Responses of a Waterhemp (*Amaranthus tuberculatus*) Population Resistant to HPPD-Inhibiting Herbicides to Foliar-Applied Herbicides

Nicholas E. Hausman, Patrick J. Tranel, Dean E. Riechers, and Aaron G. Hager*

Field and greenhouse experiments were conducted to characterize the response of a waterhemp population from McLean County, IL to foliar-applied 4-hydroxyphenylpyruvate dioxygenase (HPPD) –inhibiting herbicides and determine the population's sensitivity to herbicides from other site-of-action groups. In the field, 10 to 15–cm-tall waterhemp treated with mesotrione at 105 g ai ha^{-1}, tembotrione at 92 g ai ha^{-1}, or topromezone at 18 g ai ha^{-1} had significantly greater biomass ($\geq 10\%$) 14 d after treatment (DAT) than waterhemp harvested the day of herbicide application, indicating growth had occurred following herbicide application. Waterhemp growth stage at the time of herbicide application influenced control. Mesotrione applied at 105 g ha^{-1} alone or combined with atrazine at 560 g ai ha^{-1} provided significantly greater waterhemp control ($\geq 66\%$) when applied to small waterhemp plants (2 to 5 cm tall) compared with applications made to plants 5 to 10 or 10 to 15 cm tall. Glyphosate, glufosinate, fomesafen, lactofen, or acifluorfen provided greater waterhemp control ($\geq 68\%$) 7 and 14 DAT than mesotrione, dicamba, or 2,4-D. Control of this population with atrazine, chlorimuron, and imazethapyr did not exceed 12%. Results of a greenhouse experiment with waterhemp plants grown from field-collected seed were similar to field data, and confirm the McLean County population was poorly controlled with HPPD, photosystem II, and acetolactate synthase inhibitors.

Nomenclature: Acifluorfen; atrazine; chlorimuron; dicamba; fomesafen; glufosinate; glyphosate; imazethapyr; lactofen; mesotrione; tembotrione; topramezone; 2,4-D; waterhemp, *Amaranthus tuberculatus* (Moq.) Sauer var. *rudis* (Sauer) Costea and Tardif AMATU.

Key words: Herbicide resistance, waterhemp management.

Experimentos de campo e invernadero fueron realizados para caracterizar la respuesta de una población de *Amaranthus tuberculatus* proveniente del condado McLean en Illinois, a la aplicación foliar de herbicidas inhibidores de 4-hydroxyphenylpyruvate dioxygenase (HPPD) y determinar la sensibilidad de la población a herbicidas de grupos con otros sitios de acción. En el campo, plantas de *A. tuberculatus* de 10 a 15 cm de altura, tratadas con mesotrione a 105 g ai ha^{-1}, tembotrione a 92 g ai ha^{-1}, o topramezone a 18 g ai ha^{-1}, tuvieron una biomasa significativamente mayor ($\geq 10\%$) 14 d después del tratamiento (DAT) que *A. tuberculatus* cosechado el día de la aplicación del herbicida, indicando que hubo crecimiento después de la aplicación del herbicida. El estadio de desarrollo de *A. tuberculatus* al momento de la aplicación del herbicida influyó en el control. Mesotrione aplicado solo a 105 g ha^{-1} o combinado con atrazine a 560 g ai ha^{-1} brindó un control significativamente mayor ($\geq 66\%$) cuando se aplicó a plantas pequeñas de *A. tuberculatus* (2 a 5 cm de altura), al compararse con aplicaciones hechas a plantas de 5 a 10 ó 10 a 15 cm de altura. Glyphosate, glufosinate, fomesafen, lactofen, o acifluorfen brindaron mayor control de *A. tuberculatus* ($\geq 68\%$) 7 y 14 DAT que mesotrione, dicamba, o 2,4-D. El control de esta población con atrazine, chlorimuron, e imazethapyr no excedió 12%. Los resultados de un experimento de invernadero con plantas de *A. tuberculatus* provenientes de semillas colectadas en campo, fueron similares a los datos de campo, y confirman que la población del condado McLean fue pobremente controlada con herbicidas inhibidores de HPPD, fotosistema II, y acetolactate synthase.

Waterhemp is a small-seeded, dioecious, summer annual broadleaf weed species (Sauer 1955, 1957) common in Illinois corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr] production systems (Hager et al. 1997). Previous research has demonstrated this weed species can reduce soybean yield more than 40% (Hager et al. 2002) and corn yield more than 70% (Steckel and Sprague 2004). Once established, this species is difficult to eradicate, as individual female plants can produce in excess of one million seeds (Hartzler et al. 2004; Steckel et al. 2003) that can remain dormant in the soil seed bank for several years (Buhler and Hartzler 2001; Burnside et al. 1996). Waterhemp emergence occurs over a more prolonged period than other weed species, such as

velvetleaf (*Abutilon theophrasti* Medicus) and foxtail (*Setaria* spp.) (Hartzler et al. 1999). Waterhemp's extended emergence characteristic often necessitates an integrated management system, including utilization of soil-residual and foliar-applied herbicides, to manage this species adequately.

