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Glyphosate-Resistant Common Ragweed (*Ambrosia artemisiifolia*) in Nebraska: Confirmation and Response to Postemergence Corn and Soybean Herbicides

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Common ragweed is an important broadleaf weed in agronomic crops in the northcentral United States. A common ragweed biotype in glyphosate-resistant (GR) soybean production field in southeast Nebraska was not controlled after sequential applications of glyphosate at the labeled rate. The objectives of this study were to confirm GR common ragweed in Nebraska by quantifying the level of resistance in greenhouse and field whole-plant dose-response studies and to evaluate the response of the putative GR common ragweed to POST corn and soybean herbicides. Greenhouse whole-plant dose-response studies confirmed 7- and 19-fold resistance to glyphosate compared to the known glyphosate-susceptible (GS) biotype based on biomass reduction and control estimates, respectively. Field dose-response studies conducted in 2015 and 2016 at the putative GR common ragweed research site suggested that glyphosate doses equivalent to 15- and 40-times the labeled rate (1,260 g ae ha^{-1}) were required for 90% control and biomass reduction, respectively. Response of GR common ragweed to POST soybean herbicides in greenhouse studies indicated ≥89% control with acifluorfen, fomesafen, fomesafen plus glyphosate, glyphosate plus dicamba or 2,4-D choline, glufosinate, imazamox plus acifluorfen, and lactofen. POST corn herbicides, including 2,4-D, bromoxynil, diflufenzopyr plus dicamba, glufosinate, halosulfuron-methyl plus dicamba, mesotrione plus atrazine, and tembotrione provided ≥87% control, indicating that POST herbicides with distinct modes of action are available in corn and soybean for effective control of GR common ragweed. Results also suggested a reduced efficacy of the acetolactate synthase (ALS)-inhibiting herbicides tested in this study for control of GR and GS biotypes, indicating further research is needed to determine whether this biotype has evolved multiple herbicide resistance.

Nomenclature: 2,4-D; acifluorfen; atrazine; bentazon; bromoxynil; carfentrazone; chlorimuron; dicamba; fluthiacet; fomesafen; glufosinate; glyphosate; halosulfuron; imazethapyr; imazamox lactofen; mesotrione; primisulfuron; tembotrione; thifensulfuron; topramezone; common ragweed, *Ambrosia artemisiifolia* L.; corn, *Zea mays* L.; soybean, *Glycine max* (L.) Merr.

Key words: Acetolactate synthase inhibitors, frequency of glyphosate resistance, herbicide site of action, postemergence herbicides, resistance management.

Ambrosia artemisiifolia es una maleza de hoja ancha importante en cultivos agronómicos en el centro norte de Estados Unidos. Un biotipo de *A. artemisiifolia* resistente a glyphosate (GR) no fue controlado en un campo de producción de soja en el sureste de Nebraska, después de aplicaciones secuenciales de glyphosate a la dosis de la etiqueta. Los objetivos de este estudio fueron confirmar la existencia de *A. artemisiifolia* GR en Nebraska cuantificando el nivel de resistencia con estudios de respuesta a dosis en invernadero y en campo y evaluar la respuesta de *A. artemisiifolia* GR putativa a herbicidas POST para maíz y soja. Los estudios de respuesta a dosis en invernadero con plantas enteras confirmaron una resistencia a glyphosate 7 y 19 veces mayor al compararse con un biotipo con susceptibilidad conocida a glyphosate (GS), según los estimados de reducción de biomasa y de control, respectivamente. Los estudios de respuesta a dosis en campo realizados en 2015 y 2016 en un lugar experimental con *A. artemisiifolia* GR putativa sugirió que se requirieron dosis equivalentes a 15 y 40 veces la dosis de la etiqueta (1,260 g a ha^{-1}) para alcanzar un 90% de control y un 90% de reducción de la biomasa, respectivamente. La respuesta en estudios de invernadero de *A. artemisiifolia* GR a herbicidas POST para soja indicó ≥89% de control con acifluorfen, fomesafen, fomesafen más glyphosate, glyphosate más dicamba o 2,4-D choline, glufosinate, imazamox más acifluorfen, y lactofen. Herbicidas POST para maíz, incluyendo 2,4-D, bromoxynil, diflufenzopyr más dicamba, glufosinate, halosulfuron-methyl más dicamba, mesotrione más atrazine, y tembotrione brindaron ≥87% de control, indicando que herbicidas POST con modos de acción distintivos están disponibles en maíz y

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soja para el control efectivo de *A. artemisiifolia* GR. Los resultados también sugirieron que existe una eficacia reducida con herbicidas inhibidores de acetolactate synthase (ALS) evaluados en este estudio para el control de biotipos GR y GS, lo que indica que se necesita investigación adicional para determinar si este biotipo ha evolucionado resistencia a múltiples herbicidas.

Common ragweed is an erect summer annual broadleaf weed frequently found on roadsides, wastelands, and agronomic fields predominantly under reduced or no-till cropping systems (Bassett and Crompton 1975; Jordan et al. 2007; Saint-Louis et al. 2005). Common ragweed is native to North America and has been documented as a major cause of hay fever due to its prolific production of pollen that is allergenic and easily carried by wind (Fumanal et al. 2007; Rogers et al. 2006; Simard and Benoit 2011). High pollen production, wind pollination, and self-incompatibility promote outcrossing and high genetic diversity in common ragweed, and consequently increase the potential for evolution of herbicide resistance (Friedman and Barrett 2008; Jordan et al. 2007). Common ragweed germinates on or near the soil surface, preferably <2.5 cm deep, and most of the emergence occurs from late April to mid-May (Bassett and Crompton 1975; Gebben 1965). Common ragweed grows 1 to 2 m tall with distinct male and female flowers on the same plant, and produces 32,000 to 62,000 seeds per plant (Dickerson and Sweet 1971; Jordan et al. 2007). These characteristics, combined with long seed viability (approximately 39 years), enable common ragweed to easily establish and persist as a potential dominant weed in new habitats (Bassett and Crompton 1975).

