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Confirmation and Control of Glyphosate-Resistant Common Waterhemp (*Amaranthus rudis*) in Nebraska

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Glyphosate-resistant common waterhemp is a difficult-to-control annual broadleaf weed that has become a serious management challenge for growers in Nebraska and other states in the United States. The objectives of this study were to confirm glyphosate-resistant common waterhemp in Nebraska by quantifying level of resistance in a dose-response study, and to determine the sensitivity and efficacy of POST soybean herbicides for controlling suspected glyphosate-resistant common waterhemp biotypes. Seeds of suspected glyphosate-resistant common waterhemp biotypes were collected from seven eastern Nebraska counties. Greenhouse dose-response experiments were conducted to evaluate the response of common waterhemp biotypes to nine rates of glyphosate (0 to 16×). Common waterhemp biotypes were 3- to 39-fold resistant to glyphosate depending on the biotype being investigated and the susceptible biotype used for comparison. Results of the POST soybean herbicides efficacy experiment suggested that glyphosate-resistant biotypes, except a biotype from Pawnee County, had reduced sensitivity to acetolactate synthase (ALS)–inhibiting herbicides (chlorimuron-ethyl, imazamox, imazaquin, imazethapyr, and thifensulfuron-methyl). Glufosinate and protoporphyrinogen oxidase (PPO)–inhibiting herbicides (acifluorfen, fluthiacet-methyl, fomesafen, and lactofen) provided ≥ 80% control of glyphosate-resistant common waterhemp at 21 d after treatment (DAT). This study confirmed the first occurrence of glyphosate-resistant common waterhemp in Nebraska, and also revealed reduced sensitivity to ALS-inhibiting herbicides in most of the biotypes tested in this study.

Nomenclature: Acifluorfen; chlorimuron-ethyl; fluthiacet-methyl; fomesafen; glufosinate; glyphosate; imazamox; imazaquin; imazethapyr; lactofen; thifensulfuron-methyl; common waterhemp, *Amaranthus rudis* Sauer; soybean, *Glycine max* (L.) Merr.

Key words: Acetolactate synthase inhibitor, glutamine synthetase inhibitor, protoporphyrinogen oxidase inhibitor, resistance management.

Amaranthus rudis resistente a glyphosate es una maleza anual de hoja ancha difícil de controlar y que se ha convertido en un reto serio de manejo para productores en Nebraska y otros estados en los Estados Unidos. Los objetivos de este estudio fueron confirmar la resistencia a glyphosate de *A. rudis* en Nebraska, cuantificando el nivel de resistencia mediante estudios de respuesta a dosis, y determinar la sensibilidad y la eficacia de herbicidas POST para soja para el control de biotipos de *A. rudis* que se sospecha son resistentes a glyphosate. En siete condados del este de Nebraska, se colectaron semillas de biotipos de *A. rudis* que se sospechaba eran resistentes a glyphosate. Se realizaron experimentos de respuesta a dosis en invernadero, para evaluar la respuesta de biotipos de *A. rudis* a nueve dosis de glyphosate (0 a 16X). Biotipos de *A. rudis* fueron de 3 a 39 veces más resistentes a glyphosate, dependiendo del biotipo investigado y del biotipo susceptible usados en la comparación. Los resultados del experimento sobre la eficacia de herbicidas POST para soja sugirieron que los biotipos resistentes a glyphosate, con la excepción del biotipo proveniente del condado Pawnee, tuvieron una sensibilidad reducida a los herbicidas inhibidores de acetolactate synthase (ALS) (chlorimuron-ethyl, imazamox, imazaquin, imazethapyr, y thifensulfuron-methyl). Glufosinate y los herbicidas inhibidores de protoporphyrinogen oxidase (PPO) (acifluorfen, fluthiacet-methyl, fomesafen, y lactofen) brindaron ≥ 80% control de *A. rudis* resistente a glyphosate a 21 d después del tratamiento (DAT). Este estudio confirmó el primer caso de *A. rudis* resistente a glyphosate en Nebraska, y también reveló la sensibilidad reducida a herbicidas inhibidores de ALS en la mayoría de los biotipos evaluados en este estudio.

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Common waterhemp is a summer annual weed native to the northern United States (Bryson and DeFelice 2010) that can be found in a large range of climatic gradients from arid regions in Texas to humid/semihumid Maine (Nordby et al. 2007). It is one of the most commonly encountered and troublesome weeds in no-till agricultural fields in the midwestern United States (Hager et al. 2002; Steckel and Sprague 2004). Widespread adoption of conservation tillage and evolution of herbicide resistance are believed to aid in shifting the composition of weed flora toward small-seeded broadleaf species such as common waterhemp in corn (*Zea mays* L.)–soybean production systems (Hausman et al. 2011; Legleiter and Bradley 2008).

