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Characterization of a waterhemp (*Amaranthus tuberculatus*) population from Illinois resistant to herbicides from five site-of-action groups

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Research Article

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Abstract

Experiments were initiated to characterize a waterhemp population (CHR) discovered in a central Illinois corn field after it was not controlled by the 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor topramezone. Field experiments conducted during 2014–2015 indicated that acetolactate synthase (ALS)-, protoporphyrinogen oxidase (PPO)-, photosystem II (PSII)-, and HPPD-inhibiting herbicides and the synthetic auxin 2,4-D did not control the CHR population. Laboratory experiments confirmed target site–based resistance mechanisms to ALS- and PPO-inhibiting herbicides. Herbicide doses required to reduce dry biomass 50% (GR_{50}) were determined in greenhouse dose–response experiments, and indicated 16-fold resistance to the HPPD inhibitor mesotrione, 9.5-fold resistance to the synthetic auxin 2,4-D, and 252-fold resistance to the PSII inhibitor atrazine. Complementary results from field, laboratory, and greenhouse investigations indicate that the CHR population has evolved resistance to herbicides from five sites of action (SOAs): ALS-, PPO-, PSII-, and HPPD-inhibiting herbicides and 2,4-D. Herbicide use history for the field in which CHR was discovered indicates no previous use of 2,4-D.

Introduction

Waterhemp is a small-seeded, dioecious, summer annual broadleaf species native to the midwestern United States (Sauer 1955). This competitive weed species reduced soybean [*Glycine max* (L.) Merr.] seed yield more than 40% when not controlled for 10 wk (Hager et al. 2002) and decreased corn yield 74% with season-long interference (Steckel and Sprague 2004). Individual female waterhemp plants can produce in excess of one million seeds (Hartzler et al. 2004), which can remain dormant in the soil for years (Burnside et al. 1996; Hartzler et al. 1999).

Waterhemp populations at different locations have evolved resistances to herbicides from six site-of-action (SOA) groups, including inhibitors of acetolactate synthase (ALS) (Guo et al. 2015), photosystem II (PSII) (Patzoldt et al. 2003), protoporphyrinogen oxidase (PPO) (Shoup et al. 2003), 4-hydroxyphenylpyruvate dioxygenase (HPPD) (Hausman et al. 2011), 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) (Zelaya and Owen 2005), and synthetic auxins (Bernards et al. 2012). A survey of 59 Illinois waterhemp populations indicated that 90% contained resistance to ALS inhibitors and 25% were resistant to herbicides from multiple SOA groups (Patzoldt et al. 2002).

Growers generally control an herbicide-resistant population by applying herbicides with alternative SOAs. This strategy, however, has the potential to select for the evolution of plants with multiple-herbicide resistances. Previously, a waterhemp population resistant to EPSPS-, PSII-, PPO-, and ALS-inhibiting herbicides was reported (Bell et al. 2013), and more recently Shergill et al. (2018) confirmed resistance to herbicides from six site-of-action groups in a Missouri waterhemp population. Populations resistant to multiple-herbicide SOAs often have limited chemical control options (Bell et al. 2009; Patzoldt et al. 2005). The increase in herbicide-resistant waterhemp populations has contributed to an increased presence of this species in Illinois agronomic fields during the last two decades (Hager et al. 1997).

Several strategies have been proposed to reduce the likelihood of evolving multiple herbicide–resistant populations. Use of integrated weed management systems, including the use of dissimilar soil-residual herbicides along with foliar-applied herbicides, is essential for waterhemp control (Hager et al. 1997). Recent research revealed that combining multiple, effective herbicide SOAs in mixtures significantly reduced the selection for glyphosate-resistant waterhemp compared to annual herbicide rotation (Evans et al. 2016).

Resistance to 2,4-D or dicamba in waterhemp currently is relatively rare (Heap 2018), but commercialization of soybean and cotton (*Gossypium hirsutum* L.) varieties resistant to these synthetic auxin herbicides probably will increase their use and concomitant selection for resistance. Bernards et al. (2012) reported the first occurrence of 2,4-D resistance in a waterhemp population discovered in a warm-season grass production field that had received annual applications of 2,4-D since 1996. Recently, a Missouri waterhemp population with 3-fold resistance to 2,4-D was identified in a field dedicated to continuous soybean production and a history of 2,4-D use prior to soybean planting (Shergill et al. 2018). Multiple resistance in waterhemp, including resistance to synthetic auxins and other herbicides that control waterhemp, would reduce the number of effective herbicide combinations.

We present here the characterization of an Illinois waterhemp population exhibiting a novel, five-way combination of herbicide SOA resistances: ALS, HPPD, PPO, and PSII inhibitors, and the synthetic auxin 2,4-D. Our objectives were to: (1) measure the efficacy of corn and soybean herbicides commonly used in Illinois on the study population under field conditions; (2) investigate whether combinations of HPPD and PSII inhibitors could increase control of the population; (3) determine if resistance to ALS, PPO, and PSII inhibitors was conferred by changes in the respective target site proteins; and (4) quantify the degree of resistance to the HPPD inhibitor mesotrione, the PSII inhibitor atrazine, and the synthetic auxin 2,4-D. To address these objectives, we performed a series of field and controlled-environment studies on plants from this population.

Materials and methods

Study population and site description

In 2012 a putative multiple herbicide–resistant waterhemp population (designated CHR) was discovered in a Champaign County, IL, field after it was not controlled by topramezone. The field had an annual rotation of non-GMO corn and glyphosate-resistant soybean. Herbicide use history records indicate that herbicides from various SOA groups (HPPD, PPO, ALS, and PSII inhibitors) had been used. The soil is a Flanagan silt loam (fine, smectitic, mesic Aquic Argiudolls) with a pH of 5.5, cation exchange capacity of 19.5 mEq 100 g^{-1} soil, and organic matter content of 4.8%.

Response of CHR to herbicides: field experiments

General methods

Field experiments were conducted in 2014 and 2015 at the location where CHR was initially identified. Preplant tillage was performed each spring to prepare the seedbed for planting and control any existing vegetation. Experiments were conducted in either corn (DKC62-77RIB) or soybean (Asgrow 3231 RR2), planted in rows spaced 76 cm apart. Planting dates in 2014 were May 7 for corn and May 26 for soybean, and in 2015 May 14 for corn and May 22 for soybean. Treatments were arranged in a randomized complete block design with three replications, and each replication was a 3- by 7.6-m plot that included four crop rows. Herbicides were applied using a pressurized CO_2 backpack sprayer equipped with Teejet (TeeJet Technologies, P.O. Box 7900, Wheaton, IL) AI110025 nozzles for soil applications or AIXR110025 nozzles for foliar applications, spaced 51 cm apart on a 3-m boom calibrated to deliver 187 L ha^{-1} at 276 kPa. Crop planting preceded application of soil-applied herbicides on the same day. An assortment of herbicide active ingredients routinely applied in Illinois corn and soybean crops was selected for evaluation and included those to which CHR was hypothesized to be resistant or sensitive.