The evolution of waterhemp populations resistant to foliar-applied herbicides has effectively reduced viable herbicide options for control of waterhemp in certain agronomic crops such as corn and soybean. Prior to 2009, waterhemp had evolved resistance to herbicides from four site-of-action groups (Heap 2013): acetolactate synthase (ALS) inhibitors, photosystem II (PSII) inhibitors, protoporphyrinogen oxidase (PPO) inhibitors, and 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) inhibitors. Waterhemp resistant to multiple herbicide groups further limits control options (Foes et al. 1998; Patzoldt et al. 2005). Bell et al. (2013) identified a population of waterhemp in which individual plants demonstrated resistance to herbicides from four site-of-action groups.

Resistance to 4-hydroxyphenylpyruvate dioxygenase (HPPD) –inhibiting herbicides is a relatively recent phenomenon with the first instances reported in 2009 (Heap 2013). Waterhemp populations from McLean County, IL (Hausman et al. 2011) and Henry County, IA (McMullan and Green 2011) have been documented resistant to HPPD-inhibiting herbicides. Two biotypes of Palmer amaranth (*Amaranthus palmeri* S. Wats.) from Kansas have demonstrated reduced sensitivity to various HPPD-inhibiting herbicides (Lally et al. 2010). In greenhouse and laboratory research, waterhemp grown from seed collected at the McLean County, IL location demonstrated resistance to foliar-applied mesotrione, tembotrione, and topramezone at rates of 105, 92, and 18 g ai ha^{-1}, respectively, and resistance to the PSII inhibitor atrazine (Hausman et al. 2011; Ma et al. 2013). Additional greenhouse research revealed the Illinois waterhemp population demonstrated a 10 to 35–fold level of resistance to the HPPD inhibitor mesotrione (Hausman et al. 2011). McMullan and Green (2011) reported similar greenhouse results with the Iowa waterhemp population, which demonstrated an 8-fold level of resistance to mesotrione as well as resistance to atrazine (10-fold) and thifensulfuron (28-fold).

Previous research (Hausman et al. 2011) with the Illinois HPPD-inhibitor–resistant population was conducted solely under greenhouse conditions with herbicides representing only two site-of-action groups. The goal of the research presented herein was to characterize this particular waterhemp population further. Our objectives were (1) to quantify biomass accumulation under field conditions using a rate-response experiment with HPPD inhibitors applied alone or in combination with atrazine, (2) to characterize the response to mesotrione applied alone or with atrazine at various waterhemp growth stages under field conditions, and (3) to determine the response to various foliar-applied herbicides representing multiple site-of-action groups both in the field and greenhouse. The additional comprehensive characterization of this population's response to foliar-applied herbicides could potentially augment management decisions for other HPPD-inhibiting herbicide-resistant *Amaranthus* populations (Heap 2013).

Materials and Methods

Field Experiments. Field experiments were conducted in 2010 and 2011 at the location (McLean County, IL) from which the HPPD-inhibitor–resistant waterhemp population (designated MCR) was initially identified (Hausman et al. 2011). The soil was a Sable silty clay loam (fine–silty, mixed, superactive, mesic Typic Endoaquolls), with a pH of 6.4 and 3.2% organic matter. Preplant tillage was performed each spring to prepare the seedbed for planting and to control emerged weeds. Experiments were conducted either in corn or soybean, planted in 76-cm rows. Experiments were designed as randomized complete blocks with three replications of each treatment. Individual replications were plots measuring 3 by 7.6 m that included four crop rows. Herbicides were applied with a pressurized CO_2 backpack sprayer equipped with AIXR110025 nozzles (TeeJet Technologies, P.O. Box 7900, Wheaton, IL 60187) spaced 51 cm apart on a 3-m boom calibrated to deliver 187 L ha^{-1} at 276 kPa. Herbicides and accompanying additives that are commonly used in Illinois cropping systems were selected for evaluation. Application rate of each herbicide was based on the respective label recommendations for waterhemp control according to soil type and organic matter content (Table 1).

Table 1. Herbicides used, application rates, and source information for field (McLean County, IL) and greenhouse trials.

Common name	Trade name	Site of action[a]	Application rate(s)	Manufacturer
			g ai ha^{-1}	
Chlorimuron	Classic	ALS	11.2	DuPont Crop Protection, Wilmington, DE; http://www.dupont.com
Imazethapyr	Pursuit	ALS	70	BASF Corporation Agricultural Products, Research Triangle Park, NC; http://www.agro.basf.com
Glyphosate	RoundUp Powermax	EPSPS	840[b]	Monsanto Company, St. Louis, MO; http://www.monsanto.com
Glufosinate	Ignite	GS	450	Bayer CropScience, Research Triangle Park, NC; http://www.bayercropscience.com
Mesotrione	Callisto	HPPD	105, 210, 420	Syngenta Crop Protection, Greensboro, NC; http://www.syngentacropprotection.com
Tembotrione	Laudis	HPPD	92, 184, 368	Bayer CropScience, Research Triangle Park, NC; http://www.bayercropscience.com
Topramezone	Impact	HPPD	18, 36, 72	AMVAC Chemical Corporation, Los Angeles, CA; http://www.amvac-chemical.com
Acifluorfen	Ultra Blazer	PPO	420	United Phosphorus, Inc., King of Prussia, PA; http://www.upi-usa.com
Fomesafen	Flexstar	PPO	395	Syngenta Crop Protection, Greensboro, NC; http://www.syngentacropprotection.com
Lactofen	Cobra	PPO	140	Valent U.S.A. Corporation, Walnut Creek, CA; http://www.valent.com
Atrazine	AAtrex	PSII	560, 1680	Syngenta Crop Protection, Greensboro, NC; http://www.syngentacropprotection.com
2,4-D	2,4-D, Ester	Synthetic auxin	270[b]	Dow AgroSciences, Indianapolis, IN; http://www.dowagro.com
Dicamba	Clarity	Synthetic auxin	280[b]	BASF Corporation Agricultural Products, Research Triangle Park, NC; http://www.agro.basf.com

[a] Abbreviations: ALS, acetolactate synthase; EPSPS, 5-enolpyruvylshikimate-3-phosphate synthase; GS, glutamine synthetase; HPPD, 4-hydroxyphenylpyruvate dioxygenase; PPO, protoporphyrinogen oxidase; PSII, photosystem II.