Common waterhemp is a C_4 weed species with rapid growth habit (Horak and Loughin 2000), extended seedling emergence (Hartzler et al. 1999), and potential for prolific seed production (Duff et al. 2009; Steckel et al. 2003). It is also considered a highly competitive weed and has a detrimental effect on crop yields. In Illinois, Steckel and Sprague (2004) reported 74% corn yield reduction due to season-long interference of common waterhemp, and plants that were allowed to compete with soybean up to 10 wk after soybean unifoliate expansion reduced soybean yield by 43% (Hager et al. 2002).

Glyphosate, a broad-spectrum nonselective POST herbicide, was first commercialized in 1974 (Franz et al. 1997). The label of glyphosate lists over 100 annual broadleaf and grass weeds and almost 60 perennial weed species that can be controlled by its use (Anonymous 2012). It is the only available commercial herbicide that inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase in the shikimate pathway, resulting in insufficient aromatic amino acid production to maintain necessary protein synthesis (Herrmann and Weaver 1999). Due to selectivity issues, glyphosate was initially used for preplant, POST-directed, or postharvest application to crop lands (Green 2009). However, the use of glyphosate changed dramatically after 1996 with the commercialization of glyphosate-resistant crops (Dill et al. 2008). It has been reported that 85% of total transgenic crops grown worldwide are resistant to glyphosate (James 2013). In the United States, 94% of soybean and 89% of corn grown in 2014 were herbicide-resistant, primarily glyphosate-resistant (USDA 2014). Widespread adoption of glyphosate-resistant crops has increased farmers' reliance on glyphosate in weed management programs, replacing residual and other POST herbicides (Brookes and Barfoot 2008; Gianessi 2008; Young 2006). Consequently, glyphosate is the world's most important and most commonly used herbicide today (Dill et al. 2010; Duke and Powles 2008; Okada and Jasieniuk 2014; Powles 2008).

Overreliance on glyphosate for the past several years has created a selective advantage that was responsible for the evolution of glyphosate-resistant weeds. As of 2014, 29 weed species have evolved resistance to glyphosate in 24 countries around the world, with 16 species reported from glyphosate-resistant row crop production systems (Heap 2014b). In the United States, 14 weed species from 32 states have been confirmed resistant to glyphosate (Heap 2014b). The first glyphosate-resistant common waterhemp in the United States was confirmed in Missouri (Legleiter and Bradley 2008), but now glyphosate-resistant waterhemp biotypes occur across 13 states (Heap 2014a). In addition, numerous common waterhemp biotypes have been confirmed with resistance to herbicides with other modes-of-action, including ALS inhibitors (Horak and Peterson 1995), photosystem II inhibitors (Anderson et al. 1996), PPO inhibitors (Shoup et al. 2003), 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors (Hausman et al. 2011), and synthetic auxins or growth regulators (Bernards et al. 2012). Common waterhemp resistant to all of the above-mentioned groups (except glyphosate and PPO inhibitors) has been confirmed in Nebraska in separate biotypes (Jhala 2014).

Failure to control common waterhemp following sequential glyphosate applications has been reported in recent years by several Nebraska growers, justifying the need to confirm the existence of glyphosate-resistant common waterhemp in Nebraska. This information would be beneficial in developing effective common waterhemp management programs for soybean growers. The objectives of this study were to (1) confirm glyphosate-resistant common waterhemp in Nebraska by quantifying the level of resistance in a dose-response study, and (2) evaluate the sensitivity and efficacy of POST soybean herbicides to control suspected glyphosate-resistant biotypes collected from seven Nebraska counties.

Materials and Methods

Plant Materials. In 2012, growers from several counties in eastern Nebraska reported failure to

