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Confirmation and Control of Triazine and 4-Hydroxyphenylpyruvate Dioxygenase-Inhibiting Herbicide-Resistant Palmer Amaranth (*Amaranthus palmeri*) in Nebraska

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Palmer amaranth is a difficult-to-control broadleaf weed that infests corn and soybean fields in south-central and southwestern Nebraska and several other states in the United States. The objectives of this research were to confirm triazine and 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicide-resistant Palmer amaranth in Nebraska and to determine sensitivity and efficacy of POST-applied corn herbicides for control of resistant and susceptible Palmer amaranth biotypes. Seeds from a putative HPPD-resistant Palmer amaranth biotype from Fillmore County, NE were collected from a seed corn production field in fall 2010. The response of Palmer amaranth biotypes to 12 rates (0 to 12×) of mesotrione, tembotrione, topramezone, and atrazine was evaluated in a dose–response bioassay in a greenhouse. On the basis of the values at the 90% effective dose (ED_{90}) level, the analysis showed a 4- to 23-fold resistance depending upon the type of HPPD-inhibiting herbicide being investigated and susceptible biotype used for comparison. This biotype also had a 9- to 14-fold level of resistance to atrazine applied POST. Results of a POST-applied herbicide efficacy study suggested a synergistic interaction between atrazine and HPPD-inhibiting herbicides that resulted in > 90% control of all Palmer amaranth biotypes. The resistant biotype had a reduced sensitivity to acetolactate synthase inhibiting herbicides (halosulfuron and primisulfuron), a photosystem-II inhibitor (bromoxynil), and a protoporphyrinogen oxidase inhibitor (fluthiacet-methyl). Palmer amaranth biotypes were effectively controlled (≥ 90%) with glyphosate, glufosinate, and dicamba, whereas 2,4-D ester provided 81 to 83% control of the resistant biotype and > 90% control of both susceptible biotypes.

Nomenclature: 2,4-D; atrazine; bromoxynil; dicamba, fluthiacet-methyl; glufosinate; glyphosate; halosulfuron-methyl; lactofen; mesotrione; primisulfuron-methyl; pyrasulfotole; tembotrione; thiencarbazone-methyl; topramezone; Palmer amaranth, *Amaranthus palmeri* S. Wats.; corn, *Zea mays* L.; soybean, *Glycine max* (L.) Merr.

Key words: Dose response, herbicide resistance, resistance management, weed biomass.

Amaranthus palmeri es una maleza de hoja ancha difícil de controlar que infesta campos de maíz y soya en el centro y oeste del sur de Nebraska y en varios otros estados en los Estados Unidos. Los objetivos de esta investigación fueron confirmar la existencia de *A. palmeri* resistente a triazine y herbicidas inhibidores de 4-hydroxyphenylpyruvate dioxygenase (HPPD) en Nebraska y determinar la sensibilidad y la eficacia de herbicidas para maíz aplicados POST para el control de biotipos de *A. palmeri* susceptibles y resistentes. Semillas de *A. palmeri* con resistencia putativa a HPPD provenientes del condado Fillmore, NE fueron colectadas de un campo de producción de maíz en el otoño de 2010. La respuesta de los biotipos de *A. palmeri* a 12 dosis (0 a 12×) de mesotrione, tembotrione, topramezone, y atrazine fue evaluada en un bioensayo de respuesta a dosis en un invernadero. Con base en los valores del nivel de dosis efectiva de 90%, los análisis mostraron una resistencia de 4 a 23 veces mayor dependiendo del tipo de herbicida inhibidor de HPPD investigado y del biotipo susceptible usado como comparación. Este biotipo también tuvo un nivel de resistencia a atrazine POST de 9 a 14 veces mayor. Los resultados del estudio de eficacia de herbicidas aplicados POST sugirieron una interacción sinérgica entre atrazine y herbicidas inhibidores de HPPD que resultó en >90% de control de todos los biotipos de *A. palmeri*. El biotipo resistente tuvo una sensibilidad reducida a herbicidas inhibidores de acetolactate synthase (halosulfuron y primisulfuron), a un inhibidor del fotosistema II (bromoxynil) y a un inhibidor de protoporphyrinogen oxidase (fluthiacet-methyl). Los biotipos de *A. palmeri* fueron controlados efectivamente (≥90%) con glyphosate, glufosinate, y dicamba, mientras que 2,4-D ester brindó un control de 81 a 83% del biotipo resistente y >90% de los dos biotipos susceptibles.

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Palmer amaranth, a member of the pigweed (Amaranthaceae) family, is a native to the southwestern United States and northern Mexico (Sauer 1967). It can be characterized by rapid growth, high fecundity, competitive ability, extended emergence periodicity, and high water use efficiency (Steckel 2007; Ward et al. 2013). It is one of the most troublesome weeds in cotton (*Gossypium hirsutum* L.), corn, and soybean fields in the southern United States (Webster 2009), with majority of populations having resistance to glyphosate or acetolactate synthase (ALS)-inhibiting herbicides (or both) (Culpepper et al. 2006; Vencill et al. 2002; Wise et al. 2009).

Palmer amaranth is relatively a new weed in Nebraska. Historically, common weeds from the pigweed family reported to occur in Nebraska are tumble pigweed (*Amaranthus albus* L.), prostrate pigweed (*Amaranthus graecizans* L.), redroot pigweed (*Amaranthus retroflexus* L.), and common waterhemp (*Amaranthus rudis* Sauer) (Stubbendieck et al. 1994). They are usually found throughout Nebraska in dry prairies, cultivated and fellow fields, and roadside, industrial, and waste places (Stubbendieck et al. 1994). Recently, Palmer amaranth has been identified in Wisconsin, Michigan, Ohio, and Illinois, which has raised concerns among weed scientists and growers about the spread of this species into areas not previously reported. Because of its rapid growth habit, ability for prolific seed production, and ability to evolve herbicide-resistance, Palmer amaranth can be hard to control in corn and soybean production fields in the north-central United States.

