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Author(s): Vipin Kumar, Prashant Jha, and Amit J. Jhala

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## Confirmation of Glyphosate-Resistant Horseweed (*Conyza canadensis*) in Montana Cereal Production and Response to POST Herbicides

Vipan Kumar, Prashant Jha, and Amit J. Jhala\*

In recent years, horseweed has become an increasing problem in Montana. To confirm and characterize the level of glyphosate resistance, seeds were collected from putative glyphosate-resistant (GR) horseweed (GR-MT) plants in a wheat–fallow field in McCone County, MT. Known GR (GR-NE) and glyphosate-susceptible (GS-NE) horseweed accessions from Lincoln, NE, were included for comparison in dose–response and shikimate accumulation studies. Whole-plant glyphosate dose–response experiments conducted at the early- (5- to 8-cm diameter) and late- (12- to 15-cm diameter) rosette stages of horseweed indicated that GR-MT accessions had a 2.5- to 4.0-fold level of resistance to glyphosate relative to the GS-NE accession, on the basis of shoot dry weight ( $GR_{50}$  values). The level of resistance was 3.1- to 7.9-fold on the basis of visually assessed injury estimates ( $I_{50}$  values). At the whole-plant level, about 2.1- to 4.5-fold higher shikimate accumulation was observed in the GS-NE accession compared with the GR-MT and GR-NE accessions over a 10-d period after glyphosate was applied at  $1,260 \text{ g ae ha}^{-1}$ . In a separate greenhouse study, all three horseweed accessions were also screened with alternate POST herbicides registered for use in wheat–fallow rotations. The majority of the tested herbicides provided  $\geq 90\%$  injury at the field-use rates for all three horseweed accessions 3 wk after treatment. This is the first published report on the occurrence of GR horseweed in Montana cereal production. Increased awareness and adoption of best management practices, including the use of diversified (based on multiple sites of action) herbicide programs highlighted in this study, would aid in mitigating the further spread of GR horseweed in the cereal production fields of the U.S. Great Plains.

**Nomenclature:** Glyphosate; horseweed, *Conyza canadensis* (L.) Cronq.; wheat, *Triticum aestivum* L.

**Key words:** Glyphosate resistance, glyphosate resistance management, multiple sites of action, POST herbicides, wheat–fallow.

Horseweed, also known as marestalk, is a common winter annual broadleaf weed belonging to the Asteraceae family (Gleason and Cronquist 1963). Horseweed is a native of North America and widely distributed in the United States and Canada. It is prevalent in no-till annual cropping systems, pastures, orchards, roadsides, and industrial and waste areas of North, Central, and South America (Miller and Miller 1999). It is a highly competitive weed in agronomic crops. Byker et al. (2013) reported that the season-long interference from horseweed competition could reduce soybean [*Glycine max* (L.) Merr.] grain yield by 83% to 93%. Similarly, Ford et al. (2014) reported a 92% reduction in corn (*Zea mays* L.) grain yield at a horseweed density of 60

plants  $\text{m}^{-2}$ . Horseweed competition in cotton (*Gossypium hirsutum* L.) reduced lint yield up to 2.9-fold (Owen et al. 2011; Steckel and Gwathmey 2009). Although limited published information is available on horseweed interference in wheat, it has become an increasingly troublesome weed during fallow periods in no-till wheat–fallow rotations in the arid to semi-arid regions of the U.S. Great Plains.

Horseweed germinates throughout the year, although most plants emerge in the fall and overwinter as rosettes (Bolte 2015; Buhler and Owen 1997). It is a prolific seed producer, and a single plant can produce up to 200,000 seeds that when wind-borne can disperse over long distances (Bhowmik and Bekech 1993; Shields et al. 2006; Weaver 2001).

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\* First and second authors: Postdoctoral Research Associate and Associate Professor, Montana State University–Bozeman, Southern Agricultural Research Center, Huntley, MT 59037; third author: Assistant Professor, Department of Agronomy and Horticulture, University of Nebraska, Lincoln, NE 68583-0915. Corresponding author's Email: pjha@montana.edu

The majority of horseweed seeds emerge from the soil surface, and shallow burial (>0.5 cm) therefore reduces seedling emergence (Nandula et al. 2006). Furthermore, seeds have a short life span and will remain viable for only 2 to 3 yr (Davis and Johnson 2008). Therefore, tillage is an effective tool for managing horseweed seedbanks (Brown and Whitwell 1988). However, tillage has only limited potential for use in the dryland production systems of the Northern Great Plains due to low annual precipitation (<30 cm) and the need to conserve soil moisture. Growers in this region utilize no-till fallow for soil moisture conservation from winter precipitation (Lenssen et al. 2007), with heavy reliance on nonselective herbicides, especially glyphosate, for weed control in chemical fallow preceding winter wheat (Kumar et al. 2014).

Glyphosate is one of the most predominant and frequently used broad-spectrum, nonselective herbicides for burndown weed control in fallow or before crop planting. The high rate of adoption of glyphosate-resistant (GR) soybean, cotton, and corn in the United States has resulted in an often sole reliance on glyphosate for weed control (Green 2011; Shaw et al. 2009). Consequently, overreliance on glyphosate has led to the unprecedented evolution of 17 GR weeds in the United States (Heap 2017). GR horseweed was the first weed to develop resistance to glyphosate in U.S. corn and soybean fields (VanGessel 2001). At present, GR horseweed has been reported in 27 U.S. states (Heap 2017). In addition, horseweed populations with resistance to other herbicide families, including bipyridyliums, imidazolinone, pyrimidinylthiobenzoic acid, sulfonylureas, sulfonylaminocarbonyl-triazolinones, triazines, triazinone, ureas, and trazolopyrimidine, have been reported (Gadamski et al. 2000; Heap 2017; Mueller et al. 2003; Smisek et al. 1998).

Growers have observed greater horseweed infestations in cereal production fields in the northeastern parts of Montana, including Phillips, Valley, Garfield, McCone, Roosevelt, Richland, Dawson, and Prairie counties (Survey, Jha, unpublished data). Typically, a chemical fallow field receives two to three applications of glyphosate alone or with 2,4-D/dicamba per year to obtain season-long weed control (Kumar et al. 2014). Glyphosate is also used for burndown (before wheat planting) and postharvest in wheat stubble (Kumar and Jha 2015; Young et al. 2008). The enhanced selection pressure from

this repeated use of glyphosate has resulted in the evolution and escalating spread of GR kochia [*Kochia scoparia* (L.) Schrad.] in Montana and other Great Plains states (Heap 2017; Kumar et al. 2015). More recently, a Russian-thistle (*Salsola tragus* L.) population was confirmed resistant to glyphosate in a wheat production field in Choteau County, MT (Kumar et al. 2017).