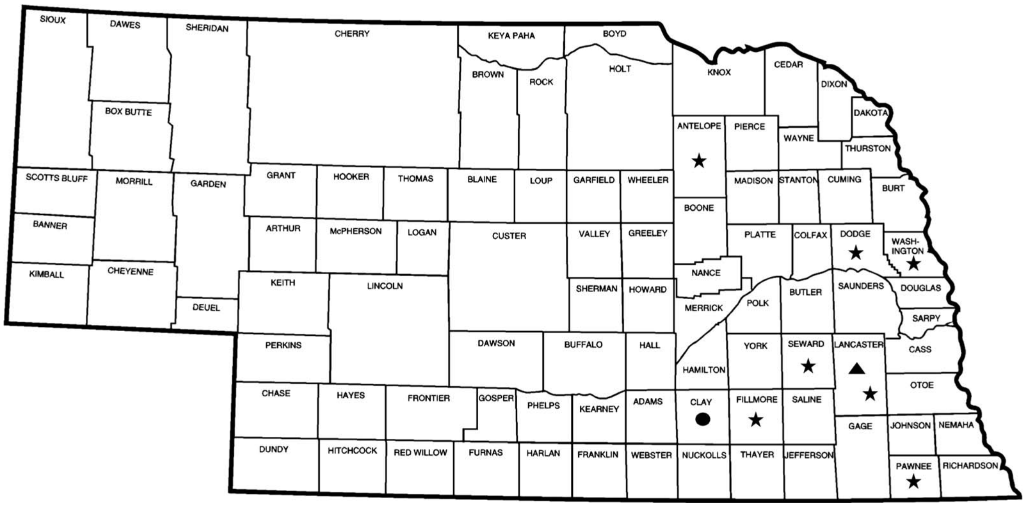

Figure 1. The eastern Nebraska counties from which suspected glyphosate-resistant common waterhemp seeds were collected in 2012 (stars). Locations from which known glyphosate-susceptible common waterhemp seeds were collected in 2006 (oval) and in 2012 (triangle).

control common waterhemp following repeated applications of glyphosate in glyphosate-resistant soybean. The fields in question had been under glyphosate-resistant corn or soybean production for at least 8 yr, mostly relying on glyphosate for weed control. In the fall of 2012, inflorescences of common waterhemp plants that survived repeated glyphosate applications were collected from fields from seven eastern Nebraska counties (Antelope, Dodge, Fillmore, Lancaster, Pawnee, Seward, and Washington) and were suspected to be glyphosate-resistant biotypes (Figure 1). Common waterhemp seeds collected in 2006 from a field near Clay Center, NE, and in 2012 from a field near Lincoln, NE, with a known history of effective control with the recommended rate of glyphosate were considered as glyphosate-susceptible biotypes: susceptible 1 (S_1) and susceptible 2 (S_2), respectively (Figure 1).

Seeds were cleaned thoroughly and stored separately in airtight polythene bags at 4 C for 2 mo to overcome seed dormancy. Seeds were germinated in plastic petri dishes (9 cm diam by 1.7-cm deep) containing one piece of water-soaked Whatman No. 4 filter paper (GE Healthcare UK Limited, Amersham Place Little Chalfont, Buckinghamshire, HP7 9NA, U.K.). The petri dishes were closed with lids to check water loss through evaporation and prevent microbial contamination. They were kept in a greenhouse; seedlings began to emerge after 5 to 6 d of incubation. The seedlings were allowed to grow in petri dishes for the next 10 d and were watered as needed. The seedlings were then transferred at cotyledon stage to germination trays containing potting mix (Berger BM1 All-Purpose Mix, Berger Peat Moss Ltd., Saint-Modeste, Quebec, Canada) by transplanting one seedling per cell. Seedlings were transplanted at the first-true leaf stage to square plastic pots (10 by 10 by 12 cm) containing a 3 : 1 mixture of potting mix to soil. Plants were supplied with adequate water and nutrients and kept in a greenhouse maintained at 28/24 C day/night temperatures. Artificial lighting was provided using metal halide lamps with 600 μmol photon m^{-2} s^{-1} light intensity to ensure a 16-h photoperiod.

Glyphosate Dose-Response Study. Greenhouse dose-response bioassays were conducted in 2012 and 2013 at the University of Nebraska–Lincoln to determine the level of resistance in suspected glyphosate-resistant biotypes. The experiments were arranged in a randomized complete block design with seven replications. Separate experiments were conducted for each biotype. A single common waterhemp plant per pot was considered as an experimental unit. Glyphosate (Touchdown HiTech®, Syngenta Crop Protection, LLC, P.O. Box 18300, Greensboro, NC 27419-8300) treatments included nine rates (0, 0.125×, 0.25×, 0.5×, 1×, 2×, 4×, 8×, and 16×), where, 1× = recommended field rate of glyphosate (1,050 g ae ha^{-1}). The 8- to 12-cm-tall common waterhemp seedlings were treated with glyphosate treatments in a single-tip chamber sprayer (DeVries Manufacturing Corp, Hollandale, MN 56045) fitted with an 8001 E nozzle (TeeJet, Spraying Systems Co., Wheaton, IL 60187) calibrated to deliver 140 L ha^{-1} spray volume at 207 kPa at a speed of 4 km h^{-1}. Each glyphosate treatment was prepared in distilled water and mixed with nonionic surfactant (Induce, Helena Chemical Co., Collierville, TN) 0.25% v/v and ammonium sulfate (DSM Chemicals North America Inc., Augusta, GA) 2.5% wt/v.

Visual control estimates were recorded at 7, 14, and 21 d after treatment (DAT) using a scale ranging from 0 to 100%, with 0% meaning no control and 100% meaning complete death or control of common waterhemp. Percentage of control was assessed on the basis of chlorosis, necrosis, and stunting in plant height compared with nontreated control plants. Aboveground biomass of each waterhemp plant was harvested at 21 DAT and oven-dried at 65 C until it reached a constant weight. The biomass data were converted into percentage of biomass reduction as compared to the nontreated control (Wortman 2014) as

$$\%\ \text{biomass reduction} = \left[(\bar{C} - B)/\ \bar{C}\right] \times 100 \quad [1]$$

where $\bar{C}$ is the mean biomass of the seven nontreated control replicates, and B is the biomass of an individual treated experimental unit.