Common ragweed interference with crop growth results in variable yield losses depending upon the density, time of emergence relative to the crop, and the type of crop infested (Jordan et al. 2007; Weaver 2001). Common ragweed is a very competitive weed in several agronomic crops, including corn and soybean (Chikoye et al. 1995; Cowbrough et al. 2003; Jordan et al. 2007). For example, Weaver (2001) reported an average yield loss of 38% in corn at a common ragweed density of ≥32 plants m^{-2}. Similarly, Coble et al. (1981) and Shurtleff and Coble (1985) reported 10% to 12% soybean yield loss with 2 to 4 common ragweed plants per ten meter row length. Weaver (2001) reported that common ragweed is more competitive in soybean than it is in corn and caused yield losses of 65% to 70% at a density of ≥30 plants m^{-2}. Season-long interference of 1 common ragweed plant per meter row of peanut (*Arachis hypogaea* L.) also resulted in 40% yield loss (Clewis et al. 2001). Therefore, management of common ragweed is imperative to reduce crop yield losses.

Prior to the commercialization of glyphosate-resistant (GR) soybean, acetolactate synthase (ALS) inhibitors such as chlorimuron-ethyl, cloransulam, or imazaquin; and protoporphyrinogen oxygenase (PPO) inhibitors, including fomesafen or lactofen, were primarily used for POST common ragweed control in soybean (Jordan et al. 2007; Rousonelos et al. 2012). However, the continuous use of GR corn and soybean in the Midwest resulted in an overreliance on glyphosate and the consequent evolution of GR weed species, including common ragweed. The first report of GR common ragweed was from Missouri in 2004, and subsequently it has been reported in 13 other states in the United States, including Alabama, Arkansas, Indiana, Kansas, Kentucky, Minnesota, Mississippi, New Jersey, North Carolina, North Dakota, Ohio, Pennsylvania, and South Dakota (Heap 2016). Additionally, common ragweed biotypes resistant to ALS and 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) inhibitors have been reported from Minnesota and Ohio in the United States and from Ontario, Canada; and biotypes resistant to ALS plus PPO inhibitors have been reported from Delaware, Ohio, and Ontario (Heap 2016; Rousonelos et al. 2012; Van Wely et al. 2015a). In recent years, management of common ragweed has become more complicated due to its evolution of resistance to herbicides belonging to distinct sites of action.

In the summer of 2014, a soybean grower reported a failure to control common ragweed following sequential applications of glyphosate at the labeled rate in a field in Gage County, Nebraska. The field had been continuously under GR corn–soybean cropping systems, with one or two glyphosate applications in each cropping season, over the last several years. This situation necessitated the need to evaluate whether this common ragweed biotype is resistant to glyphosate and to determine the level of resistance. Further, it became imperative to evaluate the efficacy of POST corn and soybean herbicides with different sites of action to determine if putative GR common ragweed has reduced susceptibility to other herbicides. Additionally, this information can be used to develop an alternate effective common ragweed control program. The objectives of this study were to confirm and quantify the level of glyphosate resistance in the putative common ragweed biotype in Nebraska and to evaluate, under greenhouse conditions, its response to POST herbicides labeled for control of broadleaf weeds in corn and soybean.

Materials and Methods

Plant Materials. Inflorescences of putative GR common ragweed were collected in the fall of 2014 from the plants that survived sequential applications of a field rate of glyphosate (1,260 g ae ha^{-1}) in a soybean production field in Gage County, Nebraska (40.44°N, 96.62°W). Before threshing, the seed heads were dried for a week at room temperature, and after cleaning, composite seed samples from about 20 plants were prepared and stored at 4 C until used in this study. Seeds from a known glyphosate-sensitive (GS) common ragweed biotype were collected from a field near Clay Center, Nebraska and used for comparison in this study.

Greenhouse Dose-Response Study. Whole-plant dose-response bioassays with putative GR and GS biotypes were conducted under greenhouse conditions at the University of Nebraska–Lincoln. During both experiments, similar growth conditions were maintained in the greenhouse with a daytime temperature of 25 ± 2 C and a nighttime temperature of 18 ± 3 C, and a relative humidity of 70% to 75%. Sodium halide lamps were used as a supplemental light source to ensure a 15-h photoperiod. Seeds of the putative GR and known GS common ragweed biotypes were germinated in plastic trays containing potting mix (Berger BM1 All-Purpose Mix, Berger Peat Moss Ltd., Saint-Modeste, Quebec, Canada). Uniform-size seedlings were transplanted to square plastic pots (10 by 10 by 12 cm) containing a 3:1 mixture of potting mix to soil after the appearance of the first true leaves. Plants were supplied with adequate nutrients weekly as needed and watered daily.

The experiments were conducted in a randomized complete block design with six replications and repeated in time. The treatments were arranged in a ten by two factorial with 10 glyphosate rates [0, 0.06×/0.12×, 0.25×, 0.50×, 1×, 2×, 4×, 8×, 16×, and 32×, where 1× is the labeled rate of 1,260 g ha^{-1} (Roundup® PowerMax, Monsanto Company, 800 North Lindberg Ave., St. Louis, MO)] and two common ragweed biotypes (GR and GS). A single common ragweed plant per pot was considered an experimental unit. Glyphosate treatments were prepared in distilled water and mixed with nonionic surfactant (Induce®, Helena Chemical Co., Collierville, TN) at 0.25% (v/v) and ammonium sulfate (DSM Chemicals North America Inc., Augusta, GA) at 2.5% (wt/v). Seedlings were treated with glyphosate at the six- to eight-leaf stage (8 to 10 cm tall) using a single-tip spray chamber (DeVries Manufacturing Corp, Hollandale, MN) fitted with an 8001E nozzle (TeeJet, Spraying Systems Co., Wheaton, IL) calibrated to deliver 190 L ha^{-1} carrier volume at 207 kPa.

Control was visually estimated and recorded at 21 d after treatment (DAT) using a 0% to 100% scale with 0% equal to no control and 100% equal to complete control or death of the treated common ragweed plant. Percent control of treated plants was assessed based on comparison with the nontreated control plants with respect to symptoms such as chlorosis, necrosis, stand loss, and stunting. Aboveground biomass of each common ragweed plant was harvested close to the base at 21 DAT and dried in an oven at 65 C for 2 d before dry weight was recorded. Aboveground biomass data were converted into percent biomass reduction compared to the nontreated control (Ganie et al. 2016; Wortman 2014) using the following equation:

$$\text{Percent biomass reduction} = \left[\left(\overline{C} - B\right)/\overline{C}\right] \times 100, \qquad [1]$$

where $\overline{C}$ is the biomass of the nontreated control replicate and B is the biomass of an individual treated experimental unit.