Foliar-applied herbicides

Various foliar-applied corn (Table 1) and soybean (Table 2) herbicide treatments were evaluated. Herbicides were applied when waterhemp plants were 8–10 cm tall at 1× and 2× label-recommended rates (Tables 3 and 4) with label-recommended spray additives. Corn growth stages at the times of application were V4 (2014) and V5 (2015), whereas soybean was at V3 each year.

Prior to application, five uniformly sized waterhemp plants per plot (15 per treatment) were marked by placing a wooden garden stake near each plant. These plants were subsequently harvested 21 d after treatment (DAT) to evaluate each treatment's effect on aboveground biomass. Additionally, 15 plants were harvested from nontreated plots the day of application to determine pretreatment aboveground biomass. Harvested plants were dried at 65 C for 7 d, and dry biomass was recorded. Herbicide efficacy was visibly evaluated and recorded 21 DAT using a scale of 0 (no control) to 100% (complete control). Ratings assessed plant injury, biomass, and stand reduction, and any recovery of treated plants.

Soil-applied herbicides

CHR responses to soil-applied corn (Table 6) and soybean (Table 7) herbicide treatments were evaluated. Herbicides were applied at 0.5, 1, and 2× label-recommended rates for soil type and organic matter content. Emerged waterhemp plants per square meter were counted 28 and 42 DAT from a consistent quadrat location in the middle of each plot. Visible estimates of weed control, presented as percent control compared with a nontreated plot, were also recorded 28 and 42 DAT.

Statistical analysis of field experiments

Linear mixed-effects models, with percent control, plant biomass, or plant density as the response variable and herbicide treatment as the independent variable, were used to quantify treatment effectiveness. Herbicide treatment was considered a fixed effect, whereas year and block nested within year were considered random effects. Statistical analysis for all field experiments was performed using PROC GLM in SAS 9.2 (Statistical Analysis Software (SAS) 9.2. SAS Institute, Inc., 100 SAS Campus Drive, Cary, NC). Treatment means for all metrics were separated by LSD utilizing PROC GLM in SAS. Differences between dry biomass of treated plants (harvested 21 DAT) and dry biomass of pretreatment plants were also calculated. Difference values were compared to determine plant recovery and regrowth following treatment.

Greenhouse herbicide dose–response experiments

Waterhemp populations

Inflorescences from 25 female CHR waterhemp plants not controlled with lactofen were collected from the field in August 2013 and dried at room temperature. Seeds were first surface sterilized by a 10-min treatment with 1:1 commercial bleach (Clorox, The Clorox Company, 1221 Broadway, Oakland, CA)–water solution, then washed twice with sterilized deionized water, suspended in 0.15% (w/w) agarose, and stored at 4 C to improve seed germination (Bell et al. 2013). Plants grown from collected seed were treated with ALS-, PSII-, and HPPD-inhibiting herbicides, and

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Table 1. Herbicides evaluated, respective site-of-action (SOA) groups, application rates, and source information for corn (*Zea mays*) herbicide field experiments, Champaign Co., IL (2014–2015).

Common name	Trade name	SOA[a]	WSSA Grouping	Application rate (g ai ha^{-1})	Manufacturer
Foliar-applied					
Mesotrione	Callisto	HPPD	27	105 210	Syngenta Crop Protection, Greensboro, NC
Tembotrione	Laudis	HPPD	27	92 184	Bayer CropScience, St. Louis, MO
Topramezone	Impact	HPPD	27	18 36	AMVAC Chemical Corp., Los Angeles, CA
Mesotrione + atrazine	Calisto + AAtrex	HPPD + PSII	27 + 5	105 + 560 210 + 560	Syngenta Crop Protection, Greensboro, NC
Tembotrione + atrazine	Laudis + AAtrex	HPPD + PSII	27 + 5	92 + 560 184 + 560	Bayer CropScience + Syngenta Crop Protection
Topramezone + atrazine	Impact + AAtrex	HPPD + PSII	27 + 5	18 + 560 36 + 560	AMVAC Chemical Corp. + Syngenta Crop Protection
Atrazine	AAtrex	PSII	5	1,680 3,360	Syngenta Crop Protection, Greensboro, NC
2,4-D	2,4-D, DMA salt	Auxin receptor	4	560[c] 1,120[c]	Nufarm Inc., Alsip, IL
Dicamba	Clarity	Auxin receptor	4	560[c] 1,120[c]	BASF Corp. Agricultural Products, Research Triangle Park, NC
Glyphosate	Roundup Powermax	EPSPS	9	840[c] 1,680[c]	Bayer CropScience, St. Louis, MO
Glufosinate	Liberty	GS	10	448 896	Bayer CropScience, St. Louis, MO
Soil-applied					
Mesotrione	Callisto	HPPD	27	105 210 420	Syngenta Crop Protection, Greensboro, NC
Isoxaflutole	Balance Flexx	HPPD	27	53 105 210	Bayer CropScience, St. Louis, MO
Atrazine	AAtrex	PSII	5	1,120 2,240 4,480	Syngenta Crop Protection, Greensboro, NC
Dicamba	Clarity	Auxin receptor	4	280[c] 560[c] 1,120[c]	BASF Corp. Agricultural Products, Research Triangle Park, NC
Saflufenacil	Sharpen	PPO	14	37 75 150	BASF Corp. Agricultural Products, Research Triangle Park, NC
Acetochlor[b]	Warrant	VLCFA	15	627 1,255 2,510	Bayer CropScience, St. Louis, MO
Acetochlor	Harness	VLCFA	15	1,098 2,196 4,392	Bayer CropScience, St. Louis, MO
S-metolachlor	Dual II Magnum	VLCFA	15	795 1,591 3,182	Syngenta Crop Protection, Greensboro, NC

[a] Abbreviations for SOA: EPSPS, enolpyruvylshikimate-3-phosphate synthase; GS, glutamine synthetase; HPPD, 4-hydrophenylpyruvate dioxygenase; PPO, protoporphyrinogen oxidase; PSII, photosystem II; VLCFA, very-long-chain fatty acid.
[b] Encapsulated formulation.
[c] Acid equivalent (g ae ha^{-1}).

survivors were crossed to generate multiple accessions. These crosses were performed to reduce variability in all subsequent dose–response experiments similar to crosses described in other research (Hausman et al. 2013; Varanasi et al. 2018). The accession 'CHR-M6' was selected from the initial crosses for greenhouse experiments because of its ample seed supply and high percent germination; note that although using the CHR-M6 accession facilitated the quantification of different resistance types occurring within the CHR population, it precluded the determination of the degree to which different resistances overlapped among individuals within the CHR source population. The response of CHR-M6 plants was compared with other sensitive and resistant waterhemp populations (described subsequently) in three separate dose–response experiments.