The United States is the largest producer of corn in the world, with a planting area of more than 37 million ha (USDA-NASS 2012). Corn is the most important crop in Nebraska, grown on about 4 million ha annually (USDA-NASS 2012). A majority of corn grown in Nebraska is hybrid corn and the use of hybrid seed requires the production of new seed each year. Therefore, corn seed production businesses have been developed to supply the need. Although soybean and corn are commonly rotated in Nebraska, corn for seed production is in a continuous corn rotation, which usually results in the repeated use of the same herbicide(s) for several years. Weed control is the most important component in seed corn production because of the lower competitive nature of inbred lines and more sensitivity of inbred lines to some herbicides (Green 1998), which reduces the herbicide options (Boerboom 1999).

Atrazine is one of the most commonly used herbicides for weed control in corn (Swanton et al. 2007). Atrazine can be applied alone or in tank mixtures with many herbicides either PRE or POST in corn (Walsh et al. 2012). Due to widespread and repeated use of atrazine, several weed species have evolved resistance. For example, atrazine-resistant Palmer amaranth has been reported in Texas, Kansas, and Georgia (Heap 2013). A Palmer amaranth biotype resistant to atrazine has also been confirmed in Nebraska (Jhala 2013). The 4-hydroxylphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides including isoxaflutole, mesotrinone, tembotrione, and topramezone are currently used extensively in corn production for weed control (Bollman et al. 2008). They are popular among seed corn and sweet corn producers because of high tolerance of corn inbreds to these herbicides, a wide weed-control spectrum, flexibility for application timings, and compatibility for tank mixes with other herbicides (Bollman et al. 2008; McMullan and Green 2011; Walsh et al. 2012; Williams and Pataky 2010). In addition, HPDD-inhibiting herbicides are also useful for control of other herbicide-resistant weeds (specifically ALS, atrazine, and glyphosate-resistant) in corn fields (Sutton et al. 2002; Vyn et al. 2006).

Palmer amaranth biotypes resistant to ALS-inhibiting herbicides, dinitroanilines, triazine, photosystem II-inhibiting herbicides, and glyphosate have been confirmed in several states of the United States (Bond et al. 2006; Culpepper et al. 2006; Norsworthy et al. 2008; Ward et al. 2013; Wise et al. 2009). Burgos et al. (2001) reported Palmer amaranth biotypes resistant to imazaquin and cross-resistant to chlorimuron, dichosulam, and pyrithiobac. Furthermore, multiple resistance in Palmer amaranth to glyphosate and pyrithiobac has been confirmed in Georgia (Sosnoskie et al. 2011).

Palmer amaranth dissemination into the north-central United States could be a tremendous hindrance to corn and soybean producers. A seed corn field in Fillmore County, NE had poor Palmer amaranth control despite application of atrazine and HPPD-inhibiting herbicides. The history of this field revealed that atrazine and HPPD-inhibiting herbicides were applied repeatedly in food-grade

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white corn. The HPPD-inhibiting herbicide-resistant weed biotypes in this part of Nebraska are of particular concern because of the proximity to intense seed corn production, which is heavily reliant on HPPD-inhibiting herbicides. The objectives of this study were to: (1) confirm triazine and HPPD-inhibiting herbicide-resistant Palmer amaranth in Nebraska by quantifying the level of resistance in a dose–response study, (2) evaluate the effect of herbicides from other mode-of-action groups commonly applied POST in corn to test the possibility of resistance, and (3) evaluate the efficacy of atrazine tank-mixed with HPPD-inhibiting herbicides and other POST-applied corn herbicides to control putative resistant and susceptible Palmer amaranth biotypes.

Materials and Methods

Plant Materials. In the fall of 2010, after consecutive years of control issues with atrazine and HPPD-inhibiting herbicides, seedheads of Palmer amaranth that survived herbicide treatments were collected from a seed corn field near Shickley, Fillmore County, NE. The seedheads were allowed to dry for a week at room temperature. Seeds were cleaned and stored at 5 C until used in this study and will be referred to as putative resistant (R) biotype. The HPPD-inhibiting herbicide-susceptible Palmer amaranth seeds were collected in 2001 from a field in Lancaster County, NE and in 2006 from a field in Clay County, NE with a known history of effective control with atrazine and HPPD-inhibiting herbicides to be used as susceptible biotypes, S_1 and S_2, respectively, for comparison with a putative resistant biotype (R) in this study.

Dose–Response Study. Greenhouse dose–response bioassays were conducted in 2011 and 2012 at the University of Nebraska-Lincoln to determine the level of resistance in suspected HPPD-inhibiting herbicide-resistant Palmer amaranth biotype (R). The S_1 and S_2 biotypes were included for comparison. Seeds from the putative resistant and susceptible biotypes were sown in plastic trays containing potting mix. The seedlings at the cotyledon to first true-leaf stage were transplanted to 10-cm-diam plastic pots containing a 3 : 1 mixture of potting mix (Metromix potting media, The Scotts Company, Marysville, OH 43041) and soil. Plants were supplied with adequate nutrients and water and were kept in a greenhouse with 30/20 C day/night temperature and 16-h photoperiod.