Field Dose-Response Study. A field dose-response study was conducted in the summers of 2015 and 2016 at the field in which the putative GR common ragweed had been reported, which was located in Gage County, southeast Nebraska (40.44°N, 96.62°W). The study was established under non-crop conditions in a natural stand of common ragweed containing about 40 plants m^{-2}. The treatments were arranged in a randomized complete block design with four replications and ten glyphosate rates, similar to the greenhouse dose-response experiments. A nontreated control was included for comparison. Individual plots were 3 m wide and 9 m long. Glyphosate treatments were applied to putative GR common ragweed plants at the four- to six-leaf stage (6 to 12 cm tall) with a CO_2-pressurized backpack sprayer calibrated to deliver 140 L ha^{-1} at 276 kPa and equipped with a four-nozzle boom fitted with AIXR 110015 flat-fan nozzles (TeeJet, Spraying Systems Co., PO Box 7900, Wheaton, IL). Control was visually estimated and recorded at 21 DAT on a 0% to 100% scale as described in the greenhouse dose-response experiment. At 21 DAT, common ragweed plants that survived glyphosate treatments were cut at the stem base, close to the soil surface, from two randomly selected 0.25-m^2 quadrats per plot. The plants were then placed in paper bags and dried in an oven for 72 h at 66 C, after which aboveground biomass was recorded. The aboveground biomass was then converted into percent biomass reduction using Equation 1.

Data for control estimates and biomass reduction from greenhouse and field dose-response studies were regressed over the glyphosate rates using a four parameter log-logistic function (Knezevic et al. 2007):

$$Y = C + \{D - C/1 + \exp[B(\log X - \log E)]\}, \quad [2]$$

where Y is the response variable (percent control or percent reduction in biomass), C is the lower limit, D is the upper limit, E is the dose resulting in 50% or 90% control (known as ED_{50} or ED_{90}) or growth reduction (known as GR_{50} or GR_{90}), B is the slope of the curve around ED_{50}, and X is the glyphosate dose. Analyses of dose-response data from greenhouse and field experiments were performed separately and the effective doses (ED_{50} or ED_{90} being doses that provided 50% or 90% control, and GR_{50} or GR_{90} being doses that resulted in 50% or 90% biomass reduction relative to the nontreated control) were determined using the *drc* package (drc 1.2, Christian Ritz and Jens Strebig, R2.5, Kurt Hornik, online) in software R (R statistical software, R Foundation for Statistical Computing, Vienna, Austria; http://www.R-project.org) (Ritz and Streibig 2005). The level of resistance from the greenhouse dose-response experiment was determined using a ratio of the ED_{90} or GR_{90} values of the putative GR and known GS biotypes. Resistance levels based on the control estimates and biomass reduction were determined separately since the ED_{90} or GR_{90} values were not the same, though the resistance levels from the field dose-response study were determined compared to the field rate of glyphosate (1,260 g ha^{-1}).

Model Goodness of Fit. The indices to check model fitness, including root mean square error (RMSE) and modeling efficiency coefficients (EF), were determined using Equations 3 and 4 in the *drc* package of R software (Mayer and Butler 1993; Roman et al. 2000; Sarangi et al. 2016):

$$\mathrm{RMSE} = \left[\frac{1}{n}\sum_{i=1}^{n}(P_i - O_i)^2\right]^{1/2} \text{ and} \quad [3]$$

$$\mathrm{EF} = 1 - \left[\sum_{i=1}^{n}(O_i - P_i)^2 \Big/ \sum_{i=1}^{n}(O_i - \bar{O}_i)^2\right], \quad [4]$$

where P_i is the predicted value, O_i is the observed value, $\bar{O}_i$ is the mean observed value, and n is the total number of observations. Smaller RMSE values indicate better fit, and EF values closer to 1 indicate more accurate predictions.

Frequency of Glyphosate Resistance. Field experiments were conducted in 2015 and 2016 at the site where the putative GR common ragweed was reported (described earlier) to determine the percent survival of the natural stand of GR common ragweed biotype. Twenty-five 0.25 m^2 quadrats were randomly established across the field. Common ragweed plants were counted from quadrats on June 10, 2015 and June 12, 2016, when more than 90% emergence had completed, and sprayed with 2× (1x = 1,260 g ha^{-1}) rate of glyphosate when most of the plants were 8 to 12 cm tall. Four weeks after the treatment, surviving plants were counted by considering plants with ≥80% injury as dead, and plants with <80% injury as survivals. Frequency of glyphosate resistance was determined by the following equation (Walsh et al. 2007):

$$\text{Frequency of resistance} = \frac{\text{Number of common ragweed plants surviving}}{\text{Number of common ragweed plants sprayed}} \times 100$$

Response to POST Corn and Soybean Herbicides. Experiments were conducted in the greenhouse at the University of Nebraska–Lincoln under the same growth conditions described in the greenhouse dose-response study. Treatments included herbicides registered for POST application in soybean (Table 1) and corn (Table 2). The goal was to determine the response of GR and GS common ragweed biotypes to herbicides with distinct sites of action. The experiments were conducted separately for corn and soybean POST herbicides in randomized complete block designs with four replications and repeated in time. Herbicide rates were selected based on the labeled rates (Tables 1 and 2) and were applied at the six- to eight-leaf stage (8 to 12 cm tall) to GR and GS common ragweed biotypes. Control was visually estimated and recorded at 21 DAT using the 0% to 100% scale described in the dose-response studies. The aboveground biomass was recorded using the same procedure explained in the greenhouse dose-response study and converted into percent biomass reduction using Equation 1.

Data were subjected to ANOVA in SAS® version 9.4 (SAS Institute Inc, Cary, NC) using the PROC GLIMMIX procedure. Before analysis, the data were tested for the normality of residuals using the PROC UNIVARIATE procedure. Control and biomass data were arcsine square-root transformed before analysis; however, back-transformed data are presented with mean separation based on the transformed data. If the ANOVA indicated that treatment effects were significant, means were separated at $P \leq 0.05$ using Fisher's Protected LSD test.