During late summer 2015, inconsistent control of a horseweed accession following two applications of glyphosate (870 g ae ha<sup>-1</sup> per application) was observed in a chemical fallow (wheat–fallow) field in McCone County in eastern Montana. In response to the control failure, seeds from the surviving horseweed plants were collected from the field and evaluated for putative resistance to glyphosate. There is a lack of published information on the effectiveness of alternative herbicides to control GR horseweed in wheat–fallow rotations. Additionally, the collected GR accession might have developed cross- or multiple resistance to other POST herbicides registered for use in wheat and/or fallow. Therefore, the objectives of this research were to (1) confirm and characterize the level of glyphosate resistance in the putative GR horseweed accession using whole-plant dose–response and shikimate accumulation assays and (2) evaluate the efficacy of POST herbicides labeled for use in wheat–fallow rotations to control GR horseweed.

## Materials and Methods

**Plant Materials.** Seeds of a putative GR horseweed accession were collected during the late fall of 2015, from a no-till fallow field near Vida in McCone County, MT. The sampled field had been in a no-till wheat fallow field for >10 yr, with a history of repeated glyphosate use. Seeds were collected from 10 randomly selected fully matured horseweed plants that had survived two glyphosate applications (870 g ae ha<sup>-1</sup> each). After threshing and cleaning, seeds from individual plants of the putative GR horseweed accession (GR-MT) were composited into one sample and stored in plastic Ziploc<sup>®</sup> bags at 4 C until used. A glyphosate-susceptible (GS) horseweed accession (referred to as GS-NE) known to be susceptible at the recommended field use rate (870 g ae ha<sup>-1</sup>) of glyphosate was collected from a soybean field at the South Central Ag Lab, University of

Nebraska–Lincoln, Clay Center, NE, in 2015. A known GR horseweed accession (referred to as GR-NE) collected from Havelock Agronomy Farm, University of Nebraska–Lincoln, Lincoln, NE, was included for comparison.

**Discriminate Dose Experiments.** Field-collected seeds from all three horseweed accessions (GR-MT, GR-NE, and GS-NE) were sown separately on the surface of 53 cm by 35 cm by 10 cm germination flats filled with a commercial potting mixture (VermiSoil™, Vermicrop Organics, 4265 Duluth Avenue, Rocklin, CA) in a greenhouse at the Montana State University Southern Agricultural Research Center near Huntley, MT. The greenhouse environment was  $26/23 \pm 3$  C day/night temperatures and 16/8 h day/night photoperiods with supplemental lighting provided by metal-halide lamps ( $550 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). Fifty seedlings from each accession were treated with the recommended field use rate ( $870 \text{ g ae ha}^{-1}$ ) of the potassium salt of glyphosate (Roundup PowerMax®, Monsanto Company, St Louis, MO) when seedlings were at the rosette stage (5- to 8-cm diameter). The glyphosate treatment was applied with 2% w/v ammonium sulfate (AMS) using a cabinet spray chamber (Research Track Sprayer, De Vries Manufacturing, RR 1 Box 184, Hollandale, MN) equipped with an even flat-fan nozzle tip (TeeJet® 8001EXR, Spraying System, Wheaton, IL) calibrated to deliver  $94 \text{ L ha}^{-1}$  of spray solution at 276 kPa.

Surviving horseweed plants were then transplanted into 10-L pots containing the previously described potting mixture and allowed to grow in the greenhouse under the conditions described earlier. Individual plants from each accession were covered with pollination bags (DelStar Technologies, 601 Industrial Drive, Middletown, DE) to prevent cross-pollination, and the progeny seeds from the selfed plants were obtained for conducting subsequent whole-plant dose–response experiments. Seeds of the GS-NE accession were generated from unsprayed plants following this same procedure.

**Whole-Plant Glyphosate Dose Response.** Previous research has shown differences in glyphosate efficacy on GR horseweed depending on the growth stage of the plants (Mellendorf et al. 2013). Therefore, two separate experiments were conducted to characterize the glyphosate resistance levels in GR horseweed accessions (GR-MT and GR-NE)

through a whole-plant glyphosate dose–response assay. The first experiment involved glyphosate applications at the early-rosette stage (seedlings with 5- to 8-cm diameter); the second experiment involved glyphosate applications at the late-rosette stage (seedlings with 12- to 15-cm diameter) of horseweed plants. Greenhouse experiments were conducted at the Montana State University (MSU) Southern Agricultural Research Center (SARC) near Huntley, MT, during the summer of 2016 and repeated in fall of 2016. The progeny seeds from selfed horseweed accessions (GR-MT, GR-NE, and GS-NE) were separately sown on the germination flats filled with a commercial potting mixture as described previously. At the 1- to 2-true leaf stage, horseweed seedlings were individually transplanted into 10-cm-diameter plastic pots containing the potting mixture described previously. Experiments were set up in a randomized complete block design with eight replications (1 plant pot<sup>-1</sup>) per treatment. Horseweed plants from each accession were treated with glyphosate at doses of 0, 217, 435, 870, 1,260, 1,740, 3,480, 5,280, 6,960, and  $8,700 \text{ g ae ha}^{-1}$  when plants were at the early-rosette stage (5- to 8-cm diameter). For the late-rosette stage (12- to 15-cm diameter), glyphosate doses of 0, 217, 435, 870, 1,260, 1,740, 3,480, 5,220, 6,960, 8,700, 17,400, and  $26,100 \text{ g ae ha}^{-1}$  were used. All treatments included AMS and were applied using a cabinet spray chamber as described previously. After glyphosate application, horseweed plants were returned to the greenhouse, watered as needed to avoid moisture stress, and fertilized (Miracle-Gro® water-soluble fertilizer [24–8–16], Scotts Miracle-Gro Products, 14111 Scottslawn Road, Marysville, OH) weekly to maintain vigorous growth. Visually assessed injury rating on a scale of 0 (no injury) to 100 (complete plant death) was recorded at 1, 2, and 3 wk after treatment (WAT). The aboveground shoot biomass was harvested at 3 WAT, and dried at 65 C for 3 d to determine the aboveground shoot dry weight per plant, expressed as a percentage of the nontreated control.