The experimental design was completely randomized with eight replications. A single Palmer amaranth plant per pot was considered an experimental unit. Herbicide treatments included 12 rates (0, 0.1×, 0.25×, 0.5×, 0.75×, 1×, 1.5×, 2×, 3×, 4×, 6×, and 12×) of mesotrione (1× = 106 g ai ha^{-1}), tembotrione (1× = 92 g ai ha^{-1}), topramezone (1× = 25 g ai ha^{-1}), and atrazine (1× = 1,121 g ai ha^{-1}) in a factorial arrangement. Seedlings were treated with herbicides at the six- to seven-leaf stage (8 to 10 cm tall). Recommended adjuvants were mixed with each herbicide treatment (Table 1). Herbicide treatments were prepared in distilled water and applied using a single-tip chamber sprayer (DeVries Manufacturing Corp, Hollandale, MN 56045) fitted with 8001E nozzle (TeeJet, Spraying Systems Co., Wheaton, IL 60187) calibrated to deliver 190 L ha^{-1} carrier volume at 207 kPa. After treatment, plants were returned to the greenhouse. Palmer amaranth control was assessed visually at 21 d after treatment (DAT) using a scale ranging from 0% (no control) to 100% (complete control). Control ratings were based on symptoms such as chlorosis, necrosis, stand loss, and stunting of plant compared with nontreated control plants.

Aboveground biomass of each Palmer amaranth biotype was harvested at 21 DAT and oven-dried for 7 d at 66 C, and dry weights were determined. The effective dose needed to suppress the population 50 and 90% (ED_{50} and ED_{90}) for each biotype and herbicide was determined using the drc package in software R (Knezevic et al. 2007). The level of resistance was calculated by dividing ED_{90} value of resistant biotype (R) by susceptible biotypes (S_1 and S_2). Where the ED_{90} values were variable for S_1 and S_2, a range of resistance levels was provided.

Efficacy of POST Herbicides. The efficacy of atrazine and HPPD-inhibiting herbicides applied alone or in tank mixes and several other POST-applied corn herbicides were evaluated for control of the R, S_1, and S_2 biotypes. The herbicide treatments included in this study are listed in Table 1. Plants were grown using the same procedures reported for the dose–response study. Herbicides were applied when plants were 10 to 12 cm tall.

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Table 1. Detail of herbicides used for POST control of Palmer amaranth biotypes.

Herbicide	Trade name	Rate	Manufacturer	Adjuvant[a]
		g ae or ai ha^{-1}		
Mesotrione	Callisto	106	Syngenta Crop Protection, Inc. Greensboro, NC 27419	COC 1% v/v + AMS 2.5% wt/wt
Mesotrione + atrazine	Callisto + Aatrex	106 + 560	Syngenta Crop Protection	COC 1% v/v + AMS 2.5% wt/wt
Topramezone	Impact	25	AMVAC, Los Angeles, CA 90023	MSO 1% v/v + AMS 2.5% wt/wt
Topramezone + atrazine	Impact + Aatrex	25 + 560	AMVAC + Syngenta Crop Protection	MSO 1% v/v + AMS 2.5% wt/wt
Tembotrione	Laudis	92	Bayer Crop Science, Research Triangle Park, NC 27709	MSO 1% v/v + AMS 2.5% wt/wt
Tembotrione + atrazine	Laudis + Aatrex	92 + 560	Bayer Crop Science + Syngenta Crop Protection	MSO 1% v/v + AMS 2.5% wt/wt
Atrazine	Aatrex	560	Syngenta Crop Protection	MSO 1% v/v + AMS 2.5% wt/wt
Glyphosate	Roundup PowerMax	860	Monsanto Company, 800 North Lindberg Ave., St. Louis, MO	AMS 2.5% wt/wt
Glufosinate	Ignite	450	Bayer Crop Science	AMS 2.5% wt/wt
2,4-D Ester	2,4-D LO-V Ester Weed Killer	560	Universal Crop Protection Alliance, LLC, Eagan, MN 55121	NIS 0.25% + AMS 2.5% wt/wt
Dicamba	Clarity	560	BASF Corporation, 26 Davis Drive, Research Triangle Park, NC 27709	COC 1% v/v + AMS 2.5% wt/wt
Halosulfuron-methyl	Permit	73	Gowan Company, P.O. Box 5569, Yuma, AZ 85364	COC 1% v/v + AMS 2.5% wt/wt
Primisulfuron-methyl	Beacon	41.6	Syngenta Crop Protection	COC 1% v/v + AMS 2.5% wt/wt
Fluthiacet-methyl	Cadet	7.2	FMC Corporation, Philadelphia, PA 19103	COC 1% v/v + AMS 2.5% wt/wt
Lactofen	Cobra	218	Valent USA Corporation, Walnut Creek, CA 94596	COC 1% v/v + AMS 2.5% wt/wt
Thiencarbazone-methyl + pyrasulfotole + bromoxynil	Huskie	235	Bayer Crop Science	NIS 0.25% + AMS 2.5% wt/wt
Bromoxynil	Buctril	200	Bayer Crop Science	NIS 0.25% + AMS 2.5% wt/wt
Primisulfuron-methyl + mesotrione + atrazine	Beacon + Callisto + Aatrex	41.6 + 92 + 560	Syngenta Crop Protection	COC 1% v/v + AMS 2.5% wt/wt

[a] Abbreviations: AMS, ammonium sulfate (DSM Chemicals North America Inc., Augusta, GA); COC, crop oil concentrate (Agridex, Helena Chemical Co., Collierville, TN); MSO, methylated seed oil (Southern Ag Inc., Suwanee, GA); NIS, nonionic surfactant (Induce, Helena Chemical Co., Collierville, TN).