[b] Acid equivalent (g ae ha^{-1}).

All herbicide applications were made when the majority of waterhemp plants were 10 to 15 cm tall unless noted otherwise.

Three separate field experiments were implemented to address the research objectives. In a rate-response experiment, biomass accumulation of MCR was quantified following treatment with one of three HPPD inhibitors (mesotrione, tembotrione, or topramezone) applied at the recommended field use rate (105, 92, and 18 g ai ha^{-1}, respectively), as well as at twice and four times the recommended rate. HPPD-inhibitor treatments were applied alone or in combination with atrazine at 560 g ai ha^{-1}. Another experiment determined the response of MCR to mesotrione (105 g ai ha^{-1}) ± atrazine (560 g ai ha^{-1}) following treatment of waterhemp plants at three discrete growth stages (2 to 5, 5 to 10, or 10 to 15 cm tall). The response of MCR to 11 herbicides, representing seven herbicide site-of-action groups, was determined in a third experiment.

Greenhouse Experiment. Inflorescences of several female waterhemp plants not controlled following foliar applications of tembotrione and mesotrione (92 and 105 g ai ha^{-1}, respectively) were collected from the McLean location in August 2009. The progeny from these harvested waterhemp plants were chosen to characterize the response of MCR to herbicides from different site-of-action groups under greenhouse conditions. One milliliter of seeds from each of the 12 inflorescences was combined in a single glass vial and shaken to create a greenhouse population representative of the field population rather than representative of the individual female plants. Seeds from this mixture were then stratified in a 0.1% agarose solution at 4 C for 30 d. The response of MCR to various foliar-applied herbicides was compared with the responses

of two other waterhemp populations (designated ACR and WCS) sensitive to HPPD-inhibiting herbicides. The ACR population demonstrates resistance to ALS-, PSII-, and PPO-inhibiting herbicides (Patzoldt et al. 2005), whereas WCS displays no resistance to herbicides from any site-of-action group (Patzoldt et al. 2002).

Waterhemp plants from all three populations were grown from seeds sown in 12- by 12-cm flats containing a commercial potting medium (Sun Gro Horticulture, 15831 Northeast 8th Street, Bellevue, WA 98008). Emerged seedlings (2 cm) were transplanted into 950-cm^3 pots (one seedling per pot) containing a 3 : 1 : 1 : 1 mixture of potting mix : soil : peat : sand that included a slow-release fertilizer (The Scotts Company, 14111 Scottslawn Road, Marysville, OH 43041). Greenhouse conditions were maintained at 28/22 C day/night with a 16-h photoperiod. Natural sunlight was supplemented with mercury halide lamps to provide 800 μmol m^{-2} s^{-1} photon flux at the plant canopy.

Uniformly sized waterhemp plants (10 to 12 cm tall) from MCR, ACR, and WCS were treated with one of eight herbicides evaluated (Table 1). Treatments were applied with a compressed-air research sprayer (DeVries Manufacturing, 28081 870th Avenue, Hollandale, MN 56045) fitted with a TeeJet 80015 EVS nozzle. The nozzle was situated 46 cm above the plant canopy, and the sprayer was calibrated to deliver 185 L ha^{-1} at 275 kPa. After herbicide application, plants were moved back to the greenhouse and arranged in a completely randomized design (CRD). Treatments applied to WCS and ACR were replicated four times, whereas treatments applied to MCR were replicated 48 times, and the experiment was conducted twice.

Data Collection. Visual estimates of percent waterhemp control were recorded on a scale of 0 (no control) to 100 (complete control). These estimates were based on waterhemp injury and growth reduction compared with the untreated control. In addition to visual estimates, four uniformly sized waterhemp plants per plot (12 per treatment) were selected prior to treatment to quantify aboveground biomass accumulation following herbicide application in the rate-response field experiment. These uniformly sized waterhemp plants (10 to 15 cm) were marked by placing a small plastic stake near each plant prior to herbicide application. All other waterhemp plants within a

15-cm diam of each marked plant were carefully removed to ensure full spray interception by the marked plants. Twelve additional plants at a similar growth stage to the marked waterhemp (10 to 15 cm) were harvested prior to herbicide application to determine pretreatment biomass. Each year, all marked waterhemp plants (including those in nontreated plots) were harvested 14 d after treatment (DAT), dried at 65 C for 7 d, and weights recorded. Mortality of treated plants was recorded in 2011. Identical measurements as were collected in field experiments (percent control, dry weight, and mortality) were made for all waterhemp plants in the greenhouse experiment, except that harvest occurred 21 DAT.