**Whole-Plant Shikimate Accumulation.** Following the methods of Perez-Jones et al. (2007), the influence of glyphosate on shikimate accumulation over time was evaluated in the selected GR (GR-MT, GR-NE) and GS (GS-NE) horseweed accessions in the fall of 2016 and repeated in the spring of 2017.

Horseweed plants from each accession were grown in 10-cm-diameter plastic pots in the greenhouse under the previously described growing conditions. At the rosette stage (8- to 10-cm diameter), plants were treated with glyphosate at 1,260 g ha<sup>-1</sup> using the spray chamber. Plants were immediately returned to the greenhouse after glyphosate application. At 1, 3, 7, and 10 d after treatment (DAT), the young leaf tissue was harvested from each treated plant. Approximately 100 mg of chopped tissue was transferred to 5-ml glass vials containing 1 ml of 0.25 N HCl plus 0.1% (v/v) polysorbate surfactant. The glass vials were stored at 25 C for 24 h. Shikimate accumulation at each sampling date was determined using the methods described by Cromartie and Polge (2000) with slight modifications. About 50-ml aliquot from each vial was pipetted into a 2-ml microcentrifuge tube, and 200 µl of periodic acid and sodium metaperiodate (0.25% [w/v] each) was added to each tube. The microcentrifuge tubes were incubated at room temperature for 90 min, and 200 µl of 0.6 N sodium hydroxide and 0.22 M sodium sulfite was then added. Shikimate accumulation was measured using an Epoch 2 Microplate Spectrophotometer (BioTek Instruments, headquartered in Winooski, VT) at 380 nm. The tissue collected from a nontreated horseweed plant from each accession was used as a reference absorbance at each harvest time. A standard curve was developed using known concentrations of shikimate. There were 10 horseweed plants (replications) for each accession.

**Response of Horseweed Accessions to POST Herbicides.** Greenhouse experiments were conducted at the MSU-SARC near Huntley, MT, to determine the effectiveness of different POST herbicides for controlling GR and GS horseweed accessions during the summer of 2016 and in the fall of 2016. Seeds of all three horseweed accessions (GR-MT, GR-NE, and GS-NE) were separately sown in germination flats containing the previously described commercial potting mixture. Horseweed seedlings at the 1- to 2-true leaf stage were individually transplanted into 10-cm-diameter plastic pots in the greenhouse under the previously described growing conditions. Horseweed seedlings (8- to 10-cm diameter) from all three accessions were treated with the recommended field use rates of POST herbicides (Table 1). Herbicide treatments were applied using

the spray chamber as described previously. Experiments were set up in a randomized complete block design with five replications (1 plant pot<sup>-1</sup>). Visual assessment on injury rating (on a scale of 0% to 100%) and the aboveground shoot biomass per plant were recorded at 3 WAT. Data on aboveground shoot biomass were expressed as a percentage of biomass reduction relative to the nontreated control for each accession.

**Statistical Analyses.** Data from all experiments were subjected to ANOVA using PROC MIXED in SAS v. 9.3 (SAS Institute, SAS Campus Drive, Cary, NC 27513) to test the main effects of experimental run, accession, treatment (glyphosate dose in the whole-plant dose response and whole-plant shikimic acid accumulation or time for shikimic acid accumulation or herbicide in the alternative POST herbicide study) and their interactions. Replication and interactions involving replication were random effects in the model. Residual analyses were performed on the injury rating and shoot dry weight reductions (percent of nontreated control) using PROC UNIVARIATE, and homogeneity of variance was checked, with all data meeting ANOVA assumptions. Comparisons for shikimate accumulation between horseweed accessions at different harvest times were made using Student's *t* test. For alternative POST herbicides, means for injury rating and shoot dry weight reduction were separated using Fisher's protected LSD test at *P* < 0.05.

For whole-plant glyphosate dose-response assays, the shoot dry weight reductions (percent of nontreated control) for each horseweed accession were regressed over glyphosate doses using a three-parameter log-logistic model (Ritz et al. 2015; Seefeldt et al. 1995):

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

where *Y* refers to the response variable (injury rating or shoot dry weight as percentage of nontreated), *D* is the upper limit, *B* is the slope of each curve, *E* is the glyphosate dose required to cause 50% response (i.e., 50% injury referred to as *I*<sub>50</sub> or 50% reduction in shoot dry weight referred as *GR*<sub>50</sub>), and *X* is the glyphosate dose. It was evident from lack-of-fit tests (*P* > 0.05) that the nonlinear regression models accurately described the injury (*P* = 0.231) and shoot dry weight (*P* = 0.421) data for each accession. Nonlinear regression parameter estimates,

Table 1. List of alternative POST herbicides for controlling glyphosate-resistant (GR-MT, GR-NE) and glyphosate-susceptible (GS-NE) horseweed accessions.<sup>a</sup>