A four-parameter log-logistic function (Equation 2) was used to determine the effective doses needed to control each common waterhemp biotype by 50 and 90% (ED_{50} and ED_{90}) using the *drc* (*drc* 2.3, Christian Ritz and Jens Strebig, R 3.1.0, Kurt Hornik, online) package in software R (R statistical software, R Foundation for Statistical Computing, Vienna, Austria) (Knezevic et al. 2007):

$$Y = c + \{d - c/1 + \exp[b(\log x - \log e)]\} \quad [2]$$

In this model, Y is the percentage of visual control or percentage of reduction in biomass, c is the lower limit, d is the upper limit, and e represents the ED_{50} and ED_{90} values. The parameter b is the relative slope around the parameter e. The level of resistance was determined by the ratio of ED_{90} value of the suspected resistant and known glyphosate-susceptible biotypes (S_1 and S_2). When the ED_{90} values were variable for S_1 and S_2, a range of resistance level was provided.

Efficacy of POST Soybean Herbicides. The efficacy of POST soybean herbicides was evaluated for control of common waterhemp biotypes. Treatments included registered POST soybean herbicides and their tank-mixes (Table 1). The study was conducted in greenhouses at the University of Nebraska–Lincoln under the same growing conditions as described in the dose-response study. Herbicide application rates were selected based on recommended labeled rates in soybean. Herbicides were applied to 8- to 12-cm-tall common waterhemp plants. Visual control estimates were recorded at 7, 14, and 21 DAT on a scale of 0 to 100%, as described in the dose-response study. Plants were cut at the soil surface at 21 DAT and oven-dried at 65 C until a constant biomass was achieved; biomass weight was then recorded. Percentage of biomass reduction of treated plants was calculated using Equation 1.

Glyphosate-resistant common waterhemp biotypes from five eastern Nebraska counties (Dodge, Lancaster, Pawnee, Seward, and Washington) and a glyphosate-susceptible biotype (S_1) of common waterhemp, collected from a field near Lincoln, NE, were selected for this experiment. Due to poor seed germination and insufficient number of plants, Antelope and Fillmore County biotypes were not included in this study. The experiment was conducted separately for each biotype in a randomized complete block design with four replications. Each experiment was repeated twice.

Data were subjected to ANOVA using the PROC GLIMMIX procedure in SAS version 9.3 (SAS Institute Inc, Cary, NC). Before analysis, data were tested for normality with the use of PROC UNIVARIATE. Visual control estimates and percent biomass reduction data were arcsine square-root transformed before analysis. However, back-transformed data are presented with mean separation based on transformed data. Where the ANOVA indicated treatment effects were significant, means were separated at $P \leq 0.05$ using Fisher's protected LSD test.

Results and Discussion

Glyphosate Dose-Response Study. There was not a significant treatment-by-experiment interaction. Therefore, data from both experiments were combined. Suspected glyphosate-resistant biotypes from seven Nebraska counties survived the labeled rate (1,050 g ae ha^{-1}) of glyphosate, whereas known

Table 1. Details of POST soybean herbicides used for control of common waterhemp biotypes in a greenhouse study conducted at the University of Nebraska–Lincoln.

Herbicide	Trade name	Rate	Manufacturer	Adjuvants[a,b]
		g ae or ai ha^{-1}		
Fluthiacet-methyl	Cadet	7.2	FMC Corporation, Philadelphia, PA; www.fmc.com	COC + AMS
Chlorimuron-ethyl	Classic	13.1	E. I. du Pont de Nemours and Company, Wilmington, DE; www.dupont.com	NIS + AMS
Imazethapyr + glyphosate	Extreme	910	BASF Corporation, Research Triangle Park, NC; www.basf.com	NIS + AMS
Fomesafen + glyphosate	Flexstar GT	1,380	Syngenta Crop Protection, Inc., Greensboro, NC; www.syngenta.com	NIS + AMS
Lactofen + glyphosate	Cobra + Roundup Powermax	140 + 1,540	Valent U.S.A. Corporation, Walnut Creek, CA; www.valent.com + Monsanto Company, St. Louis, MO; www.monsanto.com	AMS
Imazamox + glyphosate	Raptor + Roundup Powermax	26.3 + 1,540	BASF Corporation + Monsanto Company	NIS + AMS
Imazaquin + glyphosate	Scepter + Touchdown Hitech	70.6 + 1,400	BASF Corporation+ Syngenta Crop Protection, Inc.	NIS
Thifensulfuron-methyl	Harmony SG	4.4	E. I. du Pont de Nemours and Company	NIS + AMS
Glufosinate	Liberty 280	594	Bayer CropScience LP, Research Triangle Park, NC; www.cropscience.bayer.com	AMS
Lactofen	Cobra	220	Valent U.S.A. Corporation	NIS + AMS
Imazethapyr	Pursuit	70	BASF Corporation	NIS + AMS
Imazethapyr + acifluorfen	Pursuit + Ultra Blazer	70 + 245	BASF Corporation + United Phosphorus, Inc. King of Prussia, PA; www.uplonline.com	NIS + AMS
Imazamox	Raptor	44	BASF Corporation	NIS + AMS
Fomesafen	Reflex	280	Syngenta Crop Protection, Inc.	NIS + AMS
Imazamox + acifluorfen	Raptor + Ultra Blazer	44 + 175	BASF Corporation + United Phosphorus, Inc.	NIS + AMS
Chlorimuron ethyl + Thifensulfuron-methyl	Synchrony XP	7.46	E. I. du Pont de Nemours and Company	NIS + AMS
Imazethapyr + glyphosate	Tackle	2,310	Cheminova, Inc., Research Triangle Park, NC; www.cheminova.com	NIS + AMS
Acifluorfen	Ultra Blazer	420	United Phosphorus, Inc.	NIS + AMS