Statistical Analyses. Statistical analysis for field and greenhouse trials was performed with the use of PROC Mixed in SAS 9.2 (SAS Institute, Inc., 100 SAS Campus Drive, Cary, NC 27513). Fixed effects included herbicide treatment and waterhemp population (greenhouse only). Random effects included year and block within year (field only), or experimental run (greenhouse only). Mean estimates of percent waterhemp control were compared with the use of single-degree-of-freedom contrast statements or separated with the use of the SAS macro *%pdmix800* (Saxton 1998). All dry-weight data (field and greenhouse) were analyzed with the use of the SAS statement *REPEATED/GROUP*, as fit statistics revealed the heterogeneous variance structure fit the data better than the homogeneous variance structure.

Results and Discussion

Rate Response with HPPD Inhibitors Applied Alone or Combined with Atrazine. Regardless of application rate, mesotrione, tembotrione, or topramezone controlled MCR less than 40% 7 or 14 DAT (Table 2). These results from a field experiment are similar to those reported by Hausman et al. (2011), who evaluated the response of MCR to these herbicides under greenhouse conditions. The addition of atrazine increased waterhemp control 14 DAT only with tembotrione at 184 and 368 g ha^{-1} and mesotrione at 420 g ha^{-1} when compared with each HPPD inhibitor applied alone.

By 14 DAT, biomass of waterhemp treated with 1x rates of mesotrione, tembotrione, or topramezone (105, 92, and 18 g ha^{-1}, respectively) was

Table 2. Visual estimates of control and mean biomass of McLean County, IL waterhemp under field conditions (2010 and 2011).[a] Visual estimates of control sharing the same letter within a column are not significantly different at α = 0.05 (separated by the SAS macro *%pdmix800*).

Herbicide[b]	Rate	7 DAT[c]	14 DAT[c]	Biomass	Estimated difference[e]	Pr > F
	g ai ha^{-1}	———% control———		% of nontreated 14 DAT[c]	%	
Pretreatment plants[d]	–	–	–	**21**	–	–
Mesotrione	105	28 bc	21 ef	38	17	0.0065*
	210	28 bc	24 def	27	6	0.2866
	420	39 ab	38 bcde	29	8	0.0785
Tembotrione	92	26 bc	18 ef	31	10	0.0420*
	184	27 bc	23 def	31	10	0.0088*
	368	31 ab	33 cde	21	0	0.9546
Topramezone	18	24 bc	17 ef	43	22	0.0073*
	36	26 bc	19 ef	30	9	0.1449
	72	33 ab	36 bcde	22	1	0.8194
Mesotrione + atrazine	105 + 560	40 ab	36 bcde	13	−8	0.0116
	210 + 560	46 ab	46 abcd	12	−9	0.0003
	420 + 560	55 a	63 a	14	−7	0.0685
Tembotrione + atrazine	92 + 560	35 ab	31 cde	19	−2	0.5281
	184 + 560	48 ab	50 abc	12	−9	0.0014
	368 + 560	48 ab	58 ab	11	−10	0.0001
Topramezone + atrazine	18 + 560	35 ab	33 cde	16	−5	0.2763
	36 + 560	38 ab	35 bcde	14	−7	0.0049
	72 + 560	40 ab	46 abcd	12	−9	0.0018
Atrazine	560	4 c	3 f	82	61	0.0001*

* Significant at α = 0.05, treatments with positive differences indicate growth after treatment. Separated with the use of Dunnett's procedure in SAS.

[a] Trial was planted to corn.

[b] All treatments containing HPPD inhibitors included crop oil concentrate (COC) at 1% (v/v) and 28% urea ammonium nitrate (UAN) at 2.5% (v/v); COC was included with the atrazine-only treatment.

[c] Days after treatment.

[d] Plants harvested the day of spraying to assess biomass accumulation after herbicide application.

[e] Estimated difference in dry weight (as a % of the untreated) between herbicide-treated plants and pretreatment plants.

significantly greater than the biomass of pretreatment waterhemp (Table 2) indicating plant recovery and growth had occurred following herbicide application. Biomass of waterhemp plants treated with the 2x rate of tembotrione (184 g ha^{-1}) or atrazine (560 g ha^{-1}) also was significantly greater than pretreatment biomass. Although average biomass was either less or not different from pretreatment biomass for all other treatments, waterhemp control estimates recorded 7 and 14 DAT indicate generally poor control of this population.

Mortality data were collected in 2011 as an additional metric to describe the response of the MCR waterhemp population to foliar-applied HPPD-inhibiting herbicides. Mortality of the 12 waterhemp plants (those marked prior to herbicide application) did not exceed 17% when treated with 1x rates of mesotrione, tembotrione, or topramezone alone or 58% when in combination with atrazine (data not shown). Plants were considered survivors if they displayed actively growing, nonbleached tissue around the apical meristem.

These results are in general agreement with other published reports. McMullan and Green (2011) reported waterhemp control was less than 70% at the Henry County, IA location 30 DAT with mesotrione, tembotrione, or topramezone applied at twice the recommended field use rates of 105, 92, and 18 g ha^{-1}, respectively. Mortality of waterhemp generated from seed collected from the McLean location and treated with mesotrione at 315 g ha^{-1} was only 33% (Hausman et al. 2011). Lally et al. (2010) reported two putative HPPD-resistant

Table 3. Visual estimates of McLean County, IL waterhemp control, contrast statements, and 95% confidence intervals after foliar applications of mesotrione and atrazine alone or in combination under field conditions (2010 and 2011).[a] Application timings were based on waterhemp height.