Greenhouse plant culture

All plants were germinated from seeds sown in 12- by 12-cm flats containing a commercial potting medium (LC1 Sun Gro Horticulture, 15831 N.E. 8th Street, Bellevue, WA). Emerged seedlings (2 cm tall) were transplanted into 7.5-cm-deep plug inserts (one seedling per insert). One week later, plugs were transplanted into 950-cm^3 pots containing a 3:1:1:1 mixture of potting mix–soil–peat–sand that included a slow-release fertilizer (Scotts Osmocote Classic 13–13–13, The Scotts Company,

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Table 2. Herbicides evaluated, respective site-of-action (SOA) groups, application rates, and source information for soybean (*Glycine max*) herbicide field experiments, Champaign Co., IL (2014–2015).

Common name	Trade name	SOA[a]	WSSA Grouping	Application rate (g ai ha^{-1})	Manufacturer
Foliar-applied					
Chlorimuron	Classic	ALS	2	13 26	Corteva Agriscience, Indianapolis, IN
Imazethapyr	Pursuit	ALS	2	71 141	BASF Corp. Agricultural Products, Research Triangle Park, NC
Acifluorfen	Ultra Blazer	PPO	14	280 560	United Phosphorus Inc, King of Prussia, PA
Fomesafen	Flexstar	PPO	14	347 683	Syngenta Crop Protection, Greensboro, NC
Lactofen	Cobra	PPO	14	218 437	Valent U.S.A. Corp., Walnut Creek, CA
Saflufenacil	Sharpen	PPO	14	25 49	BASF Corp. Agricultural Products, Research Triangle Park, NC
Glyphosate	Roundup Powermax	EPSPS	9	840[b] 1681[b]	Bayer CropScience, St. Louis, MO
Glufosinate	Liberty	GS	10	594 1188	Bayer CropScience, St. Louis, MO
Soil-applied					
Flumioxazin	Valor	PPO	14	36 72 143	Valent U.S.A. Corp., Walnut Creek, CA
Saflufenacil	Sharpen	PPO	14	37 75 150	BASF Corp. Agricultural Products, Research Triangle Park, NC
Sulfentrazone	Spartan	PPO	14	175 350 700	FMC Corp., Philadelphia, PA
Chlorimuron	Classic	ALS	2	13 26 52	Corteva Agriscience, Indianapolis, IN
Pendimethalin	Prowl H_20	Mitotic disrupter	3	532 1064 2129	BASF Corp. Agricultural Products, Research Triangle Park, NC
Metribuzin	TriCor DF	PSII	5	280 560 1120	United Phosphorus Inc, King of Prussia, PA
Dimethenamid-*P*	Outlook	VLCFA	15	420 840 1681	BASF Corp. Agricultural Products, Research Triangle Park, NC
S-metolachlor	Dual Magnum	VLCFA	15	795 1591 3182	Syngenta Crop Protection, Greensboro, NC
Pyroxasulfone	Zidua	VLCFA	15	59 119 238	BASF Corp. Agricultural Products, Research Triangle Park, NC

[a] Abbreviations for SOA: ALS, acetolactate synthase; EPSPS, enolpyruvylshikimate-3-phosphate synthase; GS, glutamine synthetase; HPPD, 4-hydrophenylpyruvate dioxygenase; PPO, protoporphyrinogen oxidase; PSII, photosystem II; VLCFA, very-long-chain fatty acid.
[b] Acid equivalent (g ae ha^{-1}).

14111 Scottslawn Rd., Marysville, OH). Greenhouse conditions were maintained at 28 C/22 C during the 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.

Herbicide application

All herbicides were applied using a moving-nozzle, compressed-air research spray chamber (Generation III Research Sprayer; DeVries Manufacturing, 28081 870th Avenue, Hollandale, MN) with an adjustable platform and equipped with an 80015EVS even flat-spray nozzle (Teejet Technologies, Wheaton, IL). The nozzle was positioned approximately 45 cm above the plant canopy, and the sprayer was calibrated to deliver 185 L ha^{-1} at 275 kPa. Treatments were applied to all replications in order from lowest to highest dose.

Mesotrione, 2,4-D, and atrazine dose–response experiments

The response of CHR-M6 to foliar-applied mesotrione was compared to another HPPD-resistant population from Illinois (NH40) that is also resistant to ALS- and PSII-inhibiting herbicides (Hausman et al. 2011, 2013, 2016). Two populations sensitive to HPPD inhibitors (WUS and BCR) were included for comparison. WUS was collected in Brown County, OH, and is not resistant to herbicides, whereas BCR is from Brown County, IL, and is resistant to EPSPS-, ALS-, PPO-, and PSII-inhibiting herbicides (Bell et al. 2013). CHR-M6, NH40, and WUS were evaluated in atrazine dose–response experiments, whereas the response of CHR-M6 to 2,4-D was compared to WUS and a Nebraska waterhemp population (designated NE) resistant to 2,4-D (Bernards et al. 2012).

Uniformly sized plants (10 cm tall) from the respective populations (CHR-M6, NH40, BCR, WUS, and NE) were treated with

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Table 3. Waterhemp control, dry biomass, and estimated difference between dry biomass of treated plants harvested 21 d after treatment (DAT) and dry biomass of pretreatment plants in foliar-applied soybean herbicide experiments (2014–2015).

		21 DAT			
Herbicide	Rate	Control	Dry biomass	Estimated difference[a]	
	g ai ha^{-1}	%	g		(Pr>F)
Chlorimuron	13	0	11.11	10.688	<0.0001*
	26	0	11.11	10.679	<0.0001*
Imazethapyr	71	0	12.85	12.423	<0.0001*
	141	0	10.17	9.742	<0.0001*
Acifluorfen	280	35	6.18	5.752	<0.0001*
	560	40	4.09	3.665	<0.0001*
Fomesafen	347	49	3.17	2.741	0.0025*
	683	58	2.93	2.507	0.0057*
Lactofen	218	49	3.28	2.851	0.0017*
	437	55	2.63	2.203	0.0150*
Saflufenacil	25	60	4.47	4.039	<0.0001*
	49	70	1.32	0.891	0.3239
Glyphosate	840[b]	94	0.11	–0.316	0.7264
	1,681[b]	97	0.07	–0.362	0.6885
Glufosinate	594	81	0.38	–0.043	0.9620
	1,188	91	0.11	–0.321	0.7223
Nontreated	–	–	12.43	12.002	<0.0001*
Nontreated at app.[c]	–	–	.43	–	–
LSD[d]		9.4	2.5		

[a] Estimated difference in dry biomass between herbicide-treated plants and pretreatment plants.
*Significant at α = 0.05; treatments with positive differences indicate growth following herbicide application.
[b] Acid equivalent (g ae ha^{-1}).
[4] Plants harvested prior to treatment application to assess biomass accumulation following herbicide application.
[d] Separated by PROC GLM in SAS, α = 0.05.