Palmer amaranth control was visually rated at 7, 14, and 21 DAT on a scale 0 to 100%, where 0% means no control and 100% means complete control. Aboveground biomass of each plant was harvested at 21 DAT and oven-dried for 7 d at 67 C, and dry weights were recorded. Biomass data were converted to percentage of nontreated control for each Palmer amaranth biotype.

The experiment was conducted in a randomized complete block design with 19 treatments (Table 1) and eight replications. The experiment was repeated in time. Data were subjected to ANOVA using the statistical analysis software version 9.2 (SAS Institute Inc., Cary, NC). Normality, homogeneity of variance, and interactions of treatments in greenhouse repeat experiments were tested. Percent control and aboveground biomass data were arcsine square-root transformed before analysis to meet assumptions of variance analysis. However, nontransformed data are presented with mean separation on the basis of transformed data. Where the ANOVA indicated treatment effects were significant, means were separated at $P \leq 0.05$ with Fisher's protected LSD test.

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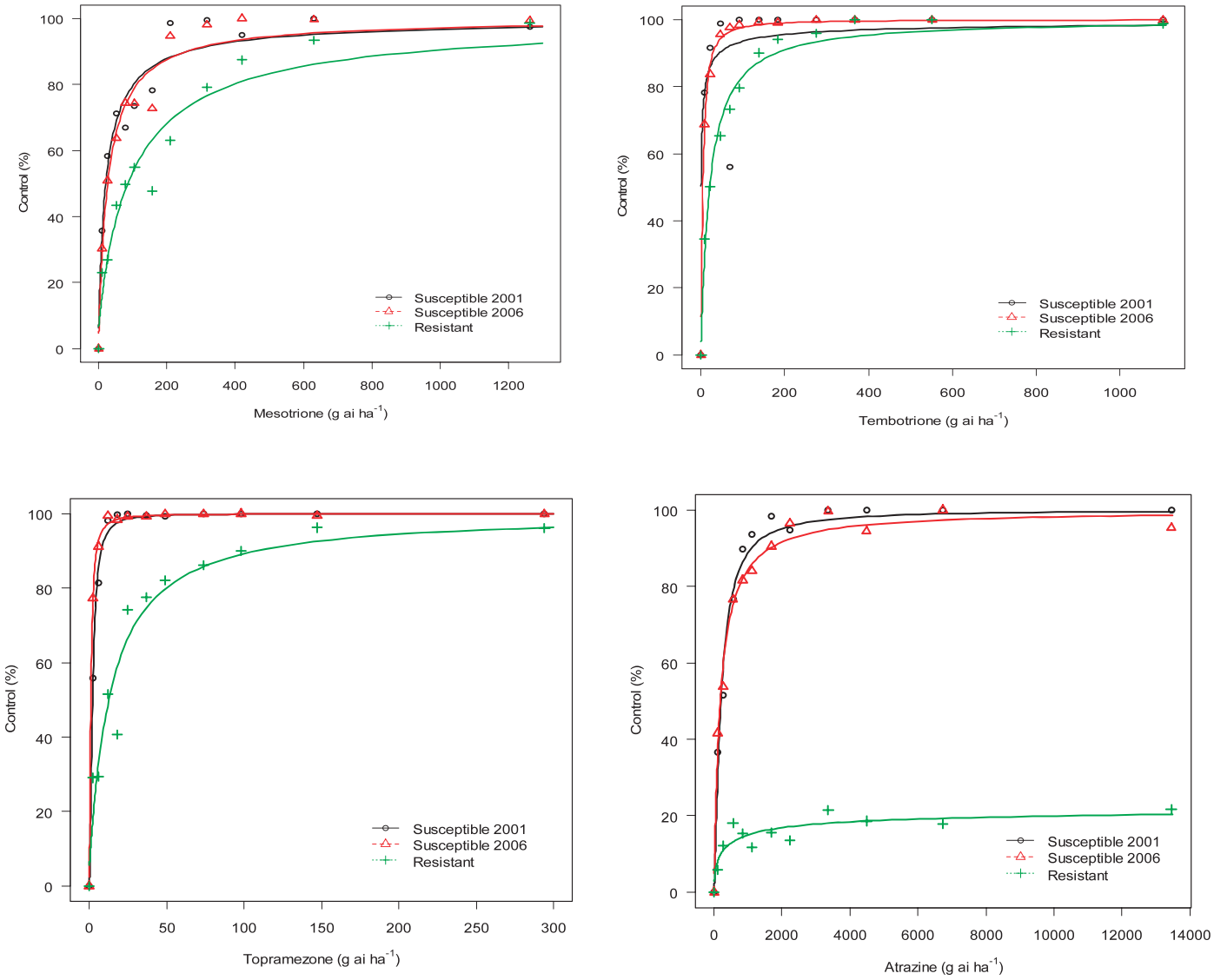

Figure 1. Control of Palmer amaranth biotypes at 21 d after treatment in a dose response to mesotrione, tembotrione, topramezone, and atrazine.

Results and Discussion

Dose–Response Study. Treatment-by-experiment interaction among dose–response studies was nonsignificant; therefore, data of both experiments were pooled and the combined data are presented. A labeled rate of mesotrione (106 g ha^{-1}) resulted in 80% control of S_1 biotype, whereas S_2 biotype required a slightly higher rate (114 g ha^{-1}) to achieve the same level of control. The putative resistant (R) Palmer amaranth biotype control was 50% with a labeled rate of mesotrione (Figure 1) and required 98 and 1,007 g ha^{-1} of mesotrione to achieve 50 and 90% control, respectively, which indicated a fourfold level of resistance on the basis of the ED_{90} value (Table 2). A similar study in Kansas reported the value of ED_{50} at 231 g ha^{-1} of mesotrione (Thompson et al. 2012). Rana et al. (2013) confirmed HPPD-inhibiting herbicide-resistant common waterhemp in Nebraska and reported 90% control of 7-cm-tall waterhemp with the 13-fold labeled rate of mesotrione at 23 DAT.