	Waterhemp height (cm)								
	2–5			5–10			10–15		
	DAT[c]								
Herbicide[b]	7	14	21[d]	7	14	21[d]	7	14	21[d]
	—% control—								
Mesotrione	69	66	47	27	21	13	12	10	13
Atrazine	8	0	0	3	0	0	3	0	0
Mesotrione + atrazine	78	80	63	53	36	30	30	26	28
Contrasts	Estimated difference[e]			95% confidence interval			Standard error		
Mesotrione									
2–5 vs. 5–10	45			29–61*			6.56		
2–5 vs. 10–15	56			40–72*			6.56		
5–10 vs. 10–15	11			−5 to 27			6.56		
Mesotrione + atrazine									
2–5 vs. 5–10	44			28–60*			6.56		
2–5 vs. 10–15	54			38–70*			6.56		
5–10 vs. 10–15	10			−6 to 26			6.56		

* Significant at $\alpha = 0.05$.

[a] Trial was planted to corn.

[b] Mesotrione and atrazine rates were 105 and 560 g ai ha^{-1}, respectively. Treatments included crop oil concentrate (COC) at 1% (v/v) and ammonium sulfate (AMS) at 2.5% (v/v).

[c] Days after treatment.

[d] 21 DAT ratings taken only in 2011.

[e] Estimated difference of mean control between application timings within mesotrione alone or mesotrione plus atrazine 14 DAT.

Palmer amaranth biotypes required 11 and 5 times more pyrasulfotole plus bromoxynil than a sensitive biotype to achieve 50% mortality. The decreased effectiveness of the HPPD inhibitors and atrazine observed within the MCR population is most likely due to elevated metabolism (Ma et al. 2013).

Response to Mesotrione Applied at Three Discrete Growth Stages. By 7 DAT, regardless of growth stage, mesotrione caused characteristic HPPD-inhibitor symptomatology on treated waterhemp plants, including bleached foliage and stunted growth (Table 3). Similar to Vyn et al. (2006) and Woodyard et al. (2009), combining atrazine with mesotrione generally increased waterhemp control compared with mesotrione alone. Control with atrazine alone did not exceed 8% regardless of growth stage, similar to greenhouse results reported by Hausman et al. (2011). Waterhemp control generally decreased by 14 DAT for all treatments, except mesotrione plus atrazine applied at the 2 to 5–cm growth stage. The decrease in control was attributed to recovery of treated plants, manifested by new, noninjured leaf tissue emerging from apical meristems and axillary buds. Contrast statements (Table 3) comparing growth-stage application timing 14 DAT demonstrated that control was significantly greater when mesotrione was applied to 2 to 5–cm–tall waterhemp compared with applications made at 5 to 10 or 10 to 15 cm (45 and 56% greater, respectively). There was no significant difference in waterhemp control between plants treated with mesotrione at 5 to 10 and 10 to 15 cm tall. Results were similar when mesotrione was combined with atrazine (Table 3).

Greater weed control when mesotrione was applied to smaller weeds has been documented previously. Johnson et al. (2002) reported increased control of common cocklebur (*Xanthium strumarium* L.) and yellow nutsedge (*Cyperus esculentus* L.) 14 DAT following early postemergence mesotrione applications (weed height 3 to 8 cm) as compared with control following later applications (weed height ≥ 15 cm). Woodyard et al. (2009) reported

Table 4. Visual estimates of McLean County, IL waterhemp control 7 and 14 d after treatment (DAT) with herbicides representing seven site-of-action groups under field conditions (2010 and 2011).[a] Ratings with the same letter within a column are not significantly different at $\alpha = 0.05$ (separated by the SAS macro *%pdmix800*).[b]

Herbicide	Site of action[b]	Additives	Rate	7 DAT	14 DAT
			g ai ha^{-1}	———% control———	
Chlorimuron	ALS	NIS + UAN	11.2	8 c	6 e
Imazethapyr	ALS	COC + UAN	70	12 c	6 de
Glyphosate	EPSPS	AMS	840[c]	88 a	89 a
Glufosinate	GS	AMS	450	81 a	68 b
Mesotrione	HPPD	COC + UAN	105	30 b	19 cd
Aciflurofen	PPO	COC + UAN	420	86 a	75 ab
Fomesafen	PPO	COC + UAN	395	93 a	89 a
Lactofen	PPO	COC + UAN	140	90 a	87 a
Atrazine	PSII	COC + UAN	1680	8 c	8 de
2,4-D	Synthetic auxin	–	270[c]	23 bc	16 cde
Dicamba	Synthetic auxin	NIS	280[c]	33 b	26 c

[a] Trial was planted to soybean.

[b] Abbreviations: ALS, acetolactate synthase; EPSPS, 5-enolpyruvylshikimate-3-phosphate synthase; GS, glutamine synthetase; HPPD, 4-hydroxyphenylpyruvate dioxygenase; PPO, protoporphyrinogen oxidase; PSII, photosystem II; NIS, nonionic surfactant at 0.25% (v/v); UAN, 28% urea ammonium nitrate at 2.5% (v/v); COC, crop oil concentrate at 1% (v/v); AMS, ammonium sulfate at 2.5% (v/v).