Results and Discussion

Dose-Response Study. Treatment by experiment interactions in the greenhouse dose-response study were not significant for either common ragweed control ($P = 0.22$) or biomass reduction ($P = 0.10$); therefore, data from both experiments were combined. The labeled rate of glyphosate (1,260 g ha^{-1}) resulted in ≥90% control of the GS common ragweed biotype, compared to ≤40% control of the putative GR biotype (Figure 1), confirming resistance. Effective glyphosate rates for 50% (ED$_{50}$) and 90% (ED$_{90}$) control of the GS common ragweed biotype were 298 and 1,287 g ha^{-1}, compared to 3,494 and 24,002 g ae ha^{-1} for the GR biotype, respectively, in the greenhouse dose-response study (Table 3). However, effective glyphosate rates for 50% (GR$_{50}$) and 90% (GR$_{90}$) biomass reduction

Table 1. Details of soybean POST herbicides used to evaluate response of the glyphosate-resistant and susceptible common ragweed biotypes in a greenhouse study conducted at the University of Nebraska–Lincoln

Herbicide common name	Application rate	Site of action[a]	Trade name	Manufacturer	Adjuvant[b]
	g ae or ai ha^{-1}				
Chlorimuron-ethyl	13.1	ALS	Classic®	E. I. du Pont de Nemours and Company, Wilmington, DE; www.dupont.com	NIS + AMS
Thifensulfuron-methyl	4.4	ALS	Harmony® SG	E. I. du Pont de Nemours and Company	NIS + AMS
Chlorimuron-ethyl + thifensulfuron	7.46	ALS	Synchrony® XP	E. I. du Pont de Nemours and Company	NIS + AMS
Imazethapyr	70	ALS	Pursuit®	BASF Corporation, Research Triangle Park, NC; www.basf.com	NIS + AMS
Imazamox	44	ALS	Raptor®	BASF Corporation	NIS + AMS
Glufosinate	594	GLS	Liberty®	Bayer CropScience LP, Research Triangle Park, NC; www.cropscience.bayer.com	AMS
Acifluorfen	420	PPO	Ultra Blazer®	United Phosphorus, Inc.	NIS + AMS
Fluthiacet-methyl	7.2	PPO	Cadet®	FMC Corporation, Philadelphia, PA; www.fmc.com	COC + AMS
Fomesafen	280	PPO	Reflex®	Syngenta Crop Protection, Inc., Greensboro, NC; www.syngenta.com	NIS + AMS
Lactofen	220	PPO	Cobra®	Valent U.S.A. Corporation, Walnut Creek, CA; www.valent.com	NIS + AMS
Fluthiacet-methyl + fomesafen	190	PPO	Marvel®	FMC Corporation + Syngenta Crop Protection, Inc.	NIS + AMS
Bentazon	950	PS II	Basagran®	BASF Corporation	NIS + AMS
Imazethapyr + acifluorfen	70 + 245	ALS + PPO	Pursuit® + Ultra Blazer®	BASF Corporation + United Phosphorus, Inc., King of Prussia, PA; www.uplonline.com	NIS + AMS
Fomesafen + glyphosate	1,380	PPO + EPSPS	Flexstar® GT	Syngenta Crop Protection, Inc.	NIS + AMS
Imazethapyr + glyphosate	910	ALS + EPSPS	Etreme®	BASF Corporation	NIS + AMS
Imazaquin + glyphosate	70.6 + 1,400	ALS + EPSPS	Scepter® + Touchdown® Hitech	BASF Corporation + Syngenta Crop Protection, Inc.	NIS
Imazethapyr + glyphosate	2,310	ALS + EPSPS	Tackle®	Cheminova, Inc., Research Triangle Park, NC; www.cheminova.com	NIS + AMS
Glyphosate + dicamba	1,681	EPSPS + SA	Roundup® Xtend	Monsanto Company, St. Louis, MO	MON 10
Glyphosate + 2,4-D choline	1,636	EPSPS + SA	Enlist® Duo	Dow AgroSciences LLC, Indianapolis, IN	AMS

[a] Abbreviations: ALS, acetolactate synthase; AMS, ammonium sulfate (DSM Chemicals North America Inc., Augusta, GA); COC, crop oil concentrate (Agridex®, Helena Chemical Co., Collierville, TN); EPSPS, 5-enolpyruvylshikimate-3-phosphate synthase; GLS, glutamine synthetase; NIS, nonionic surfactant (Induce®, Helena Chemical Co., Collierville, TN); PPO, protoporphyrinogen oxidase; PS II, photosystem II; SA, synthetic auxins.

[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).

Table 2. Details of POST corn herbicides used to evaluate the response of glyphosate-resistant and susceptible common ragweed biotypes in a greenhouse study conducted at the University of Nebraska–Lincoln

Herbicide common name	Application rate	Site of action[a]	Trade name	Manufacturer	Adjuvant[b]
	g ae or ai ha^{-1}				
Halosulfuron-methyl	70	ALS	Permit®	Gowan Company, PO Box 5569, Yuma, AZ; www.gowanco.com	COC + AMS
Primisulfuron-methyl	20	ALS	Beacon®	Syngenta Crop Protection, Inc., Greensboro, NC; www.syngenta.com	COC + AMS
Glufosinate	595	GLS	Liberty®	Bayer CropScience LP, Research Triangle Park, NC; www.cropscience.bayer.com	AMS
Mesotrione	105	HPPD	Callisto®	Syngenta Crop Protection, Inc.	COC + AMS
Tembotrione	92	HPPD	Laudis®	Bayer CropScience LP	MSO + AMS
Topramezone	18.4	HPPD	Impact®	AMVAC, Los Angeles, CA 90023; www.amvac-chemical.com	COC
Carfentrazone	8.8	PPO	Aim®	FMC Corporation, Philadelphia, PA; www.fmc.com	COC
Bromoxynil	420	PS II	Buctril®	Bayer CropScience LP	COC + AMS
2,4-D	560	SA	2,4-D LV Ester	Winfield Solutions, LLC, St Paul, MN 55164; www.winfield.com	NIS + AMS
Dicamba	280	SA	Clarity®	BASF Corporation, Research Triangle Park, NC; www.basf.com	NIS + AMS
Diflufenzopyr + dicamba	196	SA	Status®	BASF Corporation	NIS + AMS
Halosulfuron-methyl + dicamba	380	ALS + SA	Yukon®	Gowan Company	NIS + AMS
Thiencarbazone-methyl + tembotrione	91	ALS + HPPD	Capreno®	Bayer CropScience LP	NIS + AMS
Mesotrione + atrazine	105 + 2240	HPPD + PS II	Callisto® + Aatrex	Syngenta Crop Protection, Inc.	NIS + AMS
Fluthiacet-methyl + mesotrione	110	PPO + HPPD	Solstice®	FMC Corporation	NIS + AMS
S-metolachlor + glyphosate + mesotrione	2460	LCFA + EPSPS + HPPD	Halex® GT	Syngenta Crop Protection, Inc.	NIS + AMS

