

Occurrence and Characterization of Kochia (*Kochia scoparia*) Accessions with Resistance to Glyphosate in Montana

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Herbicide-resistant kochia is an increasing concern for growers in the northwestern United States. Four suspected glyphosate-resistant (Gly-R) kochia accessions (referred to as GIL01, JOP01, CHES01, and CHES02) collected in fall 2012 from four different chemical-fallow fields in northern Montana were evaluated. The objectives were to confirm and characterize the level of glyphosate resistance in kochia accessions relative to a glyphosate-susceptible (Gly-S) accession and evaluate the effectiveness of various POST herbicides for Gly-R kochia control. Whole-plant dose–response experiments indicated that the four Gly-R kochia accessions had 7.1- to 11-fold levels of resistance relative to the Gly-S accession on the basis of percent control ratings (I_{50} values). On the basis of shoot dry weight response (GR_{50} values), the four Gly-R kochia accessions exhibited resistance index (R/S) ratios ranging from 4.6 to 8.1. In a separate study, the two tested Gly-R accessions (GIL01 and JOP01) showed differential response (control and shoot dry weight reduction) to various POST herbicides 21 d after application (DAA). Paraquat, paraquat + linuron, carfentrazone + 2,4-D, saflufenacil alone or with 2,4-D, and bromoxynil + fluroxypyr effectively controlled (99 to 100%) and reduced shoot dry weight (88 to 92%) of the GIL01 accession, consistent with the Gly-S kochia accession; however, bromoxynil + MCPA and bromoxynil + pyrasulfotole provided 76% control and 83% shoot dry weight reduction of the GIL01 accession and were lower compared with the Gly-S accession. The JOP01 accession exhibited lower control or shoot dry weight reduction to all herbicides tested, except dicamba, diflufenzopyr + dicamba + 2,4-D, paraquat + linuron, and bromoxynil + pyrasulfotole, compared with the Gly-S or GIL01 population. Furthermore, paraquat + linuron was the only treatment with $\geq 90\%$ control and shoot dry weight reduction of the JOP01 kochia plants. Among all POST herbicides tested, glufosinate was the least effective on kochia. This research confirms the first evolution of Gly-R kochia in Montana. Future research will investigate the mechanism of glyphosate resistance, inheritance, ecological fitness, and alternative strategies for management of Gly-R kochia.

Nomenclature: 2,4-D; bromoxynil; carfentrazone; dicamba; diflufenzopyr; fluroxypyr; glufosinate; glyphosate; linuron; MCPA; paraquat; pyrasulfotole; saflufenacil; kochia, *Kochia scoparia* (L.) Schrad.

Key words: Glyphosate resistance, postemergence herbicides, resistance management.

Kochia scoparia resistente a herbicidas es una preocupación creciente para los productores del noroeste de Estados Unidos. Se evaluaron cuatro accesiones de *K. scoparia* (referidas como GIL01, JOP01, CHES01, y CHES02) que se sospechaban resistentes a glyphosate (Gly-R) y que fueron colectadas en el otoño 2012, en cuatro campos en barbecho químico en el norte de Montana. Los objetivos fueron confirmar y caracterizar el nivel de resistencia a glyphosate en accesiones de *K. scoparia* en relación con una accesión susceptible a glyphosate (Gly-S) y evaluar la efectividad de varios herbicidas POST para el control de *K. scoparia* Gly-R. Experimentos de respuesta a dosis en plantas enteras indicaron que las cuatro accesiones de *K. scoparia* Gly-R tuvieron 7.1 a 11 veces el nivel de resistencia en relación a la accesión Gly-S con base en evaluaciones de porcentaje de control (valores I_{50}). Con base en la respuesta del peso seco del tejido aéreo (valores GR_{50}), las cuatro accesiones de *K. scoparia* Gly-R mostraron ratios de índice de resistencia (R/S) entre 4.6 y 8.1. En un estudio aparte, las dos accesiones Gly-R evaluadas (GIL01 y JOP01) mostraron respuestas diferenciadas (reducciones en control y peso seco del tejido aéreo) a varios herbicidas POST 21 días después de la aplicación (DAA). Paraquat, paraquat + linuron, carfentrazone + 2,4-D, saflufenacil solo o con 2,4-D, y bromoxynil + fluroxypyr controlaron efectivamente (99 a 100%) y redujeron el peso seco del tejido aéreo (88 a 92%) de la accesión GIL01, en forma consistente con los resultados observados en la accesión de *K. scoparia* Gly-S. Sin embargo, bromoxynil + MCPA y bromoxynil + pyrasulfotole brindaron 76% de control y 83% de reducción en el peso seco del tejido aéreo de la accesión GIL01 y estos valores fueron menores en comparación con la accesión Gly-S. La accesión JOP01 mostró menor control o reducción en el peso seco del tejido aéreo en respuesta a todos los herbicidas evaluados, excepto dicamba, diflufenzopyr + dicamba + 2,4-D, paraquat + linuron, y