A slightly higher level of resistance (four- to six-fold) was observed for tembotrione. For example, ED_{90} value of R biotype was achieved at 179 g ha^{-1} compared with 44 and 29 g ha^{-1} of tembotrione, respectively for S_1 and S_2 biotypes (Table 2). Thompson et al. (2012) reported 50% control of

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Table 2. Values of ED_{50} and ED_{90}[a] for control of Palmer amaranth biotypes at 21 d after treatment (DAT) in a dose–response study with mesotrione, tembotrione, topramezone, and atrazine.

Palmer amaranth biotype[a]	Mesotrione		
	ED_{50} (±SE)[a]	ED_{90} (±SE)	Resistance level[b]
	g ai ha^{-1}		
S_1	21 (4)	258 (60)	-
S_2	28 (5)	260 (51)	-
R	98 (19)	1,007 (240)	4×
Tembotrione			
S_1	1 (0.9)	44 (16)	-
S_2	5 (1)	29 (3)	-
R	21 (3)	179 (27)	4 to 6×
Topramezone			
S_1	2 (0.1)	8 (1)	-
S_2	1 (0.2)	5 (0.4)	-
R	14 (2)	114 (22)	14 to 23×
Atrazine			
S_1	209 (22)	1095 (126)	-
S_2	187 (30)	1,646 (298)	-
R[c]	309 (448)	15,686 (70,769)	9 to 14×

[a] Abbreviations: ED_{50}, effective dose required to control 50% population; ED_{90}, effective dose required to control 90% population; S_1, 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicide-susceptible Palmer amaranth biotype collected from a field in 2001; S_2, HPPD-inhibiting herbicide-susceptible Palmer amaranth biotype collected from a field in 2006; R, HPPD-inhibiting herbicide-resistant Palmer amaranth biotype collected from a field in 2010; SE, standard error.

[b] Resistance level was calculated by dividing ED_{90} value of resistant biotype (R) by susceptible biotypes (S_1 and S_2). Where the ED_{90} values were variable for S_1 and S_2, a range of resistance level was provided.

[c] These values have a limited biological meaning because 90% control was never achieved.

Table 3. Values of ED_{50} and ED_{90}[a] for percent dry weight reduction of Palmer amaranth biotypes at 21 d after treatment in a dose response to mesotrione and atrazine.

Palmer amaranth biotype[a]	Mesotrione	
	ED_{50} (±SE)	ED_{90} (±SE)
	g ai ha^{-1}	
S_1	16 (3)	162 (84)
S_2	17 (2)	92 (34)
R	79 (20)	903 (229)
Tembotrione[b]		
R	13 (2)	87 (28)
Topramezon[b]		
R	7 (1)	90 (40)
Atrazine		
S_1	255 (30)	1,176 (301)
S_2	108 (30)	997 (639)
R	2,318 (1,443)	11,136 (6,934)

[a] Abbreviations: ED_{50}, effective dose required for 50% reduction of dry weight; ED_{90}, effective dose required for 90% reduction of dry weight; S_1, 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicide-susceptible Palmer amaranth biotype collected from a field in 2001; S_2, HPPD-inhibiting herbicide-susceptible Palmer amaranth biotype collected from a field in 2006; R, HPPD-inhibiting herbicide-resistant Palmer amaranth biotype collected from a field in 2010; SE, standard error.

[b] Biomass (dry weight) data of susceptible populations did not fit the log logistic model for tembotrione and topramezone, so ED_{50} and ED_{90} values of only R biotype have been presented.

Palmer amaranth biotype with 55 g ha^{-1} of tembotrione in Kansas. The susceptible biotypes (S_1 and S_2) were very sensitive to topramezone. For example, $\leq$ 8 g ha^{-1} of topramezone was sufficient to achieve 90% control. In contrast, 14 and 114 g ha^{-1} of topramezone was required to achieve 50 and 90% control of R biotype, respectively. Thus, the resistance level was 14- and 23-fold on the basis of the ED_{90} values of S_1 and S_2 biotypes, respectively (Table 2). Dose–response curves for percent biomass reduction also suggested a similar level of resistance on the basis of the ED_{50} and ED_{90} values (Table 3; Figure 2). The sensitivity of susceptible biotypes to tembotrione and topramezone was reflected in biomass (dry weight). In fact, the percent reduction in dry weight of susceptible biotypes compared with nontreated control did not fit the log logistic model for these two HPPD-inhibiting herbicides (Table 3; Figure 2).

Comparing all the herbicides evaluated in this study, the highest dose of atrazine was required for the control of R biotype. The ED_{90} values indicated that 1,095 and 1,646 g ha^{-1} of atrazine was required for control of S_1 and S_2 biotypes, respectively (Figure 1). In contrast, 90% control of R biotype was never achieved even with the highest rate of atrazine tested in this study (Figure 1; Table 2). Similarly, a recent study confirmed atrazine as well as HPPD-inhibiting herbicide-resistant Palmer amaranth biotypes in Kansas (Thompson et al. 2012).