Table 4. Waterhemp control, dry biomass, and estimated difference between dry biomass of treated plants harvested 21 d after treatment (DAT) and dry biomass of pretreatment plants in foliar-applied corn herbicide experiments (2014–2015).

		21 DAT			
Herbicide	Rate	Control	Dry biomass	Estimated difference[a]	
	g ai ha^{-1}	%	g		(Pr>F)
Mesotrione	105	66	0.26	0.033	0.8575
	210	76	0.23	0	1.0000
Tembotrione	92	57	0.42	0.188	0.3074
	184	69	0.23	0.002	0.9928
Topramezone	18	30	0.88	0.656	0.0004*
	36	49	0.47	0.245	0.1822
Mesotrione + atrazine	105 + 560	78	0.14	–0.092	0.6180
	210 + 560	88	0.15	–0.074	0.6872
Tembotrione + atrazine	92 + 560	76	0.2	–0.029	0.8746
	184 + 560	90	0.15	–0.082	0.6555
Topramezone + atrazine	18 + 560	48	0.6	0.374	0.0423*
	36 + 560	64	0.2	–0.031	0.8675
Atrazine	1,680	0	2.66	2.437	<0.0001*
	3,360	3	2.15	1.917	<0.0001*
2,4-D	560[b]	22	2.85	2.619	<0.0001*
	1,120[b]	36	1.86	1.627	<0.0001*
Dicamba	560[b]	80	0.94	0.713	0.0001*
	1,120[b]	94	0.55	0.323	0.0792
Glyphosate	840[b]	90	0.14	–0.092	0.6180
	1,680[b]	97	0.16	–0.069	0.7060
Glufosinate	448	75	0.33	0.102	0.5789
	896	90	0.12	–0.106	0.5654
Nontreated	–	–	5.07	4.841	<.0001
Nontreated at app.[c]	–	–	0.228	–	–
LSD[d]		9.7	0.51		

[a] Estimated difference in dry biomass between herbicide-treated plants and pretreatment plants.
*Significant at α = 0.05; treatments with positive differences indicate growth following herbicide application.
[b] Acid equivalent (g ae ha^{-1}).
[c] Plants harvested prior to treatment application to assess biomass accumulation following herbicide application.
[d] Separated by PROC GLM in SAS, α = 0.05.

herbicide at increasing doses equally spaced along a base 3.16 (mesotrione), 2 (2,4-D), or 2.5 (atrazine) logarithmic scale, resulting in 9, 10, and 9 herbicide doses for mesotrione, 2,4-D, and atrazine, respectively, and one nontreated control for each population (Seefeldt et al. 1995). Mesotrione doses applied to HPPD-sensitive populations (BCR and WUS) ranged from 0.1 to 1,050 g ha^{-1}, whereas the doses applied to CHR-M6 and NH40 ranged from 1 to 10,500 g ha^{-1}. Crop oil concentrate (Herbimax; Loveland Products, Inc., 3005 Rocky Mountain Avenue, Loveland, CO) at 1% (v/v) and ammonium sulfate (AMS) (N-PAK AMS; Winfield Solutions, LLC, P.O. Box 64589, St. Paul, MN) at 2.5% (v/v) were included with all treatments containing mesotrione. Doses of 2,4-D dimethylamine salt applied to the sensitive population (WUS) ranged from 4.37 to 2,240 g ae ha^{-1}, and from 140 to 17,926 g ae ha^{-1} for CHR-M6 and NE. Nonionic surfactant (Activator 90; Loveland Products, Inc., P.O. Box 1286, Greeley, CO) at 0.25% (v/v) and AMS (2.5% v/v) were included with all treatments containing 2,4-D. Doses of atrazine applied to the sensitive population (WUS) ranged from 11 to 7,002 g ai ha^{-1}, whereas doses applied to CHR-M6 and NE ranged from 72 to 43,759 g ai ha^{-1}. All treatments containing atrazine included crop oil concentrate (1% v/v) and AMS (2.5% v/v).

Immediately after herbicide application, treated plants were placed on greenhouse benches in a randomized complete block design. Each application dose was replicated eight times, and each dose–response experiment was conducted twice. At 21 DAT, all aboveground plant tissue was harvested, dried at 65 C for 7 d, and dry biomass recorded.

Statistical analysis of dose–response experiments

Dry biomass within each dose was averaged and converted to a percentage of the nontreated control. All dry biomass data generated from two runs of the experiment were pooled, as Levene's test for homogeneity of variance was not significant. Combined data were analyzed using a nonlinear regression model with the 'drc' package in R software (Knezevic et al. 2007). The dose–response model was constructed using Equation 1:

$$y = c + \left(\frac{d - c}{1 + \exp\{b[\log(x) - \log(GR50/GR90)]\}} \right). \quad [1]$$

The four-parameter, nonlinear logistic model is described as follows: b is the slope of the curve, c is the lower limit, d is the upper limit and GR_{50}/GR_{90} is 50% or 90% reduction in dry biomass, respectively, compared with nontreated plants.

Laboratory assays of resistance mechanisms

Resistance mechanisms to ALS-, PPO-, and PSII-inhibiting herbicides. To elucidate waterhemp resistance mechanisms to ALS-, PPO-, or PSII-inhibiting herbicides, genomic DNA was extracted from three CHR-M6 plants, two ALS- or PPO-resistant (positive control) populations, and two ALS- or PPO-sensitive (negative control) populations. Polymerase chain reaction

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(PCR)-based molecular markers were used to detect any polymorphisms in the ALS region encoding amino acid position 574 for each population following previously described methods (Patzoldt and Tranel 2007). An assay to detect the ΔG210 *PPX2* mutation (Lee et al. 2008; Thinglum et al. 2011) was performed utilizing an allele-specific PCR analysis. Detection of resistance mechanisms was investigated by separating the PCR products in a 1.2% agarose gel containing 5 μg ml^{-1} ethidium bromide, then comparing CHR-M6 products with products generated from the positive and negative controls.Resistance to atrazine, a symmetrical triazine, occurs through target site mutation or enhanced herbicide metabolism (Ma et al. 2013; Mengistu et al. 2000; Patzoldt et al. 2003). The Ser264 to Gly target site mutation confers resistance to both symmetrical and asymmetrical triazines (e.g., metribuzin), whereas resistance patterns resulting from enhanced metabolism are less predictable (Shukla and Devine 2008). Observations from field studies indicated soil-applied metribuzin effectively controlled CHR, suggesting a non–target site triazine resistance mechanism. To test this hypothesis, genomic DNA was extracted from three CHR-M6 plants and the entire gene encoding the atrazine target protein (*psbA*) was sequenced to determine if target site mutations were present (Foes et al. 1998).