[c] Acid equivalent (g ae ha^{-1}).

decreased common lambsquarters (*Chenopodium album* L.) control with mesotrione plus atrazine between years, partially because of increased weed height one year. Although waterhemp control with mesotrione alone or combined with atrazine was greatest following the 2 to 5–cm application timing, many treated waterhemp plants recovered and resumed growth. Additional management practices, such as interrow cultivation or utilization of a different site-of-action herbicide, would be needed to reduce additional weed interference and seed production. More waterhemp plants survived following herbicide applications made at later growth stages ($\geq$ 5 cm), reflected in the estimates of control (Table 3). The results of the present experiments demonstrate foliar-applied HPPD inhibitors are not viable options for control of this Illinois waterhemp population. Additional research was undertaken to determine what other foliar-applied herbicides remain viable options for control of this population.

Response to Herbicides from Other Site-of-Action Groups under Field Conditions. By 7 DAT, control of MCR was greatest with acifluorfen, fomesafen, lactofen, glyphosate, and glufosinate, ranging from 81 to 93% (Table 4). Poor control with mesotrione (30%) and atrazine (8%) was consistent with previously reported results

(Hausman et al. 2011). 2,4-D and dicamba provided less than 35% control, and control with the ALS inhibitors chlorimuron and imazethapyr was 12% or less. At 14 DAT, the PPO inhibitors and glyphosate provided the greatest waterhemp control (75 to 89%). Control with glufosinate (68%) was less than glyphosate and all PPO inhibitors except aciflurofen. Waterhemp control ranged from 16 to 26% with mesotrione, 2,4-D, and dicamba, whereas control with atrazine, chlorimuron, or imazethapyr was less than 10%.

The response of MCR to atrazine and ALS inhibitors was not unexpected. A survey of 59 Illinois waterhemp populations in 1998 to 1999 revealed 22% of populations contained individuals displaying resistance to both PSII and ALS inhibitors (Patzoldt et al. 2002). Obligate outcrossing coupled with production of large quantities of mobile seeds (Tranel et al. 2010) has undoubtedly aided the spread of these resistance traits throughout Illinois over the last decade. Additionally, the McLean field was treated with soil- and foliar-applied triazine herbicides for at least seven consecutive years (Hausman et al. 2011), which likely increased the selection for triazine-resistant waterhemp. Guo et al. (2015) reported that MCR contains both target and non–target-site mechanisms that confer resistance to ALS inhibitors.

Table 5. Mean visual estimates of control of HPPD-inhibitor resistant (MCR) and sensitive (ACR and WCS) waterhemp populations 7, 14, and 21 d after treatment (DAT) under greenhouse conditions. Means sharing the same letter within their respective evaluation period (7, 14, or 21 DAT) are not significantly different at α = 0.05 (separated by the SAS macro *%pdmix800*).

Herbicide	Site of action[a]	Rate	7 DAT			14 DAT			21 DAT		
		g ai ha^{-1}				—% control—					
			WCS	ACR	MCR	WCS	ACR	MCR	WCS	ACR	MCR
Chlorimuron	ALS	11.2	88 abc	2 hi	13 g	89 abcd	1 gh	10 fg	91 a	0 ef	7 ef
Imazethapyr	ALS	70	89 abc	0 i	11 gh	89 abcd	0 gh	7 gh	90 a	0 ef	4 ef
Glyphosate	EPSPS	840[b]	96 a	96 a	76 d	100 a	100 a	75 d	100 a	100 a	70 c
Glufosinate	GS	450	95 a	94 ab	92 a	100 a	96 ab	93 a	100 a	97 a	93 a
Mesotrione	HPPD	105	91 ab	88 abc	64 e	97 ab	95 abc	46 e	100 a	100 a	26 d
Lactofen	PPO	140	96 a	48 f	88 ab	100 a	24 f	81 cd	100 a	18 de	75 bc
Atrazine	PSII	1680	91 ab	4 ghi	4 i	98 ab	2 gh	2 h	100 a	1 ef	1 f
Dicamba	Synthetic auxin	280[b]	82 bcd	86 abcd	81 c	84 abcd	86 abcd	84 bc	88 ab	90 a	87 a

[a] Abbreviations: ALS, acetolactate synthase; EPSPS, 5-enolpyruvylshikimate-3-phosphate synthase; GS, glutamine synthetase; HPPD, 4-hydroxyphenylpyruvate dioxygenase; PPO, protoporphyrinogen oxidase; PSII, photosystem II.

[b] Acid equivalent (g ae ha^{-1}).

Reduced waterhemp control with glufosinate 14 DAT was attributed primarily to regrowth of treated plants (Table 4). Hoss et al. (2003) reported similar findings, with glyphosate providing greater waterhemp control than glufosinate 14 DAT because of recovery of glufosinate-treated plants. Although a small number of PPO-inhibitor–treated plants displayed some regrowth, the magnitude of recovery was less than that of glufosinate-treated waterhemp (personal observation). Hager et al. (2003) observed a similar response when a small percentage of PPO-inhibitor–treated waterhemp plants recovered from lower leaf axils 14 DAT with the same PPO-inhibiting herbicides evaluated in the present research. PPO inhibitors and glufosinate cause rapid lipid peroxidation and cell-membrane destruction, but are not extensively translocated throughout the plant (Hess 2000; Matsumoto 2002). Thorough spray coverage is essential for these herbicides to control weeds, and the high in-field waterhemp density at the McLean field location (Hausman et al. 2013) might have prevented thorough spray coverage on a small percentage of plants. Waterhemp treated with the synthetic auxins dicamba or 2,4-D displayed epinasty and leaf malformation; however, little stand reduction or mortality of treated plants had occurred by 14 DAT (Table 4). In order to eliminate the potentially confounding effects of varying climatic conditions and nonuniform spray coverage, additional research to characterize the response of the MCR population to various herbicide site-of-action groups was conducted under controlled greenhouse conditions.