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bromoxynil + pyrasulfotole, al compararse con las poblaciones Gly-S o GIL01. Adicionalmente, paraquat + linuron fue el único tratamiento con $\geq 90\%$ de control y reducción del peso seco del tejido aéreo de las plantas de *K. scoparia* JOP01. Entre los herbicidas POST evaluados, glufosinate fue el menos efectivo. Esta investigación confirma la primera evolución de *K. scoparia* Gly-R en Montana. Investigaciones futuras estudiarán el mecanismo de resistencia a glyphosate, la heredabilidad, el desempeño ecológico, y las estrategias alternativas para el manejo de *K. scoparia* Gly-R.

Kochia, a C_4 plant, is a summer annual broadleaf weed belonging to the Chenopodiaceae family. The competitiveness of kochia is attributed to its early germination in the spring, an extended period of emergence in the presence or absence of crop, and an aggressive growth habit (Christoffoleti et al. 1997; Evetts and Burnside 1972). Being a prolific seed producer, each plant can produce $> 30,000$ seeds (Stallings et al. 1995). Kochia seeds are short-lived (1 to 2 yr) in soil and exhibit low dormancy, with a majority ($> 90\%$) of nondormant seeds that are lying at or near the soil surface after dispersal in fall emerging in the following spring (Anderson and Nielsen 1996; Schwinghamer and Van Acker 2008; Zorner et al. 1984). Furthermore, kochia plants exhibit high tolerance to salt, drought, and heat stress (Friesen et al. 2009). At maturity, a plant breaks off at the soil surface and tumbles with prevailing winds, ensuring long-distance seed dispersal (Baker et al. 2010). Additionally, kochia is a facultative open pollinator, and the protogynous flowering enforces out-crossing and pollen-mediated gene flow (Mengistu and Messersmith 2002; Stallings et al. 1995).

Kochia invades cropland and noncropland areas of the semiarid to arid regions of the northwestern US and Canadian prairies (Eberlin and Fore 1984; Forcella 1985; Leeson et al. 2005). In this region of the United States, kochia is one of the most troublesome weeds in wheat (*Triticum aestivum* L.), sugar beet (*Beta vulgaris* L.), corn (*Zea mays* L.), sorghum [*Sorghum bicolor* (L.)], sunflower (*Helianthus annuus* L.), and soybean [(*Glycine max* (L.) Merr.], reducing crop yields up to 95% (Durgan et al. 1990; Mesbah et al. 1994; Weatherspoon and Schweizer 1969; Wicks et al. 1993, 1994). Kochia also is a problem weed in summer chemical-fallow fields. Winter wheat after summer chemical-fallow dominates $> 90\%$ of the dryland cropping systems of this region, including Montana, where soil moisture (< 30 cm of average annual precipitation) is often the limiting factor for continuous cropping. The purpose of chemical-fallow in wheat-fallow rotation is to prevent soil erosion and soil nutrient

depletion and, more importantly, conserve soil moisture from winter precipitation for successful establishment of winter wheat in late summer or early fall (Lenssen et al. 2007). To fulfill the aforementioned goals, chemical-fallow utilizes multiple applications of POST herbicides, predominantly glyphosate, to obtain season-long weed control in the absence of crop, tillage, or both (Fenster and Wicks 1982). Additionally, multiple herbicide applications are needed to prevent replenishment of the weed seedbank during the fallow portion of the cropping cycle, which otherwise can contribute to increased weed infestations in the following winter wheat crop (Fenster and Wicks 1982; Moyer et al. 1994).