Results and discussion

Response of CHR to herbicides: field experiments

Foliar-applied herbicides

ALS inhibitors (Group 2). Chlorimuron and imazethapyr did not control CHR 21 DAT regardless of application rate (Table 3). Plants treated with either rate of these herbicides had significant biomass increases 21 DAT compared with pretreatment plants, and biomass values were comparable to nontreated plants harvested 21 DAT. The magnitude of poor control is consistent with target site resistance to ALS-inhibiting herbicides (Patzoldt and Tranel 2007), which was investigated further as described below.

Synthetic auxins (Group 4). CHR control with two synthetic auxin herbicides differed dramatically. Dicamba controlled CHR 80% to 94% depending on rate, whereas control with 2,4-D did not exceed 36% regardless of rate (Table 4). Plants treated with 2,4-D developed injury symptoms, including minor leaf cupping and epinasty, but rapidly recovered and resumed growth. There were no differences in plant dry biomass between dicamba rates, but the 2× rate of 2,4-D reduced dry biomass more than the 1× rate. Estimated difference values indicate significant dry biomass accumulation 21 DAT with either rate of 2,4-D and the 1× rate of dicamba, but not with the 2× rate of dicamba. Control of CHR with 2,4-D was much less than that reported for a waterhemp population controlled 62% and 94% with 560 and 1,120 g ae ha^{-1} 2,4-D, respectively, 28 DAT (Robinson et al. 2012). Interestingly, control of CHR with 2,4-D is similar to that reported for MCR (see below) (16% at 14 DAT) (Hausman et al. 2016).

Atrazine (Group 5). Atrazine did not control CHR regardless of rate, and treated plants lacked injury symptoms. Estimated difference values indicate significant dry biomass accumulation 21 DAT regardless of atrazine rate. Previous studies reported 8% control of PSII inhibitor-resistant waterhemp 14 DAT (Hausman et al. 2016) and 21% control 28 DAT (Anderson et al. 1996).

PPO inhibitors (Group 14). Plants treated with PPO-inhibiting herbicides rapidly became chlorotic and eventually necrotic, but treated plants began to recover approximately 7 DAT. The PPO inhibitors that generally are applied after crop and weed emergence (acifluorfen, fomesafen, lactofen) controlled CHR 58% or less, regardless of application rate (Table 3). Saflufenacil, most commonly applied prior to soybean emergence, controlled CHR 60% to 70%. Increasing rates did not increase control of CHR with any PPO-inhibiting herbicide except saflufenacil. Additionally, differences in dry biomass were not detected among PPO inhibitors, and dry biomass did not differ by rate except with saflufenacil. Positive estimated difference values for all PPO inhibitor treatments indicate that treated plants had recovered by 21 DAT.

HPPD inhibitors (Group 27). Control of CHR with HPPD-inhibiting herbicides was variable (Table 4). Control 21 DAT was similar for 1× rates of mesotrione and tembotrione, although control did not exceed 66%. Topramezone did not control CHR more than 49% regardless of rate. Compared with the 1× rate, control increased with the 2× rate of each HPPD inhibitor, but plant dry biomass reductions for each HPPD inhibitor were not different between rates.

Regardless of rate, waterhemp plants developed characteristic foliar whitening or bleaching following application (Mitchell et al. 2001; Norris et al. 1995; van Almsick 2009). Most treated plants began to recover by 14 DAT. However, estimated difference values of pretreatment dry biomass and dry biomass harvested 21 DAT reveal few significant differences. Only plants treated with 1× topramezone produced more dry biomass 21 DAT compared with pretreatment plants.

Control of CHR with HPPD inhibitors was similar to a different HPPD-resistant waterhemp population, which was controlled less than 60% with labeled rates of three HPPD-inhibiting herbicides (McMullan and Green 2011). Control of CHR with mesotrione, tembotrione, and topramezone, however, generally was greater than control of another HPPD-resistant population from Illinois (designated MCR), for which control with the same HPPD inhibitors applied at similar rates was 27% or less (Hausman et al. 2011, 2016).

HPPD inhibitors and atrazine (Groups 27 and 5). A synergistic interaction between HPPD-inhibiting herbicides and certain PSII inhibitors has been reported but does not always overcome resistance (Hausman et al. 2011; Ma et al. 2013; Woodyard et al. 2009). Control of CHR increased when 560 g ai ha^{-1} atrazine was combined with each HPPD inhibitor, but plant dry biomass values were not different. Additionally, estimated differences in dry biomass of pretreatment and treated plants were not significant, with the exception of the 1× rate of topramezone + atrazine (Table 4). The increase in control of CHR with HPPD inhibitors in combination with atrazine is similar to the MCR population for which control with topramazone, tembotrione, and mesotrione was increased by the addition of atrazine, yet complete mortality was not achieved (Hausman et al. 2011, 2016).

Other SOAs (Groups 9 and 10). In both corn and soybean experiments glyphosate controlled CHR at least 90% regardless of rate, whereas a similar level of control with glufosinate required a 2× rate (Tables 3 and 4). Plant dry biomass, however, was similar among all rates of glyphosate and glufosinate. Estimated difference values indicate that significant dry biomass accumulation did not

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Table 5. Monthly precipitation recorded during field experiments (2014–2015) and 30-yr average for the Champaign-Urbana, IL, area (1981–2010).

Month	Precipitation		
	2014	2015	1981–2010 avg.[a]
	cm		
May	6.4	13.8	12.4
June	17	22.8	11
July	12	7.2	11.9
Total	35.4	43.8	35.3

[a]Averages retrieved from Angel J (1981–2010). Averages and Records for Champaign-Urbana, Illinois. https://www.isws.illinois.edu/statecli/cuweather/cu-averages.htm.

Table 6. Visible estimates of waterhemp control and stand counts of waterhemp density 28 and 42 d after treatment (DAT) in soil-applied corn herbicide experiments (2014–2015).