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

[b] AMS was mixed at 2.5% wt/v; COC was mixed at 2.5% v/v; NIS was mixed at 0.25% v/v.

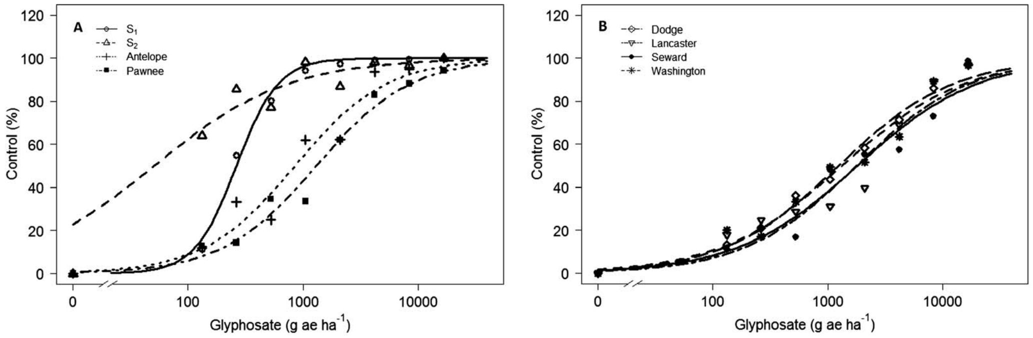

Figure 2. Control of (A) glyphosate-susceptible (S_1 and S_2) and glyphosate-resistant common waterhemp biotypes of Antelope and Pawnee Counties, and (B) Dodge, Lancaster, Seward, and Washington Counties at 21 d after treatment in glyphosate dose-response bioassay conducted at the University of Nebraska–Lincoln.

glyphosate-susceptible biotypes (S_1 and S_2) were controlled (> 90%) (Figure 2). On the basis of the values at ED_{90} level, the analysis showed a 3- to 39-fold resistance depending on the biotype being investigated and the susceptible biotype used for comparison (Table 2). Legleiter and Bradley (2008) reported 9- to 19- fold resistance in common waterhemp biotype from Missouri. A comparatively low level of resistance (≤ 10×) was observed in a biotype from Antelope and Fillmore Counties, while higher levels of resistance (> 10×) was observed in biotypes from Dodge, Lancaster, Pawnee, Seward, and Washington Counties with ED_{90} values ranging from 10,403 to > 16,800 g ae ha^{-1} (Table 2). Similarly, Light et al. (2011) reported the variability in level of resistance (3.5- to 59.7-fold) in common waterhemp biotypes from Texas and described that the differences in dose of herbicide required to control different biotypes up to a certain level might be due to their parental genotype and cross-pollination, as common waterhemp is a dioecious species and the seeds might come from open-pollinated parents of probable heterozygous origin.

Variability in visual estimates of control among all the resistant biotypes were found at elevated glyphosate rates (> 4,000 g ae ha^{-1}) (Figures 2 and 3). A similar response in glyphosate-resistant common waterhemp biotypes collected from Illinois, Iowa, and Missouri has been reported (Smith and Hallett 2006). Dose-response curves for the percentage of biomass reduction showed a similar level of resistance on the basis of ED_{90} values (Figure 3; Table 3). The ED_{50} for percentage of biomass reduction of glyphosate-resistant biotypes was slightly higher than the estimated ED_{50} values for visual control estimates (Tables 2 and 3).