Glyphosate has been widely used to control kochia in chemical-fallow fields before planting of crops (burndown) or postharvest (Donald and Prato 1991; Lloyd et al. 2011; Mickelson et al. 2004). In Montana, each field typically receives three to four applications of glyphosate in the chemical-fallow before planting of winter wheat. During summer 2012, kochia control failures with glyphosate were observed in chemical-fallow fields in Hill County and Liberty County of northern Montana, even after repeated applications of glyphosate at rates > 0.87 kg ae ha⁻¹. In response to the control failures, kochia accessions were collected from grower fields and evaluated for suspected resistance to glyphosate. Glyphosate-resistant (Gly-R) kochia was first reported in western Kansas in 2007 and was recently found in six other states in the northwestern United States and in Alberta, Canada (Beckie et al. 2013; Heap 2013; Waite et al. 2013; Westra et al. 2013). In addition to Gly-R crops (corn and soybean) and wheat-fallow rotation, evolution of Gly-R kochia is a potential concern in the noncropland areas of the region.

Furthermore, the occurrence of kochia accessions resistant to other herbicide chemistries is a serious concern for growers in the north-central and western United States (Heap 2013; Preston et al. 2009; Primiani et al. 1990). Kochia resistant to atrazine was first confirmed in 1976 in Kansas near

railroads and in corn fields, and it was subsequently reported in northwestern states, including Montana (Heap 2013). Since 1989, there has been a widespread occurrence of kochia biotypes resistant to sulfonylurea herbicides (acetolactate synthase [ALS] inhibitors), predominantly in cereal-based production systems of this region (Beckie et al. 2013; Heap 2013, Primiani et al. 1990). Dicamba (auxinic herbicide)-resistant kochia was first found in 1994 in northern Montana wheat fields, and it now occurs in North Dakota, Idaho, Nebraska, and Colorado (Cranston et al. 2001; Heap 2013; Preston et al. 2009). Furthermore, literature on POST herbicides to control Gly-R kochia is lacking. The objectives of this research were to characterize the level of glyphosate resistance in selected kochia accessions from Montana and to evaluate the effectiveness of alternative POST herbicides for use in wheat-fallow rotation to control Gly-R kochia.

Materials and Methods

Plant Materials. Putative Gly-R kochia accessions were collected in fall 2012 from four different chemical-fallow fields: two fields near Gildford and Joplin, Hill County, MT, and two fields near Chester, Liberty County, MT. The sampled grower fields were under no-till wheat-fallow rotation for > 5 yr, with a history of repeated glyphosate use. At each of the four sites, kochia seeds were collected from 5 to 10 arbitrarily selected large and mature plants that survived two to three sequential applications of glyphosate at rates > 0.87 kg ha⁻¹, with the first application made at the recommended weed size. Seeds from the field-collected plants were combined into a composite sample. Gildford and Joplin sites were within 5 km of each other, and the putative Gly-R accessions were referred to as GIL01 and JOP01, respectively. The two Chester field sites were within 3 km of each other, and the putative Gly-R accessions were referred to as CHES01 and CHES02. The glyphosate-susceptible (Gly-S) accession was obtained from a field at the Montana State University Southern Agricultural Research Center near Huntley, MT, used for long-term organic trials, and was known to be susceptible to glyphosate. To obtain additional seeds for the experiments, field-collected kochia accessions were grown in the greenhouse in 10-L pots, and

individual plants were covered with pollen bags to avoid cross-pollination. Thus, first generation seeds were harvested at maturity from self-pollinated plants for further experiments.