Herbicide	Rate	Control		Density	
		28 DAT	42 DAT	28 DAT	42 DAT
	g ai ha^{-1}	%		Plants m^{-2}	
Mesotrione	105	71	25	88	90
	210	77	38	78	110
	420	82	51	35	122
Isoxaflutole	53	54	16	160	161
	105	80	40	82	92
	210	88	61	71	89
Atrazine	1,120	13	0	510	526
	2,240	19	0	388	323
	4,480	26	0	317	341
Dicamba	280[a]	10	0	443	460
	560[a]	32	3	273	311
	1,120[a]	53	22	235	227
Saflufenacil	37	18	0	239	307
	75	45	15	262	243
	150	84	47	41	100
Acetochlor[b]	627	3	0	390	406
	1,255	22	5	386	410
	2,510	43	16	221	224
Acetochlor	1,098	79	54	87	89
	2,196	88	70	32	37
	4,392	95	85	12	22
S-metolachlor	795	23	5	286	418
	1,591	39	5	166	296
	3,182	60	25	191	218
Nontreated		–	–	467	472
LSD[c]		18	19	187	181

[a]Acid equivalent (g ae ha^{-1}).
[b]Encapsulated formulation.
[c]Separated by PROC GLM in SAS, α = 0.05.

occur following application of glyphosate or glufosinate, suggesting both remain effective for controlling CHR. Control of CHR with glyphosate and glufosinate is similar to other waterhemp populations for which control was 89% to 100% with glyphosate (Hausman et al. 2016; Robinson et al. 2012) and 82% to 95% with glufosinate (Coetzer et al. 2002; Sarangi et al. 2015).

Soil-applied herbicides

Atrazine and metribuzin (Group 5)

Monthly precipitation recorded at the field location for each season is reported in Table 5. Regardless of rate, control of CHR with atrazine 28 DAT was ≤26%; no control was observed 42 DAT (Table 6). Waterhemp density was not different from nontreated control plots 28 and 42 DAT regardless of atrazine rate. Metribuzin controlled CHR 95% and 88% at 28 and 42 DAT, respectively, at the field-recommended rate (Table 7) and reduced waterhemp density 85% compared with the nontreated 28 and 42 DAT.

Table 7. Visible estimates of waterhemp control and stand counts of waterhemp density 28 and 42 d after treatment (DAT) in soil-applied soybean herbicide experiments (2014–2015).

Herbicide	Rate	Control		Density	
		28 DAT	42 DAT	28 DAT	42 DAT
	g ai ha^{-1}	%		Plants m^{-2}	
Flumioxazin	36	35	18	262	262
	72	61	24	173	179
	143	72	44	149	152
Saflufenacil	37	46	22	212	214
	75	67	46	166	167
	150	69	48	162	167
Sulfentrazone	175	47	13	212	212
	350	69	43	191	188
	700	86	66	23	25
Chlorimuron	13	16	0	228	231
	26	13	0	375	359
	52	15	0	368	354
Pendimethalin	532	12	2	256	256
	1,064	15	11	391	338
	2,129	54	27	208	203
Metribuzin	280	79	67	125	125
	560	95	88	43	43
	1,120	98	97	6	6
Dimethenamid-*P*	420	47	19	221	214
	840	62	29	145	146
	1,681	76	58	131	130
S-metolachlor	795	20	5	272	268
	1,591	46	16	239	244
	3,182	62	39	71	72
Pyroxasulfone	59	48	17	238	236
	119	75	43	123	122
	238	84	75	62	62
Nontreated		–	–	287	271
LSD[a]		18	18	154	155

[a]Separated by PROC GLM in SAS, α = 0.05.

PPO inhibitors (Group 14)

Soil-applied PPO-inhibiting herbicides at 1× rates controlled CHR 61% to 69% 28 DAT, but 46% or less 42 DAT (Table 7). Only sulfentrazone at 2× reduced waterhemp density compared with the nontreated. This contradicts previous research demonstrating that the soil-applied PPO inhibitors flumioxazin and sulfentrazone remained efficacious on PPO-resistant waterhemp, with greater than 80% control at similar evaluation timings (Harder et al. 2012; Shoup and Al-Khatib 2004). In corn experiments (Table 6), a 1× rate of saflufenacil provided 45% and 15% control of CHR 28 and 42 DAT, respectively. Population density of saflufenacil-treated plots, however, was less than the nontreated controls.

Very-long-chain fatty acid inhibitors (Group 15)

In corn experiments, control of CHR with nonencapsulated acetochlor was different from *S*-metolachlor across rates at each evaluation. The 1× rate of nonencapsulated acetochlor controlled CHR 88% and 70% 28 and 42 DAT, respectively, whereas control with 2× *S*-metolachlor was 60% or less (Table 6). In soybean experiments, control with 2× *S*-metolachlor was 62% or less (Table 7). The label-recommended rate of *S*-metolachlor reduced waterhemp density 28 DAT in corn experiments, but not in soybean experiments or at 42 DAT in either. Control of CHR with these herbicides is similar to that previously reported for MCR, where

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control with 1× acetochlor was 83% but less than 20% with 1× *S*-metolachlor (Hausman et al. 2013).

Control with encapsulated acetochlor did not exceed 32% 28 DAT or 5% 42 DAT, with waterhemp densities not different from the nontreated control (Table 6). In soybean experiments, control with pyroxasulfone and dimethenamid-*P* applied at 1× ranged from 62% to 75% 28 DAT, but decreased to 29% to 43% 42 DAT (Table 7). The 1× and 2× rates of pyroxasulfone and the 2× rates of dimethenamid-*P* reduced waterhemp density 28 DAT, whereas only the 2× rate of pyroxasulfone reduced waterhemp density 42 DAT. Control of HPPD-resistant waterhemp with pyroxasulfone has ranged from 48% to 84% (this research), 87% to 89% for MCR (Hausman et al. 2013), and 90% to 95% for a Nebraska population 30 DAT (Oliveira et al. 2017). Control of CHR with various Group 15 herbicides was similar to that reported in subsequent studies where differential responses to Group 15 herbicides were demonstrated in field and greenhouse experiments (Strom et al. 2017).

HPPD inhibitors (Group 27)

Control across all rates of HPPD-inhibiting herbicides ranged from 54% to 88% at 28 DAT and decreased to 16% to 61% at 42 DAT (Table 6). These values are in contrast with previous reports regarding the efficacy of soil-applied HPPD inhibitors on a sensitive waterhemp population, in which 100% control was achieved 28 d after crop emergence (Vyn et al. 2006). Each rate of isoxaflutole and mesotrione did, however, reduce waterhemp density compared with the nontreated at each evaluation.

Other SOAs (Groups 2, 3, 4)

Pendimethalin and chlorimuron applied at the label-recommended rate did not control CHR more than 15% 28 DAT and less than 11% 42 DAT (Table 7), with waterhemp densities not different from nontreated plots at either evaluation time. In corn experiments, soil-applied dicamba controlled CHR 32% 28 DAT and only 3% 42 DAT with waterhemp densities not different from nontreated plots (Table 6).