Herbicide(s) <sup>b</sup>	Trade name	Application rate (g ai or ae ha <sup>-1</sup> )	Manufacturer
Bicyclopyrone + bromoxynil <sup>c</sup>	Talinor <sup>TM</sup>	37 + 175	Syngenta Crop Protection, Greensboro, NC
Bromoxynil + fluroxypyr	Starane <sup>®</sup> NXT	558 + 140	Dow AgroScience LLC, Indianapolis, IN
Bromoxynil + pyrasulfotole <sup>d,e</sup>	Huskie <sup>®</sup>	229 + 40	Bayer Crop Science, Research Triangle Park, NC
Bromoxynil + MCPA	Bromac <sup>®</sup> Advanced	560 + 560	Loveland Products, Inc., Greeley, CO
Dicamba	Rifle <sup>®</sup>	560	Loveland Products, Inc., Greeley, CO
Diflufenzopyr + dicamba + 2,4-D	Distinct <sup>®</sup> + 2,4-D LV6	(56 + 140) + 784	BASF Corporation, 26 Davis Drive, Research Triangle Park, NC; and Winfield Solutions LLC, St Paul, MN
Fluroxypyr	Starane <sup>®</sup> Ultra	156	Dow AgroScience LLC, IN
Glufosinate <sup>d</sup>	Liberty <sup>®</sup>	593	Bayer Crop Science
Halauxifen-methyl + florasulam <sup>d</sup>	Quelex <sup>TM</sup>	5.25 + 5.25	Dow AgroScience LLC, IN
Paraquat <sup>e</sup>	Gramoxone <sup>®</sup> Inteon	560	Syngenta Crop Protection
Paraquat + metribuzin <sup>e</sup>	Gramoxone <sup>®</sup> Inteon + Sencor <sup>®</sup> 75 DF	560 + 210	Syngenta Crop Protection and Bayer Crop Science
Saflufenacil <sup>d,f</sup>	Sharpen <sup>®</sup>	49	BASF Corporation, Research Triangle Park, NC
Saflufenacil + 2,4-D <sup>d,f</sup>	Sharpen <sup>®</sup> + 2,4-D LV6	49 + 784	BASF and Winfield Solutions LLC, St Paul, MN
(Thifensulfuron-methyl + tribenuron-methyl) + (clopyralid + fluroxypyr)	Affinity <sup>®</sup> TankMix + WideMatch <sup>®</sup>	(16.8 + 4.2) + (139 + 139)	DuPont, Wilmington, DE and Dow AgroScience LLC, IN
2,4-C + fluroxypyr + clopyralid	Hat Trick <sup>®</sup>	378 + 107 + 107	Loveland Products, Inc., Greeley, CO
2,4-D	2,4-D LV6	784	Winfield Solutions LLC, St. Paul, MN
2,4-D + bromoxynil + fluroxypyr <sup>d,f,g</sup>	Kochiavore <sup>TM</sup>	467 + 467 + 187	Winfield Solutions LLC, St. Paul, MN

<sup>a</sup> Abbreviations: GR-MT, glyphosate-resistant accession from McCone County, MT; GR-NE, glyphosate-resistant accession from Lincoln, NE; GS-NE, glyphosate-susceptible accession from Lincoln, NE.

<sup>b</sup> Herbicides are labeled in wheat, barley (*Hordeum vulgare* L.), or in chemical fallow. Herbicides were applied to horseweed plants at rosette stage (8- to 10-cm diameter).

<sup>c</sup> Crop oil concentrate at 1% (v/v) was included.

<sup>d</sup> Ammonium sulfate at 2% (w/v) was included.

<sup>e</sup> Nonionic surfactant at 0.25% (v/v) was included.

<sup>f</sup> Methylated seed oil at 1% (v/v) was included.

<sup>g</sup> Activator 90 at 0.25% (v/v) was included.

standard errors,  $I_{90}$  or  $GR_{90}$  (glyphosate dose needed for 90% injury or 90% reduction in shoot dry weight, respectively), and 95% confidence intervals (CI) for each accession were determined using the 'drc' package in R software (Knezevic et al. 2007). Based on the  $I_{50}$  or  $GR_{50}$  values, the resistance index (referred to as the R/S ratio) for each GR horseweed accession was estimated by dividing the  $GR_{50}$  value of a GR accession by the  $GR_{50}$  value of the GS accession.

## Results and Discussion

More than 90% of the plants from the GR-MT and GR-NE accessions survived the discriminate dose ( $870 \text{ g ae ha}^{-1}$ ) of glyphosate, whereas none of the GS-NE plants survived this discriminate dose (unpublished data).

**Whole-Plant Glyphosate Dose–Response.** *Early-Rosette Stage (5- to 8-cm diameter).* The interaction of experimental run with accession or glyphosate dose was not significant; hence, data were pooled over runs. A differential response of injury and aboveground shoot dry weight reduction (percent of nontreated control) to increasing doses of glyphosate was observed between the resistant (GR-MT and GR-NE) and susceptible (GS-NE) horseweed accessions (Figures 1 and 2). On the basis of visual assessment of injury rating, the  $I_{50}$  values for GR-MT and GR-NE accessions were 1,937 and 2,975  $\text{g ae ha}^{-1}$ , respectively, and were greater than the 619  $\text{g ae ha}^{-1}$  for the GS-NE accession (Table 2; Figure 1). Based on these  $I_{50}$  values, the GR-MT and GR-NE accessions had 3.1- and 4.8-fold resistance to glyphosate, respectively. Furthermore, the  $I_{90}$  values indicated an almost three times higher dose of glyphosate was needed to achieve 90% injury of the two GR accessions relative to the GS accession.

On the basis of the shoot dry weight response, the  $GR_{50}$  values for GR-MT and GR-NE accessions were 1,881 and 2,496  $\text{g ae ha}^{-1}$ , respectively, and were marginally higher than the 745  $\text{g ae ha}^{-1}$  rate for the GS-NE accession (Table 2; Figure 2). Based on these  $GR_{50}$  values, the GR-MT and GR-NE accessions exhibited 2.5- and 3.3-fold resistance to glyphosate, respectively. Similarly, Hanson et al. (2009) reported a horseweed accession from Central Valley, CA, with a 4.8-fold level of resistance. A GR horseweed accession from Delaware with higher

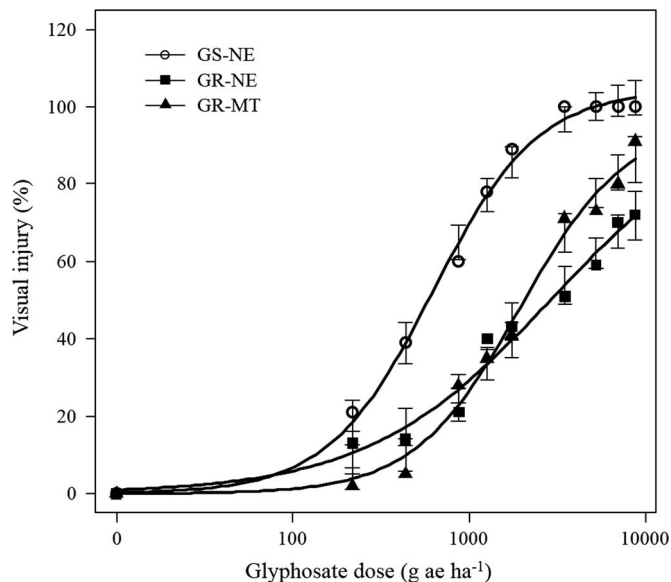


Figure 1. Injury response of glyphosate-resistant (GR; GR-MT from McCone County, MT; GR-NE from Lincoln, NE) and glyphosate-susceptible (GS-NE from Lincoln, NE) horseweed accessions treated with increasing doses of glyphosate at the early-rossette stage (5- to 8-cm diameter) in whole-plant dose–response experiments averaged over runs. Symbols represent actual values, whereas lines represent predicted values. Vertical bars indicate  $\pm$  standard error of the mean values.

levels of resistance to glyphosate (8- to 13-fold) has been reported (VanGessel et al. 2001), and similarly, GR horseweed accessions collected from cotton and soybean fields in Mississippi had 8- to 12-fold levels of resistance to glyphosate (Koger et al. 2004). In our study, the  $GR_{90}$  values for GR-MT and GR-NE horseweed accessions were 2.3 and 5.7 times higher than the GS-NE accession, respectively, suggesting that glyphosate may no longer be an effective option for controlling these GR horseweed accessions.