Whole-Plant Dose Response. Whole-plant dose-response experiments were conducted in a greenhouse at the Montana State University Southern Agricultural Research Center near Huntley, MT, during spring 2013. Seeds of putative Gly-R (GIL01, JOP01, CHES01, and CHES02) and Gly-S accessions were sown separately on the surface of 53 by 35 by 10-cm flats filled with a commercial potting medium (VermiSoil™, Vermicrop Organics, 4265 Duluth Avenue, Rocklin, CA). Single kochia seedlings were transplanted into 10-cm-diam pots containing the same potting medium as the germination trays. Experiments were arranged in a randomized complete block design with eight replications (one plant per pot), and repeated three times. The greenhouse was maintained at 26/23 ± 3 C day/night temperatures and 16/8 h day/night photoperiods, and the supplemental photoperiod was obtained with metal halide lamps (400 μmol m⁻² s⁻¹).

Actively growing 8- to 10-cm-tall kochia plants from each accession were sprayed with the potassium salt of glyphosate (Roundup PowerMax®, Monsanto Company, St Louis, MO) at doses of 0, 0.109, 0.218, 0.435, 0.87, 1.74, 2.61, 3.48, 4.35, 5.22, 6.96, 8.7, and 10.44 kg ae ha⁻¹. Ammonium sulfate (AMS) at 2.0% (wt/v) was included with all glyphosate treatments. Applications were made using a stationary cabinet spray chamber (Research Track Sprayer, De Vries Manufacturing, RR 1 Box 184, Hollandale, MN) equipped with a flat-fan nozzle tip (TeeJet 8001XR, Spraying System Co., Wheaton, IL) calibrated to deliver 94 L ha⁻¹ of spray solution at 276 kPa. After the herbicide application, plants were returned to the greenhouse, watered daily to avoid moisture stress, and fertilized (Miracle-Gro water-soluble fertilizer [24–8–16], Scotts Miracle-Gro Products Inc., 14111Scottslawn Road, Marysville, OH) weekly to maintain good growth. Percent control on a scale of 0 (no injury/control) to 100 (complete control/plant death) was determined by visual observation at 7, 14, and 21 DAA. Kochia plants were harvested at the soil level at 21 DAA and dried at 60 C for 3 d to determine shoot dry weight, calculated as percentage of the nontreated control.

Alternative POST Herbicides. On the basis of resistance confirmation from dose–response experiments, the two Gly-R accessions, including JOP01 (high tolerance to glyphosate) and GIL01 (moderate tolerance to glyphosate), were used to determine the effectiveness of different POST herbicides on control of Gly-R kochia compared with the Gly-S accession. Greenhouse experiments were conducted in a randomized complete block design with six replications (one plant per pot) for each herbicide and accession combination and repeated at least once. Herbicide treatments included: bromoxynil + MCPA (Bronate AdvancedTM, Bayer CropScience, Research Triangle Park, NC) at 0.56 + 0.56 kg ai ha⁻¹; dicamba (Rifle[®], Loveland Products Inc., Greeley, CO) at 0.28 kg ae ha⁻¹; diflufenzopyr + dicamba (Distinct[®], BASF Corp., Research Triangle Park, NC) at 0.02 + 0.07 kg ae ha⁻¹ + 2,4-D (2,4-D LV4, Winfield Solutions, St Paul, MN) at 0.28 kg ae ha⁻¹; bromoxynil + pyrasulfotole (HuskieTM, Bayer CropScience) at 0.24 + 0.03 kg ai ha⁻¹; glufosinate (Liberty[®], Bayer CropScience) at 0.59 kg ai ha⁻¹; paraquat (Gramoxone Inteon[®], Syngenta Crop Protection, Greensboro, NC) at 0.70 kg ai ha⁻¹; paraquat at 0.70 kg ai ha⁻¹ + linuron (Linex[®] 4L, NovaSource, Tessengerlo Kerley Inc., Phoenix, AZ) at 0.84 kg ai ha⁻¹; carfentrazone-ethyl + 2,4-D (RageTM D-Tech, FMC Corp., Philadelphia, PA) at 0.03 + 1.66 kg ai ha⁻¹; saflufenacil (Sharpen[®], BASF Corp.) at 0.02 kg ai ha⁻¹; saflufenacil at 0.02 kg ai ha⁻¹ + 2, 4-D at 0.28 kg ae ha⁻¹; and bromoxynil + fluroxypyr (Starane[®] NXT, Dow AgroScience LLC, Indianapolis, IN) at 0.55 + 0.14 kg ae ha⁻¹. A nontreated control for each accession was included for comparison. Nonionic surfactant at 0.25% (v/v) was added to diflufenzopyr + dicamba + 2, 4-D, bromoxynil + pyrasulfotole, paraquat, paraquat + linuron, carfentrazone + 2, 4-D, and bromoxynil + fluroxypyr. Methylated seed oil (MSO) at 1% (v/v) was added to saflufenacil and saflufenacil + 2, 4-D. AMS at 2% (wt/v) was added to saflufenacil, saflufenacil + 2,4-D, and glufosinate treatments. All herbicides were applied at their field-use rates for wheat, chemical-fallow, or both. Herbicide application procedures and plant growth conditions were similar to those described in the dose–response experiments. Herbicides were applied to 8- to 10-cm-tall kochia plants. Percent control of kochia was determined by visual assessment at 7, 14, and 21 DAA, and shoot dry