[a] Abbreviations: ALS, acetolactate synthase; AMS, ammonium sulfate (DSM Chemicals North America Inc., Augusta, GA); COC, crop oil concentrate (Agridex®, Helena Chemical Co., Collierville, TN); EPSPS, 5-enolpyruvylshikimate-3-phosphate synthase; GLS, glutamine synthetase; HPPD, 4-hydroxyphenylpyruvate dioxygenase; LCFA, long-chain fatty acid; NIS, nonionic surfactant (Induce®, Helena Chemical Co., Collierville, TN); PPO, protoporphyrinogen oxidase; PS II, photosystem II inhibitor; SA, synthetic auxins.

[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).

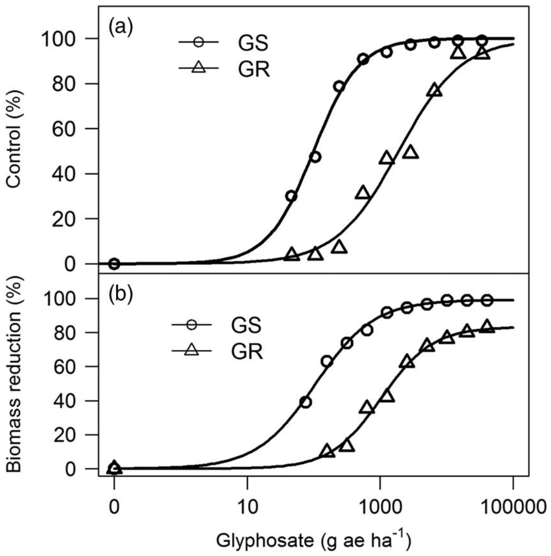

Figure 1. Dose-response curves of glyphosate-resistant (GR) and glyphosate-susceptible (GS) common ragweed biotypes from Nebraska. (a) Control at 21 days after treatment and (b) percent biomass reduction at 21 days after treatment, in a whole-plant glyphosate dose-response study conducted in the greenhouse at the University of Nebraska–Lincoln. Percent biomass reduction was calculated using the following equation: Biomass reduction $(\%) = \left[\left(\overline{C} - B\right)/\overline{C}\right] \times 100$, where C is the biomass of the nontreated control replicate and B is the biomass of an individual treated experimental unit.

were lower compared to ED_{50} and ED_{90}, respectively for both GS and GR biotypes in the greenhouse dose-response study (Tables 3 and 4). For example, GR_{50} and GR_{90} of the GS common ragweed biotypes were 106 and 1,082 g ae ha^{-1} compared to 1,045 and 7,228 g ha^{-1} for the GR biotype, respectively. Thus, the putative GR biotype showed a 7- and 19-fold level of resistance to glyphosate relative to the GS biotype, respectively, based on the ratio of GR_{90} and ED_{90} values determined from biomass reduction and control estimates (Tables 3 and 4). Similarly, Brewer and Oliver (2009) reported two GR common ragweed biotypes from Arkansas with 10- and 20-fold levels of resistance relative to known GS biotypes, based on visual estimates of control. In contrast, Pollard (2007) reported a 9.6-fold resistance to glyphosate in a GR common ragweed biotype from Missouri, based on biomass reduction. Parrish (2015) further reported two GR common ragweed biotypes from Ohio with 4.5- and 12-fold levels of glyphosate resistance relative to known GS biotypes, based on control

Table 3. Regression parameter estimates, model goodness of fit (RMSE and EF)[a], and effective glyphosate doses resulting in 50% (ED_{50}) and 90% (ED_{90}) control of glyphosate-susceptible and resistant common ragweed biotypes in greenhouse and field dose-response studies

	Glyphosate							
	Control							
	Parameter estimates[b]			Model goodness of fit		Effective doses		Resistance level[c,d]
Common ragweed biotype	*B* (±SE)	*C* (±SE)	*D* (±SE)	RMSE	EF	ED_{50} (±SE)	ED_{90} (±SE)	ED_{90} (GR)/ED_{90} (GS)
						——— g ae ha^{-1} ———		
Greenhouse dose-response								
GS	−1.5 (0.16)	0.09 (0.03)	98.9 (1.8)	7.3	0.95	298 (20)	1,287 (197)	-
GR	−1.1 (0.10)	−0.12 (0.03)	108 (9)	15.9	0.83	3,494 (328)	24,002 (4,728)	19 ×
Field dose-response								
GR	−1.12 (0.15)	7.2 (2.30)	102 (9)	19.1	0.77	2,671 (353)	19,052 (5,390)	15 ×

[a] Abbreviations: EF, modeling efficiency coefficient; GR, glyphosate-resistant common ragweed biotype collected from Gage County, Nebraska; GS, glyphosate-susceptible common ragweed biotype collected from Clay County, Nebraska; RMSE, root mean square error; SE, standard error.

[b] Regression parameters *B*, *C* and *D* represent slope, and lower and upper limits of the four-parameter log-logistic model, respectively, and were determined by using the nonlinear least-square function of the statistical software R. ED_{50}, effective glyphosate dose required for 50% control of common ragweed at 21 days after treatment; ED_{90}, effective glyphosate dose required for 90% control of common ragweed at 21 days after treatment.