*Late-Rosette Stage (12- to 15-cm diameter).* The interaction of experimental run with accession or glyphosate dose was not significant; hence, data were pooled over runs. On the basis of whole-plant glyphosate dose response, both GR horseweed accessions had 1.6 to 4.0 times higher  $I_{50}$  values compared with the  $I_{50}$  values at the early-rossette stage. The  $I_{50}$  values at the late-rossette stage were 7,799, 4,949, and 977  $\text{g ae ha}^{-1}$  for the GR-MT, GR-NE, and GS-NE horseweed accessions, respectively (Table 3; Figure 3). Based on these  $I_{50}$  values, the GR-MT and GR-NE horseweed accessions had 7.9- and 5.0-fold resistance to glyphosate, respectively. Furthermore,

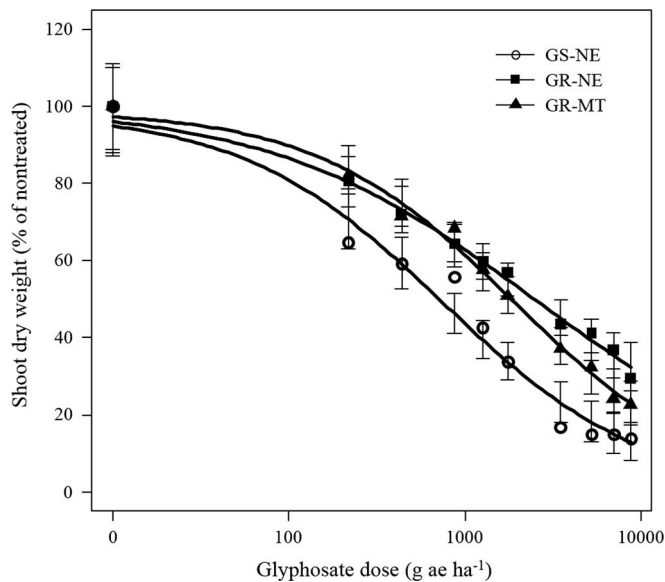


Figure 2. Shoot dry weight response of glyphosate-resistant (GR; GR-MT from McCone County, MT; GR-NE from Lincoln, NE) and glyphosate-susceptible (GS-NE from Lincoln, NE) horseweed accessions treated with increasing doses of glyphosate at the early-rosette stage (5- to 8-cm diameter) in whole-plant dose–response experiments averaged over runs. Symbols represent actual values, whereas lines represent predicted values. Vertical bars indicate  $\pm$  standard error of the mean values.

the  $I_{50}$  values for the GR-MT and GR-NE accessions at the late-rosette stage were 8.9 and 5.6 times the field use rate ( $870 \text{ g ae ha}^{-1}$ ) of glyphosate for this growth stage. Additionally, these GR accessions

required a dose of glyphosate approximately six times greater to achieve 90% injury compared with the GS-NE accession.

On the basis of shoot dry weight reduction (percentage of nontreated control), the GR-MT and GR-NE horseweed accessions had 4.0- and 3.5-fold levels of resistance to glyphosate, respectively (Table 3; Figure 4). The GR-MT and GR-NE horseweed accessions at the late-rosette stage exhibited  $GR_{50}$  values of 5.4 and 4.8 times the field use rate of glyphosate. In addition, the GR-MT and GR-NE accessions also showed higher  $GR_{90}$  values (3.6 to 6.6 times higher than the GS-NE accession) at the late-rosette stage compared with the early-rosette stage (Table 3). In contrast to our results, Koger et al. (2004) reported no effect of plant size on glyphosate resistance levels in GR horseweed accessions from Mississippi.

**Whole-Plant Shikimate Accumulation.** Data were combined over experimental runs because of a lack of significant interaction of run by accession. The main effects of horseweed accession ( $P = 0.0056$ ), harvest time ( $P < 0.05$ ), and the interaction of accession with harvest time ( $P < 0.0031$ ) were significant. The GS-NE accession accumulated higher shikimate than the GR-MT and GR-NE accessions at all harvest times (Figure 5). In general, a decrease in shikimate accumulation was observed in horseweed accessions from 1 through 10 d after glyphosate treatment. The GS-NE accession accumulated approximately 2.1, 3.1, and 2.4 times more shikimic acid than the GR-NE accession at 3, 7, and 10 d after glyphosate treatment. Similarly, the

Table 2. Regression parameter (Equation 1) estimates from the whole-plant dose–response study on the basis of visually assessed injury and shoot dry weight (percent of nontreated) of horseweed accessions treated with glyphosate at the early-rosette stage (5- to 8-cm diameter).<sup>a</sup>

Accessions	Parameter estimates ( $\pm$ SE)					
	<i>D</i>	<i>B</i>	$I_{50}$ or $GR_{50}$	95% CI	$I_{90}$ or $GR_{90}$	RI
Based on visual injury (%)						
GR-MT	96 (2.4)	-1.4 (0.2)	1,937 (43)	1,577–2,297	8,851 (121)	3.1
GR-NE	101 (3.6)	-0.8 (0.2)	2,975 (39)	2,535–3,415	9,275 (226)	4.8
GS-NE	104 (2.9)	-1.4 (0.1)	619 (27)	525–713	2,765 (87)	—
Based on shoot dry weight						
GR-MT	98 (5.7)	0.7 (0.1)	1,881 (39)	1,504–2,258	31,741 (345)	2.5
GR-NE	99 (5.6)	0.5 (0.1)	2,496 (45)	2,011–2,981	77,035 (423)	3.3
GS-NE	98 (5.7)	0.7 (0.1)	745 (17)	401–1,088	13,400 (274)	—

<sup>a</sup> Abbreviations: GR-MT, glyphosate-resistant accession from McCone County, MT; GR-NE, glyphosate-resistant accession from Lincoln, NE; GS-NE, glyphosate-susceptible accession from Lincoln, NE; *D*, upper limit of the response; *B*, relative slope around  $I_{50}$  or  $GR_{50}$ ;  $I_{50}$  and  $GR_{50}$  are effective doses ( $\text{g ae ha}^{-1}$ ) of glyphosate causing 50% injury and reduction in shoot dry weights, respectively;  $I_{90}$  and  $GR_{90}$  are effective doses ( $\text{g ae ha}^{-1}$ ) of glyphosate required for 90% injury and shoot dry weight reduction, respectively; CI, confidence interval; RI, resistance index (calculated as a ratio of  $I_{50}$  or  $GR_{50}$  of a GR accession to  $I_{50}$  or  $GR_{50}$  of the GS accession).