weight was determined at 21 DAA. For each accession, shoot dry weight data were expressed as a percentage of the nontreated control.

Statistical Analyses. Data from whole-plant dose–response experiments and alternative POST herbicide programs were subjected to ANOVA using PROC MIXED in SAS (SAS Institute Inc., Cary, NC) to test the significance of experiment run, replication, accession, treatment (glyphosate dose in the whole-plant dose–response study or herbicide in the alternative POST herbicide study), and their interactions. Residual analyses were performed on the percent control and shoot dry weight (percentage of nontreated) using PROC UNIVARIATE in SAS, which revealed that the distribution of residuals met the criteria for ANOVA. For both studies, data were pooled across experiment runs, on the basis of nonsignificant experiment run by treatment interaction, and were tested for homogeneity of variance.

For the whole-plant dose–response experiments, arcsine transformation of percent control and shoot dry weight (percentage of nontreated) data did not improve the results; therefore, nontransformed means were presented. Data for each kochia accession were regressed over glyphosate doses using the four-parameter log-logistic model (Seefeldt et al. 1995),

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

where Y refers to the response variable (percent control or shoot dry weight as a percentage of nontreated), C is the lower limit, D is the upper limit, B is the slope of each curve, E is the glyphosate dose required for 50% response (e.g., 50% control referred as I_{50} or 50% reduction in shoot dry weight referred as GR_{50}), and X is the glyphosate dose. A lack-of-fit test indicated that the nonlinear model accurately described control ($P = 0.106$) and shoot dry weight ($P = 0.631$) data. Analysis of the dose–response curves, parameter estimates, standard errors, and I_{90} or GR_{90} values (glyphosate dose required for 90% control or 90% reduction in shoot dry weight) were determined by utilizing the *drc* package in *R* software (Knezevic et al. 2007). On the basis of I_{50} or GR_{50} values, resistance index (referred as R/S ratio) for each kochia accession was calculated by dividing the I_{50}

Table 1. Regression parameters (Equation 1) for whole-plant dose response on the basis of visual assessment of percent control and shoot dry weight (percentage of nontreated) of kochia accessions from Montana treated with glyphosate.