Mechanisms of resistance and greenhouse herbicide dose–response experiments

Resistance mechanisms to ALS-, PPO-, and PSII-inhibiting herbicides

PCR-based molecular marker analysis was performed to determine if resistance to ALS-inhibiting herbicides in CHR-M6 is attributable to an amino acid substitution at position 574 of ALS. A comparison of bands generated from positive (ALS resistant) controls and CHR-M6 indicated identical band sizes, thereby revealing that plants from CHR possess a target site mutation known to confer resistance to ALS-inhibiting herbicides (Patzoldt and Tranel 2007; Tranel and Wright 2002). Resistance to PPO inhibitors was also investigated via molecular marker analysis. A comparison of the products amplified between positive (PPO resistant) controls and CHR-M6 indicated identical band sizes, thereby revealing that plants from CHR possess the ΔG210 *PPX2* mutation known to confer resistance to PPO-inhibiting herbicides (Patzoldt et al. 2006).

Sequence analysis of the entire *psb*A gene (encoding the D1 protein) did not reveal mutations known to confer target site resistance to PSII inhibitors (data not shown), suggesting that atrazine resistance in CHR-M6 might be caused by enhanced atrazine metabolism, similar to that reported previously (Ma et al. 2013).

Table 8. Estimated GR_{50} and GR_{90} values for dry biomass in pooled greenhouse dose–response experiments among waterhemp populations.[a,b]

Population	GR^{50}		GR^{90}	
		Mesotrione		
CHR-M6	32	(±4.6)	213	(±70.8)
NH40	40	(±4.9)	191	(±54)
BCR	6.6	(±1.2)	75	(±32.4)
WUS	2.1	(±0.35)	18.5	(±6)
		Atrazine		
CHR-M6	16,437	(±41,941)	294,450	(±9,685,600)
NH40	20,428	(±17,896)	3,151,800	(±492,720)
WUS	65	(±16.9)	464	(±30.7)
		2,4-D		
CHR-M6	95	(±26)	1,589	(±1,166)
NE	518	(±210)	7,131	(±6,049)
WUS	10.5	(±1.2)	54.2	(±15.05)

[a]Estimated values are expressed in g ai ha^{-1} (mesotrione and atrazine) or g ae ha^{-1} (2,4-D) and are followed by their respective standard errors in parentheses.
[b]Abbreviations: GR_{50} and GR_{90}, herbicide doses required to reduce waterhemp dry biomass 50% and 90%, respectively.

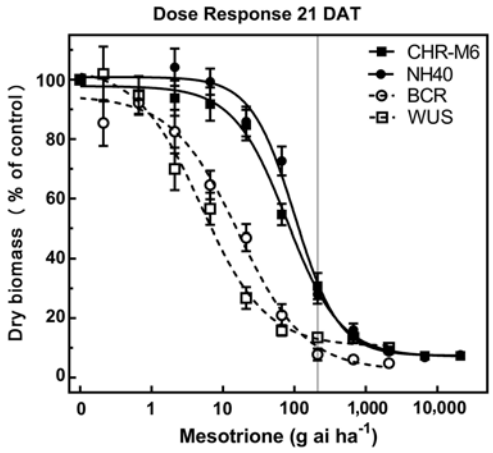

Figure 1. Mesotrione dose–response curves for CHR-M6 compared with HPPD inhibitor–sensitive populations WUS and BCR and the HPPD inhibitor–resistant NH40 populations. Aboveground dry biomass was harvested 21 d after treatment (DAT). The vertical line through response curves signifies a typical rate for field use.

Mesotrione, 2,4-D, and atrazine dose-response experiments

HPPD inhibitors caused characteristic injury (stunting and bleaching of meristematic tissue) on plants from all populations. However, compared with sensitive populations WUS and BCR, CHR-M6 and NH40 exhibited far less (data not presented). Injury to WUS and BCR generally increased over time, whereas CHR-M6 and NH40 began to recover approximately 10 DAT. By 14 DAT new, noninjured leaf tissue was present on the majority of CHR-M6 and NH40 plants.

Treatment of WUS, BCR, CHR-M6, and NH40 with a range of mesotrione doses produced response curves demonstrating decreasing dry biomass with increasing doses (Figure 1). GR_{50} and GR_{90} values (Table 8) were calculated to determine the estimated doses of mesotrione to reduce plant dry biomass 50% and 90%. Calculated resistance ratios (based on GR_{50} values) in CHR-M6 to mesotrione were 4.8- or 16-fold when compared to BCR or WUS, respectively.

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A previously confirmed HPPD inhibitor–resistant waterhemp population from Iowa exhibited an 8-fold decrease in sensitivity to mesotrione compared with a sensitive population (McMullan and Green 2011). This population required 21 g ha^{-1} mesotrione for 50% control (determined visibly), whereas CHR-M6 required 32 g ha^{-1} mesotrione to reduce dry biomass 50%. The GR_{50} of MCR was reported to be 48.5 g ha^{-1} mesotrione (Hausman et al. 2011), a value similar to that reported in this research. These data indicate that CHR-M6 exhibits a level of resistance similar to that of other waterhemp populations confirmed resistant to HPPD-inhibiting herbicides.

Unlike BCR, WUS has not been previously treated with herbicides (Bell et al. 2013). Although BCR was less sensitive to mesotrione than WUS, all plants from both populations were completely controlled (no green tissue) with 105 g ha^{-1} mesotrione (the label-recommended rate for foliar applications). In contrast, 105 g ha^{-1} mesotrione reduced dry biomass of CHR-M6 and NH40 69% and 71%, respectively, when averaged across all replications of the experiment.

Following treatment with atrazine, WUS displayed injury symptoms (leaf chlorosis followed by necrosis) commonly observed following foliar exposure to PSII-inhibiting herbicides (Hess 2000). CHR-M6 and NH40 demonstrated no injury from all but the highest atrazine doses. A distinct separation of dose–response curves between atrazine-resistant and sensitive waterhemp populations is observed in Figure 2. A majority of CHR-M6 and NH40 plants survived treatment with the highest dose of atrazine (44 kg atrazine ha^{-1}).

Using calculated GR_{50} values (Table 8), CHR-M6 was 252-fold resistant to atrazine relative to WUS, and NH40 was 1.2-fold more resistant than CHR-M6. Reports of other atrazine-resistant waterhemp populations indicate levels of resistance ranging from 10-fold (non–target site–based resistance) (McMullan and Green 2011), 38-fold (non–target site–based resistance) (Patzoldt et al. 2005), to >185-fold (target site–based resistance) (Foes et al. 1998). The magnitude of atrazine resistance in CHR-M6 and NH40, coupled with the inability to achieve plant mortality at the highest application rate, resulted in high estimated effective doses and large standard errors. An accurate estimated effective dose of atrazine could not be calculated from the data.

