkansas, which is likely a result of corn and grain sorghum [*Sorghum bicolor* (L.) Moench ssp. *bicolor*] not being widely grown in the state (USDA 2019).

Response to Dicamba

Palmer amaranth accessions were collected before dicamba-resistant crops were commercially available; therefore, dicamba-resistant Palmer amaranth was not expected. At 21 DAT, the average mortality rate of 134 accessions to an application of dicamba at half the labeled rate (280 g ha^{-1}) was 51%, and the mortality rate never exceeded 80% (Table 2). Regrowth was evident in numerous plants from all accessions at 21 DAT (data not shown). Increasing the dicamba application rate from 280 g to 560 g ha^{-1} decreased the variability in mortality rates and increased the average mortality rate by 31 percentage points to 82%. Furthermore, 33% of the 127 accessions screened had a mortality rate of 63% to 80%, but some plants within these accessions were severely injured and apparent regrowth was not likely. Overall, the Palmer amaranth accessions in this study were sensitive to dicamba at 560 g ha^{-1} but not 280 g ha^{-1}. The lack of control with dicamba at 280 g ha^{-1} is concerning because the ability to make timely applications in the field is sometimes difficult, resulting in treatment of larger-than-labeled weeds or weeds partially covered by the crop canopy. If the dicamba-resistant technology is not managed properly, the probability is high for shifting the sensitivity of an accession toward one that will be more difficult to control. In fact, a dicamba-resistant Palmer amaranth accession has been reported in Kansas (Peterson et al. 2019), and previous research has shown that low-dose selection for resistance to dicamba in Palmer amaranth can occur rapidly (Tehranchian et al. 2017).

Response to Glufosinate

All accessions used were susceptible to glufosinate (594 g ha^{-1}). Of the 185 accessions screened, 93%, 98%, and 100% of the accessions had a mortality rate of at least 99%, 95%, and 90%, respectively (Table 2). Averaged across accessions, glufosinate killed 99.5% of the treated plants. Resistance to this SOA in Palmer amaranth has not yet been documented, and our findings indicate glufosinate remains an effective, viable option for the control of Palmer amaranth. Glufosinate use in glufosinate-resistant crops is currently an underused weed management system in Arkansas (Riar et al. 2013). In the coming decade, glufosinate use is expected to increase as crops with multiple herbicide-resistance traits, including glufosinate resistance, are introduced to the market. For glufosinate to remain an effective tool, growers need to take a proactive resistance-management approach by combining or rotating effective SOAs with glufosinate.

Response to Glyphosate plus Imazethapyr

In Arkansas, glyphosate and ALS-resistant Palmer amaranth are already widespread (Bond et al. 2006; Salas et al. 2016; Singh et al. 2018). Here, we were interested in evaluating Palmer amaranth accessions for multiple-herbicide resistance to both glyphosate and imazethapyr. Thus, glyphosate at 860 g ha^{-1} was applied in combination with imazethapyr at 79 g ha^{-1} to plants at the 4- to 6-leaf stage. As expected, 98% of the 140 accessions screened at 21 DAT had less than 80% mortality (Table 2). Most accessions had mortality rates ranging from 6% to 22%, which highlight the severity of glyphosate and imazethapyr resistance in Palmer amaranth accessions in Arkansas. In addition, the ALS-inhibiting herbicides pyrithiobac and trifloxysulfuron are not reliable options for controlling glyphosate-resistant Palmer amaranth in Arkansas, meaning that multiple resistance to Group 2 and Group 9 herbicides is common (Norsworthy et al. 2008). Hence, that 209 of 215 Palmer amaranth accessions showed multiple resistance to glyphosate and pyrithiobac was not surprising, as has been reported in the Mississippi Delta region of eastern Arkansas (Bagavathiannan and Norsworthy 2016).