Overall results of the dose–response curves suggested that both susceptible Palmer amaranth biotypes (S_1 and S_2) were effectively controlled with

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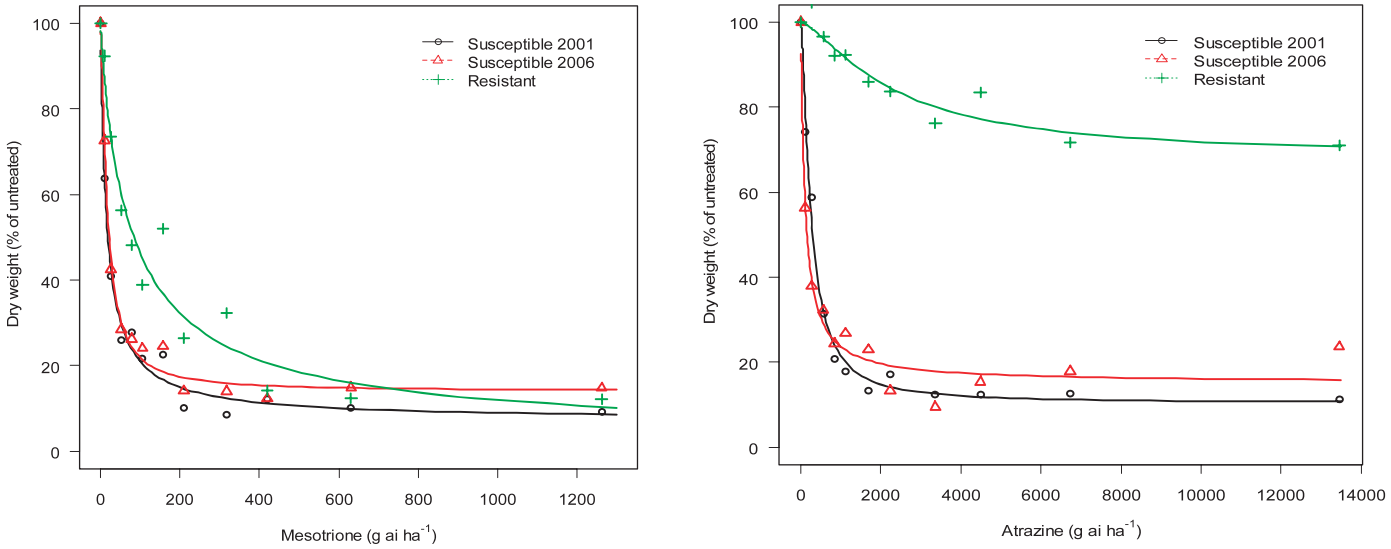

Figure 2. Percent dry weight reduction of Palmer amaranth biotypes at 21 d after treatment in a dose response to mesotrione and atrazine.

labeled rates of atrazine and HPPD-inhibiting herbicides. The dose–response analysis showed a 4- to 23-fold resistance in R biotype, depending upon the type of HPPD-inhibiting herbicide and susceptible biotype used for comparison (Table 2). For example, among HPPD-inhibiting herbicides, Palmer amaranth biotype has shown higher level of resistance to topramezone followed by tembotrione and mesotrione on the basis of ED_{90} values (Table 2). Biomass reduction data supported the visual control observations at 21 DAT and suggested a similar level of resistance at the ED_{50} and ED_{90} levels (Table 3). Although level of resistance to HPPD-inhibiting herbicides would not be usually considered high, labeled POST application rates were not adequate for satisfactory control. For example, at the recommended labeled use rates, mesotrione, tembotrione, and topramezone provided approximately 55, 80, and 65% control of the R biotype, respectively. The R biotype had 9- to 14-fold level of resistance to POST-applied atrazine. The level of atrazine resistance is likely much higher, but was difficult to determine, since the highest dose only provided 20% control (Figure 1) and 25% biomass reduction (Figure 2).

Efficacy of POST Herbicides. Treatment-by-experiment interaction among greenhouse studies was significant; therefore, data for both experiments are presented separately. The results suggested that both susceptible biotypes (S_1 and S_2) were sensitive to HPPD-inhibiting herbicides applied alone or in a tank mix with atrazine and provided 41 to 98% control at 7 DAT and usually $>$ 90% control at 21 DAT with a 71 to 91% reduction in biomass at 21 DAT (Tables 4 and 5). Atrazine applied alone provided 45 and 73% control and similarly, bromoxynil provided 37 and 42% control of S_1 and S_2 biotypes, respectively in experiment 1 (Table 4). The similar results were observed in experiment 2 with $\leq$ 66% control of S_1 and S_2 biotypes with atrazine and bromoxynil (Table 5). This suggested that HPPD-inhibiting herbicide-susceptible Palmer amaranth biotypes (S_1 and S_2) had reduced sensitivity to atrazine and bromoxynil. A reduced susceptibility of Palmer amaranth and several other weed species to atrazine is not a new phenomenon; there have been reports of atrazine resistance in Palmer amaranth in Texas and Kansas (Heap 2013).

The R biotype response to HPPD-inhibiting herbicides applied alone was similar to the dose–response study and resulted in unacceptable control (Tables 4 and 5). Atrazine and bromoxynil provided poor control ($\leq$ 8%) of the R biotype in experiment 1 (Table 4), and $\leq$ 40% control in experiment 2 (Table 5) with $<$ 30% biomass reduction; confirming reduced sensitivity to photosystem II inhibitors (triazine and nitrile). Historically bromoxynil applied alone is not effective for control of Palmer amaranth and it was reflected in this study because control of susceptible biotypes

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Table 4. Effects of POST herbicide treatments on control and biomass reduction of Palmer amaranth biotypes at 7 and 21 d after treatment in experiment 1.