Accession ^a	Regression parameters (\pm SE)					R/S ^b
	<i>d</i>	<i>C</i>	<i>b</i>	I ₅₀ or GR ₅₀	I ₉₀ or GR ₉₀	
Based on % control						
Gly-S	100.6 (0.8)	0.2 (2.2)	-2.3 (0.1)	0.33 (0.01)	0.84 (0.06)	
GIL01	93.5 (1.4)	0.5 (1.1)	-4.5 (0.3)	2.48 (0.05)	4.02 (0.17)	7.5
JOP01	102.5 (5.0)	-0.2 (1.4)	-2.0 (0.2)	3.64 (0.50)	11.17 (2.30)	11.0
CHES01	101.2 (5.2)	-0.6 (1.6)	-1.6 (0.1)	2.84 (0.22)	10.79 (1.98)	8.6
CHES02	92.4 (1.6)	5.0 (1.3)	-3.1 (0.3)	2.35 (0.07)	4.73 (0.35)	7.1
Based on shoot dry weight						
Gly-S	100.2 (1.9)	13.2 (1.0)	1.5 (0.1)	0.27 (0.01)	1.12 (0.16)	
GIL01	95.6 (1.5)	19.0 (1.2)	2.3 (0.2)	1.25 (0.05)	3.14 (0.30)	4.6
JOP01	97.5 (1.3)	25.8 (2.0)	2.2 (0.2)	2.19 (0.09)	5.90 (0.70)	8.1
CHES01	94.1 (2.1)	28.9 (2.3)	1.8 (0.3)	1.62 (0.10)	5.27 (1.05)	6.0
CHES02	95.6 (1.5)	15.8 (1.4)	2.1 (0.2)	1.24 (0.05)	3.12 (0.35)	4.6

^a Abbreviations: Gly-S, glyphosate-susceptible accession, Huntley, MT; GIL01, glyphosate-resistant (Gly-R) accession, Gildford, MT; JOP01, Gly-R accession, Joplin, MT; CHES01, Gly-R accession no. 1, Chester, MT; CHES02, Gly-R accession no. 2, Chester, MT; I₅₀ and GR₅₀ are effective doses (kg ae ha⁻¹) of glyphosate required for 50% control and shoot dry weight reduction, respectively; I₉₀ and GR₉₀ are effective doses (kg ae ha⁻¹) of glyphosate required for 90% control and shoot dry weight reduction, respectively.

^b R/S is calculated as a ratio of I₅₀ or GR₅₀ of a Gly-R accession to I₅₀ or GR₅₀ of the Gly-S accession.

or GR₅₀ value of a Gly-R accession by the I₅₀ or GR₅₀ value of the Gly-S accession. Independent *t* tests were performed to determine whether the I₅₀ or GR₅₀ values between accessions were significantly different at $\alpha = 0.05$.

For the alternative POST herbicide experiments, percent control and shoot dry weight (percentage of nontreated) data were arcsine-transformed before analysis to improve homogeneity of variance and normality of residuals. Nontransformed means are presented in tables based on the interpretations of the transformed data. Means were separated using Fisher's protected LSD test at $P < 0.05$.

Results and Discussion

Whole-Plant Dose Response. The Gly-S and putative Gly-R kochia accessions (GIL01, JOP01, CHES01, and CHES02) were treated with a discriminating dose of glyphosate at 0.87 kg ha⁻¹ (field-use rate of glyphosate). Whereas the Gly-S kochia plants were completely controlled, putative Gly-R accessions survived the discriminating dose of glyphosate at 21 DAA. The level of glyphosate resistance in those Gly-R accessions was further characterized by whole-plant dose-response experiments. Glyphosate doses ≤ 0.87 kg ha⁻¹ did little to no injury to all four Gly-R accessions 21 DAA.

On the basis of visually assessed percent control, I₅₀ values for the CHES02, GIL01, CHES01, and JOP01 accessions were 2.35, 2.48, 2.84, and 3.64 kg ha⁻¹, respectively, and were higher than the 0.33 kg ha⁻¹ for the Gly-S accession (Table 1; Figure 1). Additionally, the I₉₀ values indicated that 4.8 to 13.3 times more dose of glyphosate would be needed to obtain 90% control of the four Gly-R accessions relative to the Gly-S accession. On the basis of visually assessed percent control response (I₅₀ values), the four Gly-R kochia accessions exhibited R/S ratios of 7.1 to 11.0.