Response to Herbicides from other Site-of-Action Groups under Greenhouse Conditions. Mean visual estimates of control for WCS, ACR, and MCR by treatment are presented in Table 5. MCR response to mesotrione in the greenhouse was analogous to field results. Injury was greatest by 7 DAT (64%), and thereafter declined over subsequent evaluations (26% by 21 DAT). By 14 DAT, plants were demonstrating signs of recovery from initial injury, manifested by emergence of new, noninjured leaf tissue near the apical meristem and resumption of growth. Conversely, MCR plants treated with glyphosate, glufosinate, or dicamba did not demonstrate signs of recovery from 7 to 14 DAT. Atrazine, chlorimuron, and imazethapyr provided the least control of MCR, ranging from 1 to 7% 21 DAT, whereas control with glyphosate, lactofen, dicamba, and glufosinate ranged from 70 to 93%. MCR dry weight data revealed the highest amount of accrued plant biomass occurred in the plants treated with atrazine, imazethapyr, or chlorimuron (Figure 1).

MCR biomass following glyphosate application was significantly higher than WCS and ACR; however, MCR dry weight in this treatment accounted for approximately only 10% of the untreated (Figure 1). The overall magnitude of this difference was small in comparison to the response difference of MCR and ACR with WCS to atrazine
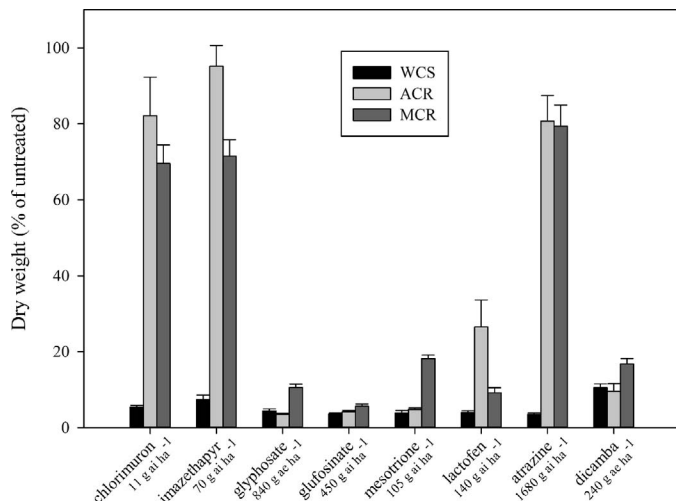
Figure 1. Responses of HPPD-inhibitor resistant (MCR) and sensitive (ACR and WCS) waterhemp populations following foliar applications of herbicides representing seven site-of-action groups under greenhouse conditions. Data are dry weights collected 21 d after treatment (DAT), and presented as a percent of the untreated (± 1 SEM).

or ALS inhibitors. MCR had the same mean biomass as WCS and ACR following treatment with glufosinate or dicamba (Figure 1). Visual estimates of dicamba injury and injury symptomatology (epinasty and leaf malformation) were generally similar among all three biotypes (Table 5). Estimates for dicamba control of MCR were much greater in the greenhouse than the field, perhaps because more emphasis was placed on evaluating individual plants rather than total biomass reduction in the field.

All MCR plants treated with mesotrione (96 plants in total across two runs of the greenhouse experiment) survived to 21 DAT (when plants were harvested), though the amount of injury varied from plant to plant. Conversely, all replicates (eight plants in total across two runs of the greenhouse experiment) of WCS and ACR were completely controlled with mesotrione by 21 DAT (Table 5). Additionally, the accumulated biomass of MCR following treatment with mesotrione was greater than WCS and ACR. Biomass accumulation of MCR and WCS plants treated with lactofen was significantly less (Figure 1) compared with ACR, a population known to be resistant to PPO-inhibiting herbicides (Patzoldt et al. 2005).

These results demonstrate MCR is less sensitive to HPPD, PSII, and ALS inhibitors and support the conclusions reached by Hausman et al. (2011), Ma

et al. (2013), and Guo et al. (2015). Furthermore, results indicate a high percentage of plants survive when labeled use rates of HPPD inhibitors are applied to 10 to 15–cm-tall waterhemp. Mesotrione alone or in combination with atrazine applied at the 2 to 5–cm application timing increased control of MCR, although numerous plants survived. In general, PPO inhibitors, glufosinate, and glyphosate provided the greatest control of MCR in both field and greenhouse experiments. HPPD inhibitor resistance is present in multiple states (Heap 2013) and could increase with additional selection intensity (Allen et al. 2011).