Treatment with 2,4-D caused characteristic synthetic auxin injury (epinasty, leaf strapping, stunting) on plants from all populations. The dose required to cause injury to plants in the sensitive population was much less than that required to cause injury to CHR-M6 and NE (data not presented). At higher doses (2,240–17,926 g ae ha^{-1}), injury symptoms not commonly observed with synthetic auxin herbicides, including leaf chlorosis and necrosis, were observed on CHR-M6 and NE plants. Complete mortality of CHR-M6 or NE was not achieved with most doses, and treated plants began to recover by 21 DAT. Failure to achieve complete control resulted in high standard errors of calculated GR values. Dry biomass of WUS, CHR-M6, and NE plants treated with a range of 2,4-D rates decreased with increasing doses (Figure 3). The calculated GR_{50} values (Table 8) for CHR-M6 produce a resistance ratio of 9.5-fold compared with WUS, whereas NE is 5-fold more resistant than CHR-M6. The resistance ratio for CHR-M6 compared with WUS increases to 30-fold when GR_{90} values are used to calculate ratios.

The Nebraska 2,4-D–resistant waterhemp population was reported to be 10-fold resistant to 2,4-D (Bernards et al. 2012), whereas the Missouri population (Shergill et al. 2018) was 3-fold resistant. Resistance ratios of other weed species resistant to

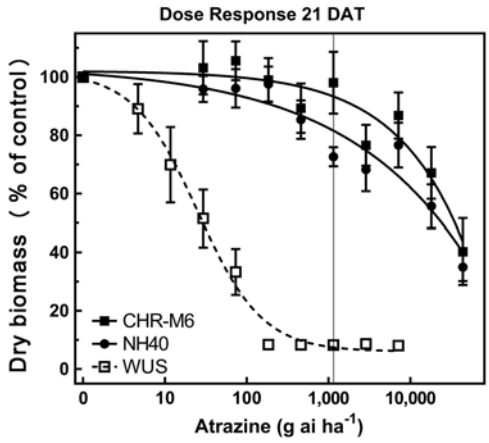

Figure 2. Atrazine dose–response curve for CHR-M6 compared with atrazine-resistant population NH40, and the sensitive population WUS. Aboveground dry biomass was harvested 21 d after treatment (DAT). The vertical line through response curves signifies a typical rate for field use.

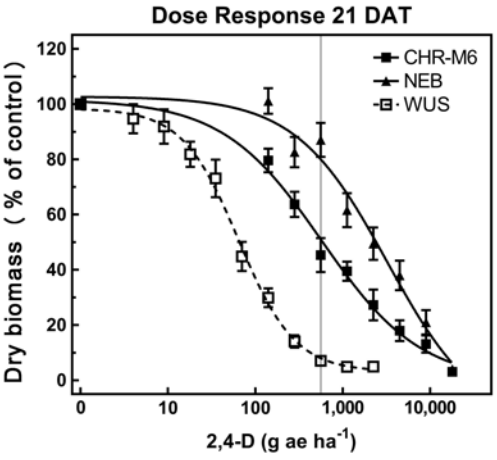

Figure 3. 2,4-D dose–response curve for CHR-M6 compared with 2,4-D-resistant population NE, and the sensitive population WUS. Aboveground dry biomass was harvested 21 d after treatment (DAT). The vertical line through response curves signifies a typical rate for field use.

2,4-D have varied, including 2.5-fold in wild radish (*Raphanus raphanistrum* L.) (Walsh et al. 2004), 18-fold in wild mustard [*Brassica kaber* (DC.) Wheeler] (Heap and Morrison 1992), 25-fold for prickly lettuce (*Lactuca serriola* L.) (Burke et al. 2009), and 29-fold for globe fringebrush [*Fimbristylis miliacea* (L.) Vahl] (Karim et al. 2004).

The rate of 2,4-D commonly used in Illinois (1,120 g ae ha^{-1}) reduced dry biomass of CHR-M6 and NE by 60% and 39%, respectively. The same rate reduced the dry biomass of WUS by 95%. These data illustrate a distinct difference in response to 2,4-D between the sensitive population (WUS) and CHR-M6.

Implications and future research

Our results indicate that the CHR population is resistant to herbicides from five site-of-action groups, including inhibitors of ALS, PPO, PSII, and HPPD, and the synthetic auxin 2,4-D. Unexpectedly, CHR has evolved resistance to 2,4-D, which, based on herbicide use history, had not previously been applied. The dioecious biology of waterhemp and high intraspecific genetic

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variability favor the evolution of resistance and multiple resistance (Tranel et al. 2011), but may not solely be responsible. Cross-resistance, that is, resistance to a herbicide through indirect selection by another (Beckie and Tardif 2012), might have resulted in 2,4-D resistance in CHR. Resistance to 2,4-D in the Nebraska waterhemp population is due to enhanced metabolism, probably mediated by cytochrome P450 enzymes (Figueiredo et al. 2018). Although the mechanism of 2,4-D resistance within CHR remains unknown, it is possible that selection for P450-mediated resistance, as occurred in MCR (Ma et al. 2013), resulted in cross-resistance to 2,4-D.

Resistance to 2,4-D in CHR could have been introduced via migration. Waterhemp pollen can travel long distances by wind (Liu et al. 2012), which could allow pollen migration into CHR and introduce genes from a neighboring resistant population (Liu et al. 2012), similar to glyphosate resistance (Sarangi et al 2017). Finally, seed might have migrated into CHR via wildlife (de Vlaming and Proctor 1968; Myers et al. 2004), equipment (Heijting et al. 2008), or water (Li and Qiang 2009; Norsworthy et al. 2014).

Based on the present research, glyphosate and glufosinate are among the few remaining foliar-applied herbicides that control CHR. *Amaranthus* species have not yet evolved resistance to glufosinate (Heap 2018), but numerous waterhemp populations have evolved resistance to glyphosate (Heap 2018), highlighting the urgent need to implement management practices that preserve the efficacy of glufosinate and glyphosate for managing CHR. This research also revealed limited soil-applied options for managing CHR. The PSII inhibitor metribuzin was effective, but CHR is resistant to atrazine. Nonencapsulated acetochlor also controlled CHR, but a differential response was documented with other Group 15 active ingredients. Further research is needed to investigate potential resistance to soil-applied herbicides in CHR and will continue to characterize the response of the population and resistance mechanisms to various foliar- and soil-applied herbicides. Overall, a population with this magnitude of multiple resistance can pose significant challenges for its effective management, which highlights the necessity for continued efforts in herbicide discovery and the implementation of weed management programs not solely dependent upon herbicides.

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