On the basis of shoot dry weight dose-response, GR₅₀ values for the CHES02, GIL01, CHES01, and JOP01 accessions were 1.24, 1.25, 1.62, and 2.19 kg ha⁻¹, respectively, and were higher compared with the Gly-S accession (0.27 kg ha⁻¹) (Table 1; Figure 2). Furthermore, GR₉₀ values for the Gly-R accessions were 2.8 to 5.3 times greater than the Gly-S accession, and 3.6 to 6.8 times the field-use rate of glyphosate. On the basis of GR₅₀ values, the four Gly-R accessions had R/S ratios of 4.6 to 8.1, further suggesting that increasing the rate of glyphosate is not a viable option to control these Gly-R kochia plants.

Furthermore, a differential tolerance to glyphosate was evident between the four Gly-R accessions. JOP01 accession had a higher I₅₀ or GR₅₀

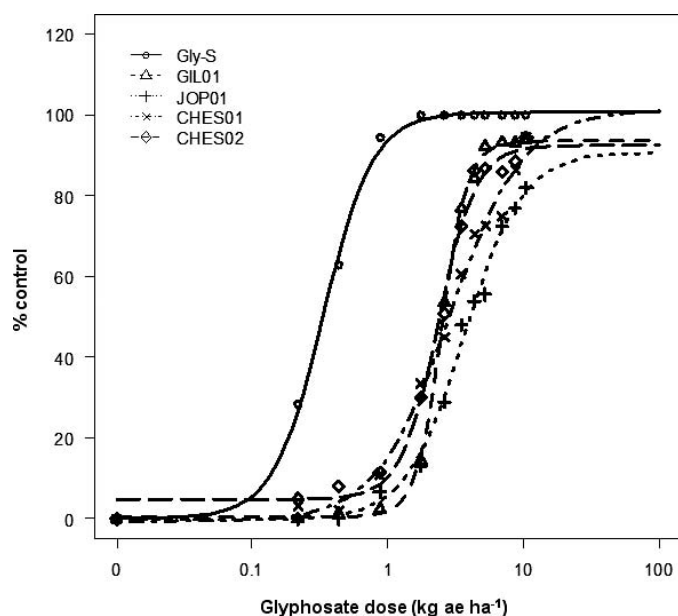


Figure 1. Control response based on visual assessment of four glyphosate-resistant (Gly-R) kochia accessions (GIL01 from Gildford, MT; JOP01 from Joplin, MT; CHES01 and CHES02 from Chester, MT) and a glyphosate-susceptible accession (Gly-S from Huntley, MT) in a whole-plant dose-response experiment.

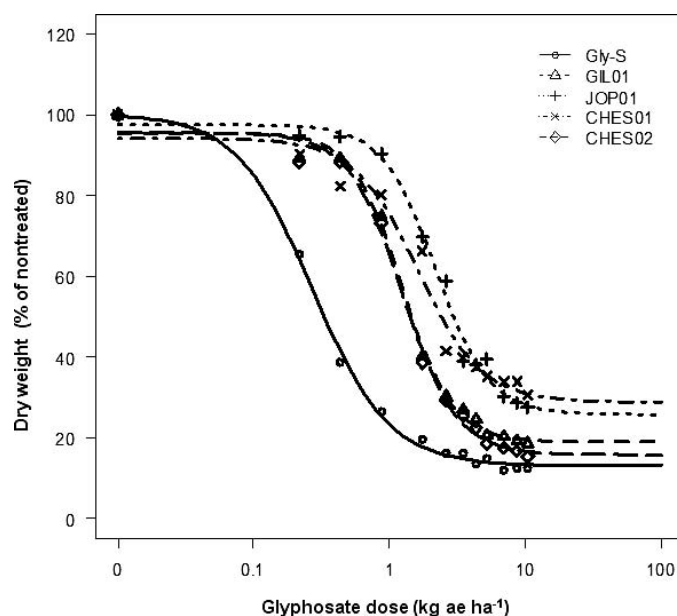


Figure 2. Shoot dry weight response of four glyphosate-resistant (Gly-R) kochia accessions (GIL01 from Gildford, MT; JOP01 from Joplin, MT; CHES01 and CHES02 from Chester, MT) and a glyphosate-susceptible accession (Gly-S from Huntley, MT) in a whole-plant dose-response experiment.