Efficacy of POST Soybean Herbicides. There was not a significant treatment-by-experiment interaction for common waterhemp control and biomass reduction; therefore, data from both experiments were combined. Glyphosate-resistant biotypes were

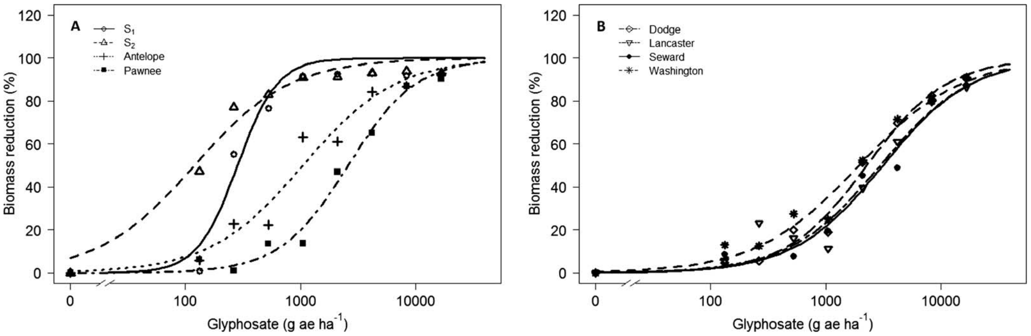

Figure 3. Biomass reduction (%) of (A) glyphosate-susceptible (S_1 and S_2) and glyphosate-resistant common waterhemp biotypes of Antelope and Pawnee Counties, and (B) Dodge, Lancaster, Seward, and Washington Counties at 21 d after treatment in glyphosate dose-response bioassay conducted at the University of Nebraska–Lincoln

Table 2. Estimates of the glyphosate dose in a dose-response study resulting in 50% (ED_{50}) and 90% (ED_{90}) control of common waterhemp biotypes collected from seven eastern Nebraska counties.

Common waterhemp biotype[a]	Glyphosate[b]		
	ED_{50}[a]	ED_{90}[a]	Resistance level[c]
	g ae ha^{-1}		
S_1	263 ± 15	659 ± 90	—
S_2	51 ± 23	956 ± 366	—
Antelope	852 ± 98	6,391 ± 1,547	7 to 10×
Dodge	1,246 ± 161	15,582 ± 4,609	16 to 24×
Fillmore	303 ± 36	2,419 ± 639	3 to 4×
Lancaster	1,790 ± 232	> 16,800	22 to 32×
Pawnee	1,308 ± 147	10,403 ± 2,629	11 to 16×
Seward	1,813 ± 312	> 16,800	27 to 39×
Washington	1,341 ± 173	> 16,800	21 to 30×

[a] Abbreviations: ED_{50}, effective dose of glyphosate required to control 50% biotype at 21 d after treatment; ED_{90}, effective dose of glyphosate required to control 90% population at 21 d after treatment; S_1, glyphosate-susceptible common waterhemp biotype collected in 2006 from a field near Clay Center, NE; S_2, glyphosate-susceptible common waterhemp biotype collected in 2012 from a field near Lincoln, NE.

[b] Values represent mean ± SE in g ae ha^{-1}.

[c] Resistance level at ED_{90} value of respective common waterhemp biotype divided by ED_{90} value of susceptible biotypes (S_1 and S_2). A range of resistance level was provided due to a difference in ED_{90} values for S_1 and S_2.

Table 3. Estimates of the glyphosate dose resulting in 50% (ED_{50}) and 90% (ED_{90}) reduction in shoot biomass of common waterhemp biotypes collected from seven eastern Nebraska counties.

Common waterhemp biotypes[a]	Glyphosate[b]		
	ED_{50}[a]	ED_{90}[a]	Resistance level[c]
	g ae ha^{-1}		
S_1	283 ± 10	683 ± 65	—
S_2	120 ± 10	986 ± 147	—
Antelope	1,051 ± 74	8,198 ± 1,365	8 to 12×
Dodge	2,275 ± 122	13,371 ± 1,700	14 to 20×
Fillmore	205 ± 17	3,202 ± 572	3 to 5×
Lancaster	2,984 ± 244	> 16,800	22 to 32×
Pawnee	2,599 ± 131	11,683 ± 1,372	12 to 17×
Seward	3,152 ± 220	> 16,800	23 to 32×
Washington	1,948 ± 129	> 16,800	19 to 27×

[a] Abbreviations: ED_{50}, effective dose of glyphosate required for 50% reduction in shoot biomass of common waterhemp biotype at 21 d after treatment; ED_{90}, effective dose of glyphosate required for 90% reduction in shoot biomass of common waterhemp biotype at 21 d after treatment; S_1, glyphosate-susceptible common waterhemp biotype collected in 2006 from a field near Clay Center, NE; S_2, glyphosate- susceptible common waterhemp biotype collected in 2012 from a field near Lincoln, NE.

[b] Values represent mean ± SE in g ae ha^{-1}.