Herbicide	Rate	Control at 7 DAT[a,b,c]			Control at 21 DAT[b,c]			Reduction in biomass[b,c,d]		
		S_1	S_2	R	S_1	S_2	R	S_1	S_2	R
	g ae or ai ha^{-1}	%--------								
Nontreated control[d]	-	0	0	0	0	0	0	-	-	-
Mesotrione	106	93 bcde	90 bcd	62 e	99 a	99 a	58 d	88 a	87 a	70 cd
Mesotrione + atrazine	106 + 560	96 bcd	95 abc	94 bc	99 a	99 a	99 a	91 a	91 a	94 a
Topramezone	25	93 cde	91 bcd	71 de	99 a	99 a	58 d	89 a	86 a	79 bc
Topramezone + atrazine	25 + 560	96 abcd	94 bc	90 bc	99 a	99 a	99 a	91 a	91 a	95 a
Tembotrione	92	92 def	90 bcde	84 cd	99 a	99 a	96 ab	88 a	85 a	87 ab
Tembotrione + atrazine	92 + 560	98 abc	95 abc	90 bc	99 a	99 a	98 a	91 a	91 a	94 a
Atrazine	560	70 h	75 e	10 fg	45 e	73 c	7 e	44 bc	68 b	11 e
Glyphosate	860	100 a	100 a	100 a	99 a	99 a	96 ab	89 a	89 a	93 a
Glufosinate	450	95 bcd	93 bc	91 bc	99 a	99 a	99 a	91 a	91 a	94 a
2,4-D Ester	560	84 fgh	79 de	65 e	97 ab	94 b	83 bc	86 a	84 a	72 cd
Dicamba	560	85 efg	87 cde	72 de	97 ab	98 ab	92 abc	87 a	89 a	85 abc
Halosulfuron-methyl	73	75 gh	37 f	6 g	74 d	42 d	3 e	55 b	43 c	7 e
Primisulfuron-methyl	41.6	82 gh	49 f	12 fg	87 c	52 d	8 e	82 a	34 c	11 e
Fluthiacet-methyl	7.2	94 bcd	96 abc	67 e	95 bc	95 ab	57 d	87 a	87 a	55 d
Lactofen	218	99 ab	97 ab	90 bc	99 a	99 a	75 cd	91 a	90 a	79 bc
Thiencarbazone-methyl + pyrasulfotole + bromoxynil	235	96 abcd	94 abc	73 de	99 a	99 a	78 cd	92 a	89 a	75 bc
Bromoxynil	200	54 i	44 f	16 f	37 e	42 d	8 e	32 c	39 c	10 e
Primisulfuron-methyl + mesotrione + atrazine	41.6 + 92 + 560	93 bcd	94 abc	95 ab	99 a	99 a	99 a	91 a	89 a	94 a

[a] Abbreviations: DAT, days after treatment; S_1, HPPD-inhibiting herbicide-susceptible Palmer amaranth biotype collected from a field in 2001; S2, HPPD-inhibiting herbicide-susceptible Palmer amaranth biotype collected from a field in 2006; R, suspected HPPD-inhibiting herbicide-resistant Palmer amaranth biotype collected from a field in 2010.

[b] The data were arc-sine square-root transformed for homogenous variance before analysis; however, data presented are the means of actual values for comparison based on interpretation from the transformed data.

[c] Means within columns with no common letter(s) are significantly different according to Fisher's protected LSD test where $P \leq 0.05$.

[d] The percent control (0%) data of nontreated control were not included in analysis. Biomass reduction was calculated on the basis of comparison with the biomass weight of nontreated control.

was also < 45% (Tables 4 and 5). Surprisingly, poor control (< 22%) of the R biotype was observed for ALS-inhibiting herbicides (halosulfuron and primisulfuron), and protoporphyrinogen oxidase (PPO)-inhibiting herbicide (fluthiacet-methyl) at 21 DAT (Tables 4 and 5). Usually fluthiacet-methyl is not very effective for control of Palmer amaranth or any species of pigweed family. Thus, the R biotype of Palmer amaranth has shown decreased sensitivity to three herbicide modes of action including HPPD, ALS, and phosystem II (triazine and nitrile); however, more research is required to determine the level of resistance to ALS and bromoxynil. It has to be noted that atrazine rate was 560 g ha^{-1} in this study compared with 1,121 g ha^{-1} (as a 1× rate) in a dose–response study that is also responsible for reduced control of R biotype with atrazine. The evolution of multiple herbicide resistance has been reported previously in Palmer amaranth (Nandula et al. 2012; Sosnoskie et al. 2011;). Similarly, tall waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer], a closely related species to Palmer amaranth, has been confirmed resistant in Iowa to three herbicide mechanisms of action: HPPD, photosystem II, and ALS inhibitors (McMullan and Green 2011).

Control of the R biotype of Palmer amaranth was usually inadequate when atrazine or HPPD-inhibiting herbicides were applied alone (Tables 4 and 5). However, tank mixing atrazine and HPPD inhib-

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Table 5. Effects of POST herbicide treatments on control and biomass reduction of Palmer amaranth biotypes at 7 and 21 d after treatment in experiment 2.