Response to S-metolachlor

The average mortality rate of 121 accessions to *S*-metolachlor at 1,064 g ha^{-1} was 96%; 74% of these accessions had a mortality rate of at least 95% (Table 2). Palmer amaranth with a low level of metabolic resistance to *S*-metolachlor, but not other Group 15 herbicides, has been reported in Arkansas (Brabham et al. 2019). Brabham et al. (2019) reported the average LD_{90} values for the two susceptible and two resistant accessions were 190

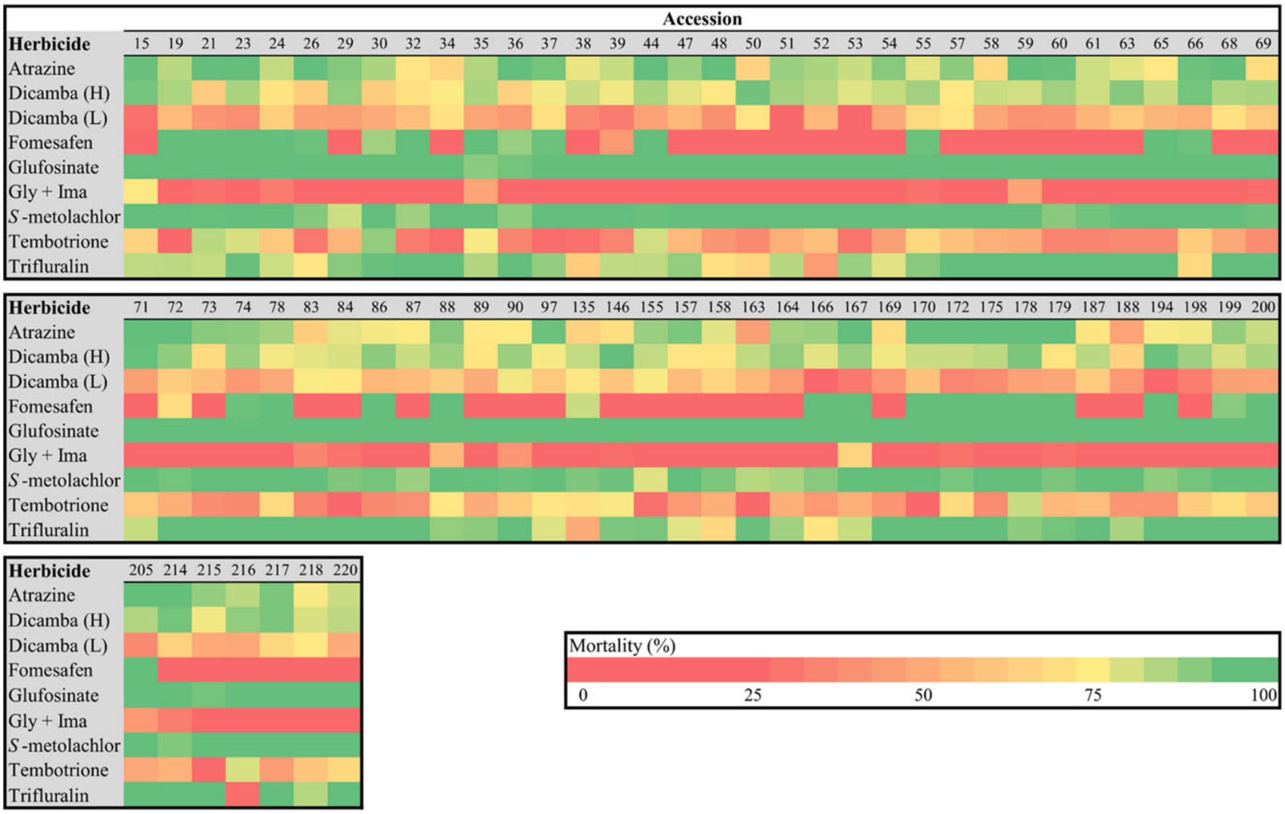

Figure 1. Mortality (%) heatmap of Palmer amaranth accessions screened for sensitivity to different herbicides. Accessions known to contain a target-site resistance mechanism to fomesafen were given a 0% mortality value on the basis of data from Varanasi et al. (2018). Abbreviations: Dicamba (H): dicamba high rate; Dicamba (L): dicamba low rate; Gly + Ima; glyphosate + imazethapyr.

and 1,168 g ha^{-1}, respectively. The two resistant accessions from Brabham et al. (2019) were used in the current study and had an average 91% mortality rate.