[c] Resistance level at ED_{90} value of respective common waterhemp biotype divided by ED_{90} value of susceptible biotypes (S_1 and S_2). A range of resistance level was provided due to a difference in ED_{90} values for S_1 and S_2.

sensitive ($\geq$ 90% control) to PPO-inhibiting herbicides, including acifluorfen, fomesafen, and lactofen applied alone or in tank-mixes with glyphosate or ALS inhibitors (Tables 4 and 5). A similar response was observed with the glyphosate-susceptible biotypes. Fluthiacet-methyl provided 90% control of glyphosate-resistant common waterhemp biotypes, but glyphosate-susceptible biotypes were less sensitive and resulted in < 56% control at 21 DAT (Tables 4 and 5). Jhala et al. (2014) reported that fluthiacet-methyl is usually not very effective on *Amaranthus* species. Therefore, a variable response can be expected. However, shoot regrowth was observed at 21 DAT in most of the plants treated with PPO inhibitors (data not shown).

Most common waterhemp biotypes tested in this study had reduced sensitivity to ALS-inhibiting herbicides, which could be attributed to the predominance of ALS inhibitor–resistant common waterhemp in the Midwest due to heavy reliance on herbicides of this chemistry in the past (Heap 2014a). However, a dose-response study is required to quantify level of resistance to ALS inhibitors in common waterhemp biotypes tested in this study. Reduced sensitivity of common waterhemp to different groups of herbicides is not a new phenomenon in the Midwest. For example, Horak and Peterson (1995) reported an eight-fold level of resistance to ALS inhibitors in two common waterhemp biotypes in Kansas. Lovell et al. (1996) found a 490-fold level of resistance to ALS inhibitors in common waterhemp biotypes from the same counties in Kansas. Additionally, multiple-herbicide–resistant common waterhemp has been reported in Illinois, Iowa, Kansas, Minnesota, and Missouri (Bell et al. 2013; Foes et al. 1998; Heap 2014a; Legleiter and Bradley 2008; Patzoldt et al. 2005; Shoup et al. 2003).

Glufosinate provided $\geq$ 82% control of glyphosate-resistant as well as susceptible common

Table 4. Efficacy of POST soybean herbicides for control and biomass reduction of common waterhemp biotype of glyphosate-susceptible (S_1) and glyphosate-resistant biotypes of Dodge and Lancaster Counties at 21 d after treatment.[a,b,c,d]

		S_1		Dodge		Lancaster	
Herbicide	Rate	Control at 21 DAT	Reduction in biomass	Control at 21 DAT	Reduction in biomass	Control at 21 DAT	Reduction in biomass
	g ae or ai ha^{-1}	%					
Nontreated control	—	0	—	0	—	0	—
Fluthiacet-methyl	7.2	56 h	54 g	91 cd	54 c	92 bc	87 a
Chlorimuron-ethyl	13.1	2 l	2 j	13 fgh	26 d	13 de	18 bc
Imazethapyr + glyphosate	910	91 cde	87 cde	21 efg	19 def	11 e	14 cd
Fomesafen + glyphosate	1,380	87 ef	85 de	97 abc	64 bc	98 ab	88 a
Lactofen + glyphosate	140 + 1,540	90 cde	88 bcde	96 abc	75 b	98 ab	91 a
Imazamox + glyphosate	26.3 + 1,540	98 ab	95 a	12 gh	14 ef	18 de	16 bc
Imazaquin + glyphosate	70.6 + 1,400	95 bc	94 ab	23 ef	21 de	15 de	7 d
Thifensulfuron-methyl	4.4	35 i	33 h	28 e	24 d	12 de	15 bcd
Glufosinate	594	90 cde	85 de	84 d	67 b	82 c	85 a
Lactofen	220	82 fg	80 ef	97 abc	89 a	95 ab	90 a
Imazethapyr	70	9 jk	7 i	6 h	11 f	17 de	13 cd
Imazethapyr + acifluorfen	70 + 245	89 def	87 cde	94 bc	87 a	98 ab	92 a
Imazamox	44	17 j	13 i	9 h	11 f	12 de	13 cd
Fomesafen	280	78 g	75 f	99 a	88 a	99 a	93 a
Imazamox + acifluorfen	44 + 175	88 def	87 cde	98 ab	88 a	99 a	93 a
Chlorimuron ethyl + thifensulfuron-methyl	7.46	4 kl	8 i	23 ef	25 d	19 de	15 bcd
Imazethapyr + glyphosate	2,310	99 a	92 abc	26 e	25 d	22 d	26 b
Acifluorfen	420	94 cd	92 abc	99 a	90 a	99 a	90 a

[a] Abbreviations: S_1, glyphosate-susceptible common waterhemp biotype collected in 2006 from a field near Clay Center, NE; DAT, days after treatments.

[b] Data were arcsine square-root transformed before analysis; however, back-transformed actual mean values are presented based on the interpretation from the transformed data.