Table 3. Regression parameter (Equation 1) estimates from the whole-plant dose–response study on the basis of visually assessed injury and shoot dry weight (percent of nontreated) of horseweed accessions treated with glyphosate at the late-rosette stage (12- to 15-cm diameter).<sup>a</sup>

Populations	Parameter estimates ( $\pm$ SE)					
	<i>D</i>	<i>B</i>	<i>I</i> <sub>50</sub> or <i>GR</i> <sub>50</sub>	95% CI	<i>I</i> <sub>90</sub> or <i>GR</i> <sub>90</sub>	RI
Based on visual injury (%)						
GR-MT	103 (4.7)	-2.3 (0.3)	7,799 (97)	6,894–8,704	19,571 (298)	7.9
GR-NE	102 (4.3)	-1.1 (0.1)	4,949 (65)	4,365–5,534	19,857 (331)	5.0
GS-NE	98 (1.3)	-2.4 (0.2)	977 (33)	910–1,043	3,108 (119)	—
Based on shoot dry weight						
GR-MT	98 (4.4)	0.7 (0.1)	4,775 (86)	4,069–5,481	53,504 (352)	4.0
GR-NE	99 (4.6)	0.6 (0.1)	4,216 (51)	3,696–4,736	97,325 (452)	3.5
GS-NE	99 (4.3)	1.2 (0.1)	1,181 (29)	901–1,460	14,720 (229)	—

<sup>a</sup> Abbreviations: GR-MT, glyphosate-resistant accession from McCone County, MT; GR-NE, glyphosate-resistant accession from Lincoln, NE; GS-NE, glyphosate-susceptible accession from Lincoln, NE; *D*, upper limit of the response; *B*, relative slope around *I*<sub>50</sub> or *GR*<sub>50</sub>; *I*<sub>50</sub> and *GR*<sub>50</sub> are effective doses (g ae ha<sup>-1</sup>) of glyphosate causing 50% injury and reduction in shoot dry weights, respectively; *I*<sub>90</sub> and *GR*<sub>90</sub> are effective doses (g ae ha<sup>-1</sup>) of glyphosate required for 90% injury and shoot dry weight reduction, respectively; CI, confidence interval; RI, resistance index (calculated as a ratio of *I*<sub>50</sub> or *GR*<sub>50</sub> of a GR accession to *I*<sub>50</sub> or *GR*<sub>50</sub> of the GS accession).

shikimate accumulation by GS-NE horseweed accession was 3.4, 4.5, and 3.6 times greater than the GR-MT accession at 3, 7, and 10 d after glyphosate treatment. Shikimate accumulations in GR horseweed

accessions from Arkansas, Delaware, and Mississippi were 7.0-, 1.4-, and 4.0-fold lower, respectively, than the GS accession when treated with 5.3 mg ae L<sup>-1</sup> of glyphosate solution in a leaf-disk assay (Koger et al. 2005).

A lower level of shikimate accumulation has been observed in other GR weeds following glyphosate treatment. For instance, a GR rigid ryegrass (*Lolium rigidum* Gaudin) biotype from California accumulated 10-fold less shikimic acid than the susceptible biotype at 11 d after glyphosate treatment at 2.24 kg ha<sup>-1</sup> (Simarmata et al. 2003). Another GR rigid ryegrass biotype with altered glyphosate translocation patterns accumulated two times less shikimate than a susceptible population at 4 DAT with glyphosate at 0.42 kg ha<sup>-1</sup> (Perez-Jones et al. 2007). A GR goosegrass [*Eleusine indica* (L.) Gaertn.] biotype also accumulated approximately 2-fold less shikimic acid than the susceptible biotype at 2 d after glyphosate treatment (Tran et al. 1999). In contrast, Mueller et al. (2013) reported no significant differences in shikimate accumulation among the GR and GS populations of horseweed at 2 and 4 d after glyphosate treatment. The differential shikimate accumulation between GR and GS accessions observed in our study can possibly be attributed to target-site mutations or altered translocation patterns, as previously reported in other GR weed species (Cross et al. 2015; Perez-Jones et al. 2007; Simarmata et al. 2003; Wakelin and Preston 2006).

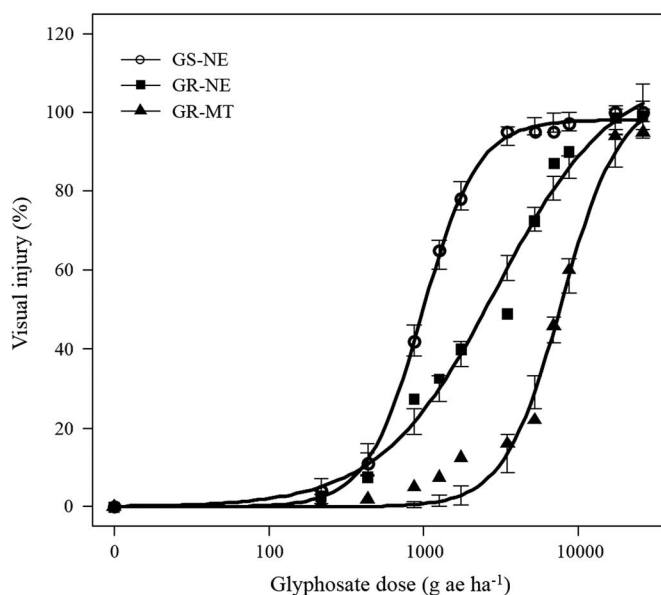


Figure 3. Injury response of glyphosate-resistant (GR; GR-MT from McCone County, MT and GR-NE from Lincoln, NE) and glyphosate-susceptible (GS-NE from Nebraska, NE) horseweed accessions treated with increasing doses of glyphosate at the late-rosette stage (12- to 15-cm diameter) in whole-plant dose–response experiments averaged over runs. Symbols represent actual values, whereas lines represent predicted values. Vertical bars indicate  $\pm$  standard error of the mean values.