Herbicide	Rate	Control at 7 DAT[a,b,c]			Control at 21 DAT[b,c]			Reduction in biomass[b,c,d]		
		S_1	S_2	R	S_1	S_2	R	S_1	S_2	R
	g ae or ai ha^{-1}	%								
Nontreated control[d]	-	0	0	0	0	0	0	-	-	-
Mesotrione	106	41 gh	42 cd	38 f	85 b	90 bc	66 gf	74 bc	76 abc	63 bc
Mesotrione + atrazine	106 + 560	76 c	80 b	60 de	99 a	99 a	91 bcd	83 ab	86 a	83 a
Topramezone	25	45 fg	47 cd	43 f	98 a	98 a	73 efg	74 bc	81 ab	71 abc
Topramezone + atrazine	25 + 560	77 c	75 b	58 de	99 a	99 a	87 cde	83 ab	84 ab	80 a
Tembotrione	92	42 g	46 cd	47 ef	90 b	96 ab	88 cde	75 abc	71 bc	76 ab
Tembotrione + atrazine	92 + 560	78 c	80 b	65 d	99 a	99 a	97 ab	81 abc	87 a	79 ab
Atrazine	560	55 ef	56 c	23 gh	58 cd	66 d	21 k	71 c	78 abc	7 e
Glyphosate	860	99 a	99 a	96 a	99 a	99 a	99 a	81 abc	85 ab	82 a
Glufosinate	450	89 b	80 b	82 bc	99 a	99 a	99 a	85 a	87 a	74 ab
2,4-D Ester	560	74 c	85 b	70 cd	91 b	96 ab	81 def	72 c	79 abc	53 c
Dicamba	560	70 c	71 b	71 cd	92 b	88 c	91 bcd	74 bc	77 abc	73 ab
Halosulfuron-methyl	73	55 ef	38 d	14 h	65 c	48 e	21 k	44 d	32 d	4 ef
Primisulfuron-methyl	41.6	58 de	34 d	19 h	69 c	42 e	21 k	45 d	26 d	8 e
Fluthiacet-methyl	7.2	78 c	84 b	58 de	47 d	69 d	38 i	47 d	64 c	31 d
Lactofen	218	93 b	95 a	84 b	98 a	99 a	84 de	77 abc	78 abc	73 ab
Thiencarbazone-methyl + Pyrasulfotole + bromoxynil	235	69 cd	75 b	47 ef	99 a	99 a	57 gh	81 abc	83 ab	53 c
Bromoxynil	200	30 h	39 d	36 fg	23 e	36 e	40 hi	32 e	39 d	26 d
Primisulfuron-methyl + mesotrione + atrazine	41.6 + 92 + 560	77 c	80 b	59 de	99 a	99 a	96 abc	81 abc	86 a	79 a

[a] Abbreviations: DAT, days after treatment; S_1, HPPD-inhibiting herbicide-susceptible Palmer amaranth biotype collected from a field in 2001; S2, HPPD-inhibiting herbicide-susceptible Palmer amaranth biotype collected from a field in 2006; R, suspected HPPD-inhibiting herbicide-resistant Palmer amaranth biotype collected from a field in 2010.

[b] Data were arc-sine square-root transformed for homogenous variance before analysis; however, data presented are the means of actual values for comparison on the basis of interpretation from the transformed data.

[c] Means within columns with no common letter(s) are significantly different according to Fisher's protected LSD test where $P \leq 0.05$.

[d] Percent control (0%) data of nontreated control were not included in analysis. Biomass reduction was calculated on the basis of comparison with the biomass weight of nontreated control.

itors resulted in $\geq$ 98 and 87% control of R biotype in experiments 1 and 2, respectively, with a similar level of biomass reduction at 21 DAT (Tables 4 and 5). This was due to the synergistic interaction between atrazine and HPPD-inhibiting herbicides. Several studies reported a synergistic effect between HPPD and photosystem II inhibitors, probably due to complementary modes of action of these herbicides (Abendroth et al. 2006; Armel et al. 2005; Hugie et al. 2008). For example, Walsh et al. (2012) reported the synergistic effect of mesotrione and atrazine on susceptible and resistant wild radish (*Raphanus raphanistrum* L.) populations. Abendroth et al. (2006) reported synergism on Palmer amaranth with a combination of mesotrione and photosystem II inhibitors (atrazine, bromoxynil, and metribuzin). Similarly, Sutton et al. (2002) demonstrated synergism of mesotrione and atrazine on redroot pigweed.

Other than tank mixtures of atrazine and HPPD-inhibiting herbicides, Palmer amaranth biotypes were effectively controlled ($\geq$ 90%) with a few POST herbicides including glyphosate, glufosinate, and dicamba (Tables 4 and 5). 2,4-D ester provided 81 to 83% control of R biotype and 91 to 97% control of both susceptible biotypes. As shown in this study and historically, glyphosate has been very effective for control of Palmer amaranth (Corbett et al. 2004; Parker et al. 2005); however, because of evolution of glyphosate-resistant Palmer amaranth

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in the southern United States, glyphosate should not be considered as a single management option (Whitaker et al. 2010).

The suspected resistant Palmer amaranth biotype from a seed corn production field in Nebraska is resistant to POST-applied HPPD-inhibiting herbicides (mesotrione, tembotrione, and topramezone) and atrazine. Palmer amaranth is known to evolve resistance to several groups of herbicides with different sites of action (Nandula et al. 2012; Sosnoskie et al. 2011; Ward et al. 2013). Atrazine and HPPD-inhibiting herbicides are usually applied in tank mixtures to expand the weed-control spectrum by corn producers. Weed management in seed corn production will be complicated with the evolution of triazine and HPPD-resistant Palmer amaranth biotypes. Therefore, integrated weed management strategies including crop rotation, tillage, and systems approach utilizing residual followed by POST herbicides with different sites of action will be required for control of triazine and HPPD-inhibiting herbicide-resistant Palmer amaranth biotypes for sustainable seed corn production in Nebraska.

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