[c] Resistance level in the greenhouse dose-response study was calculated by dividing the ED_{90} value of the GR common ragweed biotype by that of the GS biotype.

[d] In the field dose-response study, resistance level was determined by dividing the ED_{90} values of the GR common ragweed by the labeled rate of glyphosate (1,260 g ae ha^{-1}), because the GS biotype was not available for comparison.

estimates, and 4- and 7-fold levels of resistance based on biomass reduction. The RMSE values for control estimates for the greenhouse dose-response study were 7.3 and 15.9 and the EF values were 0.95 and 0.83, respectively for the GS and GR common ragweed biotypes, indicating a good fit of the model (Table 3).

Results from the field dose-response study suggested relatively higher levels of resistance in the putative GR common ragweed biotype. The effective glyphosate rates determined from the field dose-response study for 50% and 90% control were 2,671 and 19,052 g ha^{-1} compared to 1,312 and 50,596 g ha^{-1} required for aboveground biomass

Table 4. Regression parameter estimates, model goodness of fit (RMSE and EF)[a], and effective glyphosate doses resulting in 50% (GR_{50}) and 90% (GR_{90}) biomass reduction of glyphosate-susceptible and resistant common ragweed biotypes in greenhouse and field dose-response studies

	Glyphosate							
	Biomass reduction							
	Parameter estimates[b]			Model goodness of fit		Effective doses		Resistance level[c,d]
Common ragweed biotype	*B* (±SE)	*C* (±SE)	*D* (±SE)	RMSE	EF	GR_{50} (±SE)	GR_{90} (±SE)	GR_{90}(GR)/GR_{90} (GS)
						——— g ae ha^{-1} ———		
Greenhouse dose-response								
GS	−0.98 (0.14)	−0.09 (0.03)	99 (2)	6.0	0.96	106 (11)	1,082 (226)	-
GR	−1.13 (0.23)	−0.08 (0.03)	83 (5)	17.5	0.78	1,045 (194)	7,228 (3,357)	7×
Field dose-response								
GR	−1.02 (0.2)	−0.09 (0.04)	81 (4)	13.9	0.79	1,312 (658)	50,596 (20,691)	40×

[a] Abbreviations: EF, modeling efficiency coefficient; GR, glyphosate-resistant common ragweed biotype collected from Gage County, Nebraska; GS, glyphosate-susceptible common ragweed biotype collected from Clay County, Nebraska; RMSE, root mean square error; SE, standard error.

[b] Regression parameters *B*, *C*, and *D* represent slope and lower and upper limits of the four-parameter log-logistic model, respectively, and were determined by using the nonlinear least-square function of the statistical software R. GR_{50}, effective glyphosate dose required for 50% biomass reduction of common ragweed relative to the nontreated control at 21 days after treatment; GR_{90}, effective glyphosate dose required for 90% biomass reduction of the common ragweed relative to the nontreated control treatments at 21 days after treatment.

[c] Resistance level in the greenhouse dose-response study was calculated by dividing the GR_{90} value of the GR common ragweed biotype by that of the GS biotype.

[d] In the field dose-response study, resistance level was determined by dividing the GR_{90} values of the GR common ragweed biotype by the labeled rate of glyphosate (1,260 g ae ha^{-1}), because the GS biotype was not available for comparison.

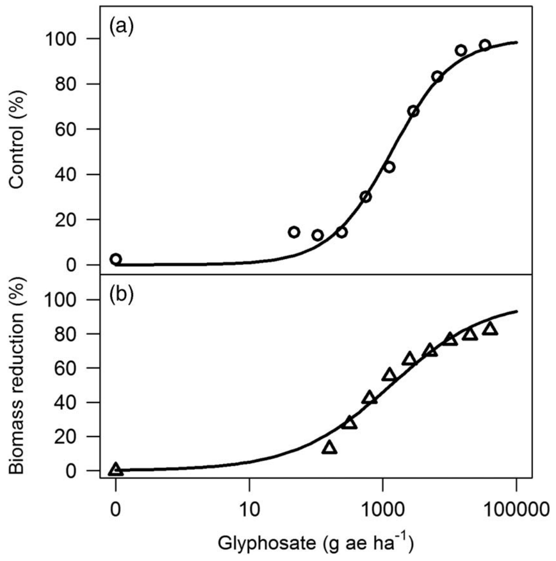

Figure 2. Dose-response curves of a glyphosate-resistant common ragweed biotype from Nebraska. (a) Control at 21 days after treatment and (b) percent biomass reduction at 21 days after treatment, in a whole-plant glyphosate dose-response study conducted at the putative glyphosate-resistant common ragweed field research site in Gage County, Nebraska. Percent biomass reduction was calculated using the following equation: Biomass reduction $(\%) = \left[\left(\bar{C} - B\right)/\bar{C}\right] \times 100$, where C is the biomass of the nontreated control replicate and B is the biomass of an individual treated experimental unit.

reduction, respectively (Figure 2, Tables 3 and 4). Van Wely et al. (2015b) reported that 1,606 and 7,675 g ha^{-1} of glyphosate were required for 50% and 95% control of GR common ragweed at 28 DAT under field conditions in Ontario, Canada. The comparison of ED_{90} and GR_{90} values from the field dose-response study with the labeled rate of glyphosate (1,260 g ha^{-1}) revealed that the glyphosate rate required to achieve 90% control and biomass reduction was 15- and 40-times the labeled rate, respectively (Tables 3 and 4). In a field dose-response study in Ontario, Van Wely et al. (2015b) reported two GR common ragweed biotypes with 2- to 28-fold levels of resistance relative to a known GS biotype based on biomass reduction and control estimates. The RMSE values for control and biomass reduction for the field dose-response study were 19.1 and 13.9 and the EF values were 0.77 and 0.79, respectively, indicating a good fit of the model (Tables 3 and 4). Sarangi et al. (2016) also reported RMSE values ranging from 5.4 to 11.6 and EF values of 0.83 to 0.97 for validation of a four parameter log-logistic model for common waterhemp (*Amaranthus rudis* Sauer) plant height in response to water stress.