Worryingly, an additional 14 of the 121 accessions screened in the current study had mortality rates of not more than 91%, indicating the spread of *S*-metolachlor resistance is still in the early stages. In Arkansas, *S*-metolachlor and other Group 15 herbicides are heavily relied upon to obtain season-long control of Palmer amaranth. Our findings highlight the need for growers to reduce the exposure of Palmer amaranth accessions to *S*-metolachlor by mixing with other effective SOAs or at least alternating between Group 15 herbicides. In other experiments carried out in pecan [*Carya illinoinensis* (Wangenh.) K. Koch] orchards, the use of PRE *S*-metolachlor provided more than 99% control of glyphosate-resistant Palmer amaranth accessions (Mohseni-Moghadam et al. 2013). In North Carolina, *S*-metolachlor had better efficacy in controlling glyphosate-resistant Palmer amaranth accessions than did pendimethalin in soybean cropping systems (Whitaker et al. 2010). Those findings suggest *S*-metolachlor is still an effective chemical alternative for controlling Palmer amaranth and, likewise, remains an option for controlling Palmer amaranth in most fields throughout Arkansas.

Response to Tembotrione

Like atrazine, the selection pressure for resistance to HPPD-inhibiting herbicides in Arkansas is presumed to be low; nonetheless, resistance can be found in nearby states (Heap 2020). Furthermore, HPPD-inhibitor resistance is often associated with atrazine resistance and is metabolic (Küpper et al. 2018; Ma et al. 2013; Nakka et al. 2017). Given that most Palmer amaranth accessions in this study were susceptible to atrazine, we expected effective Palmer amaranth control with tembotrione at 92 g ha^{-1}. However, we observed inconsistent control of Palmer amaranth accessions at 21 DAT. The average mortality rate of 154 accessions was 51%, and 93% of these accessions had a mortality rate less than 80% (Table 2). The typical plant response to tembotrione within an accession varied greatly and mimicked a bell-shaped curve that ranged from healthy to dead plants (data not shown). Moreover, Singh et al. (2018) reported that 33% of the 172 Palmer amaranth accessions collected in Arkansas from 2008 to 2016 exhibited reduced sensitivity to mesotrione (105 g ha^{-1}). The observed variability in mortality rates in the current study and as reported by Singh et al. (2018) indicates that difficult-to-control accessions with triketone herbicides already exist in Arkansas.

Response to Trifluralin

In Arkansas, before the advent of glyphosate-resistant crops, trifluralin was a commonly used herbicide for Palmer amaranth control, but it is no longer widely used (Gossett et al. 1992; Kniss 2018; Webster and Coble 1997). The average mortality rate of 119 accessions sprayed with trifluralin at 1,120 g ha^{-1} was 87%, with 41% of the accessions having a mortality rate of at least 95% (Table 2). However, the mortality rate of 22% of accessions was less than 80%, indicating accessions with reduced sensitivity to trifluralin can be found in Arkansas. The first confirmed case of trifluralin resistance in Arkansas was reported in an accession collected in 2016 (Heap 2020). However, trifluralin resistance in Palmer amaranth was already believed to be prevalent in Arkansas

(J.K. Norsworthy, personal communication) and was documented in 1998 in the neighboring state of Tennessee (Heap 2020). Nevertheless, only a trifluralin-resistant Palmer amaranth accession with cross-resistance to benefin, isopropalin, pendimethalin, and ethalfluralin has been confirmed in South Carolina (Gossett et al. 1992). Palmer amaranth resistance to trifluralin in Arkansas needs to be confirmed and characterized in the low-susceptibility accessions.

Prevalence of Difficult-to-Control Accessions

Based on our results, control of Palmer amaranth with commonly used SOAs is becoming increasingly difficult in Arkansas. Accessions with three-way resistance to glyphosate, ALS-, and PPO-inhibiting herbicides appear to be common (Bond et al. 2006; Salas et al. 2016; Singh et al. 2018; Varanasi et al. 2018). For example, resistance of the accessions used in this study to fomesafen was previously determined by Varanasi et al. (2018), who reported 141 accessions had a target-site mutation that conferred resistance to fomesafen. In our study, glyphosate + imazethapyr did not control any of these accessions (data not shown). In addition, tembotrione (average mortality rate, 51%) had poor efficacy on most of these accessions, and nearly one-fifth of the accessions have suspected resistance to trifluralin (Figure 1).

Of the remaining herbicide SOAs, most Palmer amaranth accessions were sensitive to glufosinate, atrazine, dicamba (high rate), and *S*-metolachlor (Figure 1). However, within each herbicide, except glufosinate, control of some accessions was less than expected. This highlights the need to use a multitactic approach for Palmer amaranth management to protect the efficacy of the remaining effective SOAs and to mitigate and delay the dispersion of accessions with reduced sensitivity to these SOAs.

Acknowledgments. This research received no specific grant. No conflicts of interest have been declared.

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