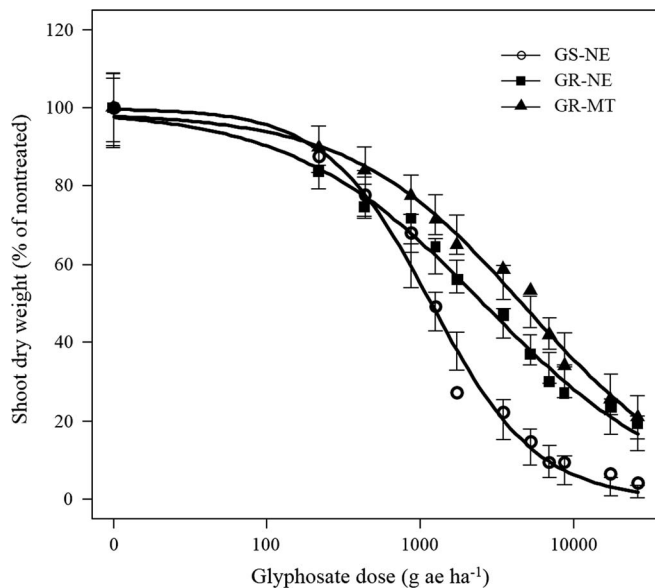


Figure 4. Shoot dry weight response of glyphosate-resistant (GR; GR-MT from McCone County, MT; GR-NE from Lincoln, NE) and glyphosate-susceptible (GS-NE from Lincoln, NE) horseweed treated with increasing doses of glyphosate at the late-rosette stage (12- to 15-cm diameter) in whole plant dose-response experiments averaged over runs. Symbols represent actual values, whereas lines represent predicted values. Vertical bars indicate  $\pm$  standard error of the mean values.

### Effectiveness of Alternative POST Herbicides.

*Visual Assessment of Percent Injury.* The interaction of experimental run by accession or herbicide treatment was not significant; hence, results were combined over two runs. The interaction of herbicide treatment by accession was significant ( $P < 0.0001$ ) on percent visual injury at 3 WAT, indicating a differential response of these accessions to the POST herbicides (labeled in wheat-fallow rotation) evaluated in this study. A majority of the alternate POST herbicide programs tested were effective on all three horseweed accessions. The premix of bromoxynil with pyrasulfotole or MCPA; diflufenzopyr + dicamba + 2,4-D; glufosinate; paraquat alone or in combination with metribuzin; saflufenacil alone or in combination with 2,4-D; thifensulfuron + tribenuron in combination with clopyralid + fluroxypyr; and 2,4-D alone provided effective visual injury ( $\geq 90\%$ ) of all three horseweed accessions (Table 4). In another study conducted by Mellendorf et al. (2013), visual injury of GR horseweed plants with paraquat ( $840 \text{ g ai ha}^{-1}$ ) and saflufenacil ( $25 \text{ g ai ha}^{-1}$ ) was  $\geq 90\%$  at 2 WAT. Similarly, in a field

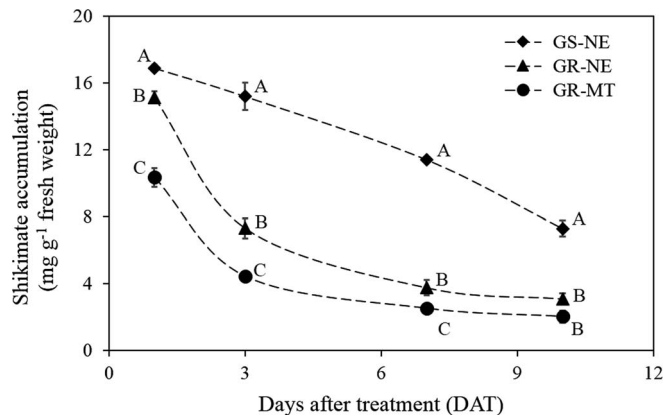


Figure 5. Shikimate accumulation of glyphosate-susceptible (GS-NE from Lincoln, NE; filled diamond) and glyphosate-resistant (GR-NE from Lincoln, NE: closed triangles; GR-MT from McCone County, MT: closed circles) horseweed accessions as affected by time averaged over runs. Vertical bars represent standard errors of the mean ( $n = 10$ ). Similar uppercase letters indicate no differences between accessions within a harvest time according to Fisher's protected LSD test at  $P < 0.05$ .

study, the percent visual injury of GR horseweed with glufosinate at  $580 \text{ g ai ha}^{-1}$  was 95% at 2 WAT (Steckel et al. 2006).

For the GS-NE horseweed accession, injury with halauxifen-methyl + florasulam and 2,4-D + bromoxynil + fluroxypyr was  $\geq 93\%$  at 3 WAT. In contrast, injury with bromoxynil + bicyclopyrone, bromoxynil + fluroxypyr, dicamba alone, and fluroxypyr alone ranged between 82 to 88% at 3 WAT (Table 4).

For the GR-NE accession, control with fluroxypyr, halauxifen + florasulam, and 2,4-D + bromoxynil + fluroxypyr was comparable to the GS-NE accession (Table 4). However, higher visual injury with bromoxynil + bicyclopyrone, bromoxynil + fluroxypyr, and dicamba was observed for the GR-NE (96% to 100%) than the GS-NE accession. In another study, injury of GR horseweed with dicamba at 140 and  $280 \text{ g ae ha}^{-1}$  was 93% and 98%, respectively, at 4 WAT (Everitt and Keeling 2007). Kruger et al. (2010) also observed effective control ( $>97\%$ ) of GR horseweed with dicamba at  $280 \text{ g ai ha}^{-1}$ .

For the GR-MT accession, injury with bromoxynil + bicyclopyrone, bromoxynil + fluroxypyr, and fluroxypyr alone was comparable to the GS-NE accession (Table 4). However, visual injury with halauxifen + florasulam (85%) and 2,4-D + bromoxynil + fluroxypyr (82%) was lower compared with the GS-NE and GR-NE accessions.