Frequency of Glyphosate Resistance. The results suggested that 82% of 1,750 common ragweed plants treated in 2015 and 84% of 2,125 plants treated in 2016 survived 2× (where × is 1,260 g ha^{-1}) rate of glyphosate 4 wk after treatment. Therefore, the frequency of glyphosate resistance in common ragweed biotype ranged from 82% to 84%.

Response to POST Soybean Herbicides. Treatment by experiment interactions for common ragweed control ($P = 0.09$) and biomass reduction ($P = 0.12$) were not significant; therefore, data were combined over two runs. Acifluorfen, fomesafen, fomesafen plus glyphosate, glyphosate plus dicamba or 2,4-D choline, glufosinate, imazamox plus acifluorfen, and lactofen provided 89% to 99% control of GR common ragweed at 21 DAT (Table 5). In recent years, glufosinate and PPO inhibitors have been widely used for controlling GR weeds: for instance, glufosinate provided 99% control of GR giant ragweed (*Ambrosia trifida* L.) (Kaur et al. 2014) and ≥80% control of GR common waterhemp (Sarangi et al. 2015). Previous studies have reported >90% control of common ragweed with fomesafen or lactofen (Chandi et al. 2012; Taylor et al. 2002) and glyphosate plus fomesafen (Van Wely et al. 2015b).

ALS inhibitors (Table 1) were not effective for control of GR or GS common ragweed biotypes and resulted in only 11% to 62% control (Table 5). Similar to the results of this study, Van Wely et al. (2015b) reported <61% control of common ragweed with ALS inhibitors, including chlorimuron-ethyl, cloransulam, imazethapyr, and thifensulfuron. Conversely, common ragweed biotypes showed a differential response to bentazon (PS II inhibitor) and fluthiacet-methyl (PPO inhibitor). Bentazon resulted in 26% and 99% control and fluthiacet-methyl provided 40% and 72% control of GR and GS biotypes, respectively (Table 5).

Results of the biomass reduction were mostly in consensus with the control estimates in both GR and GS biotype. Glufosinate, fomesafen, lactofen, and glyphosate plus fomesafen or 2,4-D choline resulted in a biomass reduction ranging from 80% to 94% without statistical difference among them (Table 5). Similarly, Van Wely et al. (2015b) reported >90%

Table 5. Response of glyphosate-resistant and susceptible common ragweed biotypes to POST soybean herbicides at 21 days after treatment in a greenhouse study conducted at the University of Nebraska–Lincoln

		Glyphosate-resistant biotype[a–d]		Glyphosate-susceptible biotype	
Herbicide	Rate	Control at 21 DAT	Reduction in biomass	Control at 21 DAT	Reduction in biomass
	g ae or ai ha^{-1}	%			
Chlorimuron-ethyl	13.1	28 f	38 jk	21 cd	46 bc
Thifensulfuron-methyl	4.4	26 f	37 kj	18 cd	50 b
Chlorimuron-ethyl + thifensulfuron-methyl	7.46	62 bc	65 fg	30 dc	58 b
Imazethapyr	70	26 f	35 k	28 cd	48 b
Imazamox	44	38 ef	40 jk	11 d	28 c
Glufosinate	594	99 a	90 abc	98 a	89 a
Acifluorfen	420	98 a	83 bcde	95 a	85 a
Fluthiacet-methyl	7.2	40 de	46 ij	72 b	81 a
Fomesafen	280	90 a	92 ab	95 a	81 a
Lactofen	220	95 a	94 a	99 a	86 a
Fluthiacet-methyl + fomesafen	190	74 b	80 de	99 a	85 a
Bentazon	950	26 f	34 k	99 a	85 a
Imazethapyr + acifluorfen	70 + 245	53 c	55 hi	71 b	58 b
Imazamox + acifluorfen	44 + 175	89 a	81 cde	90 a	83 a
Fomesafen + glyphosate	1,380	99 a	88 abcd	99 a	87 a
Imazethapyr + glyphosate	910	73 b	75 ef	81 ab	85 a
Imazaquin + glyphosate	70.6 + 1,400	51 cd	58 gh	34 c	56 b
Imazethapyr + glyphosate	2,310	63 bc	66 fg	98 a	89 a
Glyphosate + dicamba	1,681	99 a	80 de	80 ab	84 a
Glyphosate + 2, 4-D choline	1,636	99 a	84 abcde	99 a	83 a
P-value		<0.0001	<0.0001	<0.0001	<0.0001

[a] Abbreviations: DAT, day after treatment.

[b] Data were arcsine square-root transformed before analysis; however, back-transformed actual mean values are presented based on 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 the nontreated control were not included in the analysis. Reduction in biomass was calculated based on the average biomass of the nontreated control.

biomass reduction in common ragweed with glyphosate plus fomesafen or acifluorfen under field conditions. Additionally, most ALS inhibitors resulted in <65% biomass reduction of both GR and GS biotypes (Table 5). The reduced efficacy of ALS inhibitors for common ragweed control is not surprising since common ragweed biotypes with resistance to ALS inhibitors have been previously reported (Chandi et al. 2012; Patzoldt et al. 2001; Van Wely et al. 2015a). In Nebraska, other weed species, including common waterhemp, kochia [*Kochia scoparia* (L.) Schrad.], horseweed [*Conyza canadensis* (L.) Cronq.], and Palmer amaranth (*Amaranthus palmeri* S. Wats.) have evolved resistance to ALS inhibitors due to repeated applications of these herbicides in corn–soybean cropping systems (Jhala et al. 2014; Sarangi et al. 2015). More research is needed to confirm whether this GR common ragweed biotype is resistant to ALS inhibitors and to determine the mechanisms of resistance.