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

[d] Percent control data (0%) of nontreated control were not included in the analysis. Reduction in biomass was calculated on the basis of average biomass of nontreated control.

waterhemp biotypes with biomass reduction varying from 67 to 93% at 21 DAT (Tables 4 and 5). A recent study in Nebraska reported 99% control of glyphosate-resistant giant ragweed (*Ambrosia trifida* L.) in glufosinate-resistant soybean when 2,4-D was applied preplant followed by PRE and in-crop glufosinate treatments (Kaur et al. 2014). Similarly, Aulakh et al. (2012) reported 84% control of ≤ 10-cm-tall Palmer amaranth (*Amaranthus palmeri* S. Watts) with glufosinate in no-till cotton (*Gossypium hirsutum* L.). Therefore, integrating glufosinate-tolerant soybean in corn–soybean cropping systems might be an additional tool for controlling glyphosate-resistant weeds (Johnson et al. 2014).

This study showed that most of the suspected glyphosate-resistant common waterhemp biotypes collected from eastern Nebraska counties have a high level of resistance to glyphosate, as well as reduced sensitivity to ALS-inhibiting herbicides applied at labeled rates. The occurrence of glyphosate-resistant common waterhemp biotypes will be a significant detriment to corn and soybean producers in Nebraska. Legleiter et al. (2009) reported that herbicide programs containing PRE followed by POST herbicides resulted in greater control of glyphosate-resistant common waterhemp and reduced weed seed production, and provided the highest soybean yield and net income. The PPO-inhibiting herbicides resulted in > 90% control of glyphosate-resistant biotypes in this study. Although, resistance to PPO inhibitors has not been confirmed in common waterhemp in Nebraska, repeated use of these herbicides may result in resistance. In fact, a

Table 5. Efficacy of POST soybean herbicides for control and biomass reduction of glyphosate-resistant common waterhemp biotype of Pawnee, Seward, and Washington Counties at 21 d after treatment.[a,b,c,d]

Herbicide	Rate	Pawnee Control at 21 DAT	Pawnee Reduction in biomass	Seward Control at 21 DAT	Seward Reduction in biomass	Washington Control at 21 DAT	Washington Reduction in biomass
	g ae or ai ha^{-1}	%					
Nontreated control	—	0	—	0	—	0	—
Fluthiacet-methyl	7.2	89 cde	87 cdef	92 b	89 a	92 a	91 a
Chlorimuronethyl	13.1	74 fg	72 hi	9 cd	10 c	21 bc	21 bc
Imazethapyr + glyphosate	910	95 abc	91 abc	16 c	14 bc	27 bc	27 b
Fomesafen + glyphosate	1,380	95 abc	91 abc	98 a	91 a	95 a	92 a
Lactofen + glyphosate	140 + 1,540	95 abc	93 ab	97 ab	89 a	93 a	94 a
Imazamox + glyphosate	26.3 + 1,540	77 fg	79 gh	11 cd	18 b	28 b	17 cd
Imazaquin + glyphosate	70.6 + 1,400	81 efg	82 efg	17 c	12 bc	8 d	7 e
Thifensulfuron-methyl	4.4	77 fg	77 ghi	11 cd	9 cd	21 bc	21 bc
Glufosinate	594	92 bcd	92 abc	91 b	89 a	94 a	93 a
Lactofen	220	97 ab	88 bcde	95 ab	91 a	96 a	93 a
Imazethapyr	70	60 h	57 j	7 cd	10 c	14 cd	12 de
Imazethapyr + acifluorfen	70 + 245	98 a	88 bcde	99 a	91 a	95 a	94 a
Imazamox	44	84 def	81 fg	4 d	4 d	7 d	8 e
Fomesafen	280	97 ab	91 abc	99 a	95 a	97 a	96 a
Imazamox + acifluorfen	44 + 175	98 a	95 a	98 a	91 a	95 a	92 a
Chlorimuron ethyl + thifensulfuron-methyl	7.46	72 gh	69 i	11 cd	13 bc	26 bc	23 bc
Imazethapyr + glyphosate	2,310	89 cde	84 defg	14 c	13 bc	25 bc	28 b
Acifluorfen	420	99 a	90 abcd	99 a	93 a	95 a	94 a

[a] Abbreviations: DAT, days after treatment.

[b] Data were arcsine square-root transformed before analysis; however, back-transformed actual mean values are presented based on the interpretation from the transformed data.

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

[d] Percent control data (0%) for nontreated control were not included in the analysis. Reduction in biomass was calculated on the basis of average biomass of nontreated control.

common waterhemp biotype resistant to ALS inhibitors, glyphosate, PPO inhibitors, and triazine herbicides has been confirmed in Illinois (Bell et al. 2013), which leaves no POST soybean herbicide that can effectively control this biotype in glyphosate-resistant soybean. Management of glyphosate-resistant common waterhemp in Nebraska would require long-term integrated strategies such as crop rotation, rotational use of herbicide-resistant crop technologies, or use of herbicides with different mechanisms of action, as well as cultural and mechanical methods of weed control.

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