Table 4. Visual injury estimates and shoot dry weight reduction (relative to nontreated control) of horseweed accessions at 3 wk after treatment with various POST herbicides.<sup>a</sup>

Herbicide (s)	Application rate (g ai or ae ha <sup>-1</sup> )	Visual injury <sup>b</sup>			Shoot dry weight reduction <sup>b</sup>		
		GS-NE	GR-NE	GR-MT	GS-NE	GR-NE	GR-MT
		%			%		
Bicyclopyrone + bromoxynil <sup>c</sup>	37 + 175	85 dB	96 aA	82 bcB	77 eB	87 abcA	77 eB
Bromoxynil + fluroxypyr	558 + 140	82 eB	100 aA	85 bcB	77 eB	87 abcA	80 deB
Bromoxynil + pyrasulfotole <sup>d,e</sup>	229 + 40	98 abcA	97 abcA	97 aA	89 abA	90 aA	89 bA
Bromoxynil + MCPA	560 + 560	96 abcA	97 abcA	91 dB	85 cA	87 abcA	78 eB
Dicamba	560	82 eB	98 abA	98 aA	76 eB	88 abA	89 bA
Diflufenzopyr + dicamba + 2,4-D	(56 + 140) + 784	99 abcA	100 aA	98 aA	90 abA	88 abA	90 abA
Fluroxypyr	156	88 dA	85 eA	83 cdA	81 dA	81 fA	76 eB
Glufosinate <sup>d</sup>	593	100 aA	100 aA	100 aA	90 abA	88 abA	90 abA
Halauxifen-methyl + florasulam <sup>d</sup>	5.25 + 5.25	93 cdA	92 bcA	85 bcB	89 abA	86 abcdA	78 eB
Paraquat <sup>e</sup>	560	100 abcA	92 bcA	100 aA	92 aA	89 abA	89 bA
Paraquat + metribuzin <sup>e</sup>	560 + 210	96 abcA	97 abcA	100 aA	89 abA	87 abcA	90 bA
Saflufenacil <sup>d,f</sup>	49	99 abA	97 abcA	100 aA	90 abA	88 abcA	91 abA
Saflufenacil + 2,4-D <sup>d,f</sup>	49 + 784	100 aA	100 aA	100 aA	90 abA	91 aA	94 aA
(Thifensulfuron-methyl + tribenuron-methyl) + (clopyralid + fluroxypyr)	(16.8 + 4.2) + (139 + 139)	96 abcAB	90 deB	98 aA	89 abA	82 defB	90 abA
2,4-D + fluroxypyr + clopyralid	378 + 107 + 107	98 abcA	95 abcAB	90 bB	89 abA	86 abcdAB	83 dB
2,4-D	784	93 bcA	91 cdeA	96 aA	88 bcA	82 defA	85 cdA
2,4-D + bromoxynil + fluroxypyr <sup>d,f,g</sup>	467 + 467 + 187	97 abcA	94 abcA	82 cdB	90 abA	87 abcB	78 eC

<sup>a</sup> Abbreviations: GR-MT, glyphosate-resistant (GR) accession from McCone County, MT; GR-NE, glyphosate-resistant accession from Lincoln, NE; GS-NE, glyphosate-susceptible accession from Lincoln, NE. Herbicides were applied to horseweed plants at the rosette stage (8- to 10-cm diameter).

<sup>b</sup> For visual injury or shoot dry weight reduction data, means for a horseweed accession within a column followed by similar lowercase letters are not different based on Fisher's protected LSD test at  $P < 0.05$ ; means for an herbicide treatment within a row followed by similar uppercase letters are not different based on Fisher's protected LSD test at  $P < 0.05$ .

<sup>c</sup> Crop oil concentrate at 1% (v/v) was included.

<sup>d</sup> Ammonium sulfate at 2% (w/v) was included.

<sup>e</sup> Nonionic surfactant at 0.25% (v/v) was included.

<sup>f</sup> Methylated seed oil at 1% (v/v) was included.

<sup>g</sup> Activator 90 at 0.25% (v/v) was included.

*Shoot Dry Weight Reduction.* The interaction of herbicide treatment by accession was significant ( $P < 0.0001$ ) for percent shoot dry weight reduction. In general, the response of all three horseweed accessions for shoot dry weight reduction was consistent with the percent control assessment for a majority of the POST herbicides tested. For example, bromoxynil in combination with pyrasulfotole or MCPA; diflufenzopyr + dicamba + 2,4-D; glufosinate; paraquat alone or in combination with metribuzin; saflufenacil alone or in combination with 2,4-D; thifensulfuron + tribenuron in combination with clopyralid + fluroxypyr; and 2,4-D alone reduced shoot dry weight of all three horseweed accessions by 82% to 94% (Table 4). Bromoxynil in combination with bicyclopyrone or fluroxypyr provided 77% to 80% reduction in shoot dry weight of the GS-NE and GR-MT accessions compared with 87% reduction of the GR-NE accession. The shoot dry weight reductions of the GR-MT accession with fluroxypyr, halauxifen + florasulam, 2,4-D + fluroxypyr + clopyralid, and 2,4-D + bromoxynil + fluroxypyr were 76%, 78%, 83%, and 78%, and were lower than both the GS-NE and GR-NE accessions.

These results confirm the first report of GR horseweed in a no-till wheat–fallow system in Montana. Along with previous cases of GR kochia and Russian-thistle (Heap 2017), the evolution of GR horseweed will be an additional challenge for Montana cereal producers. Importantly, an escalating spread of GR horseweed in the cereal fields of Montana can be expected because of horseweed’s prolific seed production and wind-mediated seed dispersal (Bhowmik and Bekech 1993; Shields et al. 2006), if not managed proactively.

Growers’ awareness and adoption of best management practices (Norsworthy et al. 2012) are critical to prevent further development of GR horseweed populations in this region. Growers should be proactive in managing the horseweed seedbank in wheat using crop competition and herbicide mixtures (multiple sites of action) investigated in this study, which needs to be validated under field conditions. All possible efforts should be made to prevent seed production by GR horseweed plants in the field. The ongoing work on the underlying mechanism of resistance in GR-MT, genetic inheritance, and the fitness cost (if any) associated with glyphosate resistance will determine the potential spread of GR alleles in horseweed populations in the U.S. Great Plains.

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