Response to POST Corn Herbicides. Treatment by experiment interactions for common ragweed control ($P > 0.10$) and biomass reduction ($P > 0.16$) were not significant; therefore, data were combined over the experimental runs. Bromoxynil, 2,4-D, diflufenzopyr plus dicamba, glufosinate, halosulfuron-methyl plus dicamba, mesotrione plus atrazine, tembotrione, and topramezone controlled GR and GS common ragweed biotypes 87% to 99% at 21 DAT (Table 6). Everman et al. (2007) also reported ≥90% control of common ragweed with glufosinate applied

Table 6. Response of glyphosate-resistant and susceptible common ragweed biotypes to POST corn herbicides at 21 days after treatment in a greenhouse study conducted at the University of Nebraska–Lincoln

		Glyphosate-resistant biotype[a–d]		Glyphosate-susceptible biotype	
Herbicide	Rate	Control at 21 DAT	Reduction in biomass	Control at 21 DAT	Reduction in biomass
	g ae or ai ha^{-1}	%			
Halosulfuron-methyl	70	68 bcde	79 abc	17 c	68 d
Primisulfuron-methyl	20	15 g	37 efg	18 c	27 f
Glufosinate	595	99 a	80 abc	99 a	90 ab
Mesotrione	105	84 bcd	76 bcd	78 b	86 ab
Tembotrione	92	87 abc	86 ab	98 a	84 abc
Topramezone	18.4	87 abc	63 d	96 a	83 abcd
Carfentrazone	8.8	58 e	34 fg	32 c	50 e
Bromoxynil	420	99 a	80 abc	99 a	90 ab
2,4-D	560	92 ab	75 bcd	98 a	84 abc
Dicamba	280	66 c	77 bc	94 a	77 bcd
Diflufenzopyr + dicamba	196	99 a	82 abc	95 a	81 abcd
Halosulfuron-methy + dicamba	380	87 abc	69 cd	91 ab	80 abcd
Thiencarbazone-methyl + tembotrione	91	24 g	46 bcd	99 a	36 ef
Mesotrione + atrazine	105 + 2240	99 a	91 a	99 a	91 ab
Fluthiacet-methyl + mesotrione	110	72 cde	78 abc	87 ab	82 abcd
S-metolachlor + glyphosate + mesotrione	2460	84 bcd	84 ab	99 a	94 a
P-value		<0.0001	<0.0001	<0.0001	<0.0001

[a] Abbreviations: DAT, day 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%) of the nontreated control were not included in the analysis. Reduction in biomass was calculated on the basis of the average biomass of the nontreated control.

at 470 g ai ha^{-1} in cotton (*Gossypium hirsutum* L.). Despite having a similar site of action, 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors tembotrione and topramezone resulted in 98% and 96% control of GS common ragweed, respectively, in contrast to 78% control with mesotrione applied alone with no difference in response of GR biotype (84% to 87% control) (Table 6). Zollinger and Ries (2006) reported 52% control of common ragweed with mesotrione compared to 94% and 97% control with tembotrione and topramezone, respectively. Tank-mixing mesotrione with atrazine provided 99% control of the GR and GS common ragweed biotypes in this study; this is likely due to the synergistic effect of photosystem II (PS II) and HPPD inhibitors as reported in the literature (Hugie et al. 2008; Walsh et al. 2012; Woodyard et al. 2009). For instance, Whaley et al. (2006) reported 37% to 49% control of common ragweed with mesotrione; however, control improved to >83% and 95% when tank-mixing atrazine at 280 and 560 g ha^{-1}, respectively.

Variable control was observed with dicamba, fluthiacet-methyl plus mesotrione, and *S*-metolachlor plus glyphosate plus mesotrione. For example, dicamba resulted in 66% and 94% control, and fluthiacet-methyl plus mesotrione provided 72% and 87% control of GR and GS biotypes, respectively, compared to 84% and 99% control with *S*-metolachlor plus glyphosate plus mesotrione. However, Chandi et al. (2012) reported ≥99% control with thifensulfuron early-POST fb dicamba POST in corn. ALS-inhibiting herbicides such as halosulfuron-methyl provided 17% to 68% control compared to 15% to 18% control with primisulfuron-methyl (Table 6), whereas, Taylor et al. (2002) reported that halosulfuron, primisulfuron, prosulfuron, or cloransulam-methyl provided ≥98% control. Surprisingly, thiencarbazone-methyl plus tembotrione provided 24% and 99% control of the GR and GS common ragweed biotype, respectively, though differences in the biomass reduction were not prominent. Most herbicide treatments that provided effective common ragweed control resulted in 63% to 94% biomass reduction of GR and GS biotypes, with few statistical differences among them (Table 6).

Phenoxy-based herbicide tank-mixtures are anticipated to be available for use in multiple-herbicide-resistant soybean in the near future (Craigmyle et al. 2013; Miller and Norsworthy 2016; Wright et al. 2010). In this study, glyphosate plus 2,4-D choline or dicamba provided 99% control of GR common ragweed. Similarly, Chahal et al. (2015) reported >90% control of 10 cm tall GR common waterhemp and GR giant ragweed with 2,4-D choline plus glyphosate applied at 1,640 g ha^{-1}. Chahal and Johnson et al. (2012) further reported ≥95% control of GR horseweed with 2,4-D amine plus glyphosate, while in a recent study, Miller and Norsworthy (2016) reported >90% control of GR Palmer amaranth with 2,4-D choline and glyphosate dimethylamine. Craigmyle et al. (2013) reported improved weed control efficacy as a result of tank-mixing glufosinate with 2,4-D compared to the efficacy of either of these herbicides applied alone.

Practical Implications. This is the first report of GR common ragweed in Nebraska. Greenhouse dose-response studies confirmed a 7- to 19-fold level of resistance compared to the known GS biotype, while a field dose-response study conducted at the putative GR common ragweed research site revealed that 15- and 40-times the labeled rate of glyphosate was predicted to be required for 90% control and biomass reduction, respectively. The evolution of GR common ragweed in Nebraska will make weed control more challenging for corn and soybean growers in eastern Nebraska as GR common waterhemp, giant ragweed, horseweed, and Palmer amaranth have been confirmed and are widely distributed in the area. The response of GR common ragweed to POST corn and soybean herbicides suggested that alternate POST herbicide options are available for effective management of GR common ragweed. Since both GR and GS common ragweed biotypes exhibited reduced sensitivity to labeled rates of ALS inhibitors, dose-response studies are needed to evaluate whether GR common ragweed from Nebraska is also resistant to ALS inhibitors. Field studies are needed to evaluate common ragweed management programs based on the integration of herbicides with different sites of action applied PRE and/or POST with non-chemical options including crop rotation, minimum tillage, reduced row spacing, and the use of cover crops.

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