Acknowledgments

Literature Cited

Allen J, Hinz J, Essner R, Fischer J, Van Wert S (2011) Introducing a new soybean event with glyphosate and HPPD tolerance. Proc North Central Weed Sci Soc 66:144

Bell MS, Hager AG, Tranel PJ (2013) Multiple resistance to herbicides from four site-of-action groups in waterhemp (*Amaranthus tuberculatus*). Weed Sci 61:460–468

Buhler DD, Hartzler RG (2001) Emergence and persistence of seed of velvetleaf, common waterhemp, woolly cupgrass, and giant foxtail. Weed Sci 49:230–235

Burnside OC, Wilson RG, Weisberg S, Hubbard KG (1996) Seed longevity of 41 weed species buried 17 years in eastern and western Nebraska. Weed Sci 44:74–86

Foes MJ, Liu LX, Tranel PJ, Wax LM, Stoller EW (1998) A biotype of common waterhemp (*Amaranthus rudis*) resistant to triazine and ALS herbicides. Weed Sci 46:514–520

Guo J, Riggins CW, Hausman NE, Hager AG, Riechers DE, Davis AS, Tranel PJ (2015) Non-target site resistance to ALS inhibitors in waterhemp (*Amaranthus tuberculatus*). Weed Sci 63:399–407

Hager AG, Wax LM, Bollero GA, Stoller EW (2003) Influence of diphenylether herbicide application rate and timing on common waterhemp (*Amaranthus rudis*) control in soybean (*Glycine max*). Weed Technol 17:14–20

Hager AG, Wax LM, Simmons FW, Stoller EW (1997) Waterhemp management in agronomic crops. Univ Ill Bull 855:12

Hager AG, Wax LM, Stoller EW, Bollero GA (2002) Common waterhemp (*Amaranthus rudis*) interference in soybean. Weed Sci 50:607–610

Hartzler RG, Battles B, Nordby D (2004) Effect of common waterhemp (*Amaranthus rudis*) emergence date on growth and fecundity in soybean. Weed Sci 52:242–245

Hartzler RG, Buhler DD, Stoltenberg DE (1999) Emergence characteristics of four annual weed species. Weed Sci 47:578–584

Hausman NE, Singh S, Tranel PJ, Riechers DE, Kaundun SS, Polge ND, Thomas DA, Hager AG (2011) Resistance to HPPD-inhibiting herbicides in a population of waterhemp (*Amaranthus tuberculatus*) from Illinois, United States. Pest Manag Sci 67:258–261

Hausman NE, Tranel PJ, Riechers DE, Maxwell DJ, Gonzini LC, Hager AG (2013) Responses of an HPPD inhibitor-resistant waterhemp (*Amaranthus tuberculatus*) population to soil-residual herbicides. Weed Technol 27:704–711

Heap IM (2013) International Survey of Herbicide Resistant Weeds. http://www.weedscience.org. Accessed April 10, 2013

Hess DF (2000) Light-dependent herbicides: an overview. Weed Sci 48:160–170

Hoss NE, Al-Khatib K, Peterson DE, Loughin TM (2003) Efficacy of glyphosate, glufosinate, and imazethapyr on selected weed species. Weed Sci 51:110–117

Johnson BC, Young BG, Matthews JL (2002) Effect of postemergence application rate and timing of mesotrione on corn (*Zea mays*) response and weed control. Weed Technol 16:414–420

Lally NG, Thompson CR, Peterson D (2010) Palmer amaranth differential response to pyrasulfotole & bromoxynil. Proc North Central Weed Sci Soc 65:68

Ma R, Kaundun SS, Tranel PJ, Riggins CW, McGinness DL, Hager AG, Hawkes T, McIndoe E, Riechers DE (2013) Distinct detoxification mechanisms confer resistance to mesotrione and atrazine in a population of waterhemp. Plant Physiol 163:363–377

Matsumoto H (2002) Inhibitors of Protoporphyrinogen oxidase: a brief update. Pages 151–161 *in* Boger P, Wakabayashi K, Hirai K eds. Herbicide Classes in Development. New York, NY: Springer-Verlag

McMullan PM, Green JM (2011) Identification of a tall waterhemp (*Amaranthus tuberculatus*) biotype resistant to HPPD-inhibiting herbicides, atrazine, and thifensulfuron in Iowa. Weed Technol 25:514–518

Patzoldt WL, Tranel PJ, Hager AG (2002) Variable herbicide responses among Illinois waterhemp (*Amaranthus rudis* and *A. tuberculatus*) populations. Crop Prot 21:707–712

Patzoldt WL, Tranel PJ, Hager AG (2005) A waterhemp (*Amaranthus tuberculatus*) biotype with multiple resistance across three herbicide sites of action. Weed Sci 53:30–36

Sauer J (1955) Revision of the dioecious *amaranths*. Madrono 13:5–46

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

Saxton AM (1998) A macro for converting mean separation output to letter groupings in Proc Mixed. Proc. Ann SAS Users Group Int 23:1243–1246

Steckel LE, Sprague CL (2004) Common waterhemp (*Amaranthus rudis*) interference in corn. Weed Sci 52:359–364

Steckel LE, Sprague CL, Hager AG, Simmons FW, Bollero GA (2003) Effects of shading on common waterhemp (*Amaranthus rudis*) growth and development. Weed Sci 51:898–903

Tranel PJ, Riggins CW, Bell MS, Hager AG (2010) Herbicide resistances in *Amaranthus tuberculatus*: a call for new options. J Agric Food Chem 59:5808–5812

Vyn JD, Swanton CJ, Weaver SE, Sikkema PH (2006) Control of *Amaranthus tuberculatus var. rudis* (common waterhemp) with pre and postemergence herbicides in *Zea mays* L. (maize). Crop Prot 25:1051–1056

Woodyard AJ, Bollero GA, Riechers DE (2009) Broadleaf weed management in corn utilizing synergistic postemergence herbicide combinations. Weed Technol 23:513–518