value compared with GIL01, CHES01, and CHES02 accessions (Table 2). Overall, the resistance hierarchy of Gly-R kochia accessions was: JOP01 > CHES01 ≥ GIL01 = CHES02. Resistance indices (R/S ratio) of 3.9 to 6.0 based on GR₅₀ values or 5.4 to 6.9 based on I₅₀ values have been reported in Gly-R kochia accessions from southern Alberta, Canada (Beckie et al. 2013). In the same study, a Gly-R accession from Hays, KS, had an R/S ratio of 3.7 or 5.8 on the basis of shoot dry weight or plant survival response, respectively. Also, Waite et al. (2013) reported a differential response of kochia accessions to glyphosate in Kansas, with I₅₀ values (on the basis of visually assessed percent control) ranging from 470 to 2,149 g ha⁻¹. It seems that the resistance levels in some of those Gly-R kochia accessions from Montana were higher than those reported in Gly-R accessions from western Kansas and southern Alberta, Canada.

Alternative POST Herbicides. *Visual Assessment of Percent Control.* The interaction of herbicide treatment by accession was significant ($P < 0.0001$) on kochia control at 21 DAA. For the Gly-S kochia accession, bromoxynil + MCPA, bromoxynil + pyrasulfotole, paraquat, paraquat + linuron, carfentrazone + 2, 4-D, saflufenacil + 2, 4-

D, and bromoxynil + fluroxypyr provided the most effective control (97 to 100%) 21 DAA (Table 3). Control with diflufenzopyr + dicamba + 2,4-D (87%) was superior to dicamba alone (80%); however, it did not differ from the saflufenacil alone treatment (88% control). Also, Wicks et al. (1993) reported > 95% control of 7.5- to 10-cm-tall kochia plants treated with bromoxynil (0.4 kg ha⁻¹) and paraquat (0.3 kg ha⁻¹). In a study conducted by Nandula and Manthey (2002) on a dicamba-resistant kochia accession, bromoxynil (0.28 kg ha⁻¹) + MCPA (0.28 kg ha⁻¹), carfentrazone (0.02 kg ha⁻¹), and fluroxypyr (0.14 kg ha⁻¹) provided > 88% control. Although the dicamba rate of 0.28 kg ha⁻¹ has not been previously evaluated, control of 2- to 8-cm-tall kochia plants (dicamba-susceptible) treated with dicamba at 0.14 kg ha⁻¹ varied from 68 to 90% depending on the biotype tested (Tonks and Westra 1997). Kochia control as high as 95% with dicamba applied at 0.84 kg ha⁻¹ has been reported (Lloyd et al. 2011). In our study, control of the Gly-S accession was least (70%) with glufosinate. For the GIL01 accession, control with POST herbicides was not different from the Gly-S accession, except for bromoxynil + MCPA, bromoxynil + pyrasulfotole,

Table 2. Independent *t* tests of I_{50} (on the basis of visual assessment) and GR_{50} (based on shoot dry weight) values from the whole-plant dose response of kochia accessions from Montana.^a

Accessions	Based on visual control		Based on dry weight	
	I_{50}	<i>P</i> > <i>F</i>	GR_{50}	<i>P</i> > <i>F</i>
Gly-S vs. GIL01	0.33 vs. 2.48	< 0.0001	0.27 vs. 1.25	< 0.0001
Gly-S vs. JOP01	0.33 vs. 3.64	< 0.0001	0.27 vs. 2.19	< 0.0001
Gly-S vs. CHES01	0.33 vs. 2.84	< 0.0001	0.27 vs. 1.62	< 0.0001
Gly-S vs. CHES02	0.33 vs. 2.35	< 0.0001	0.27 vs. 1.24	< 0.0001
GIL01 vs. JOP01	2.48 vs. 3.64	< 0.0001	1.25 vs. 2.19	< 0.0001
GIL01 vs. CHES01	2.48 vs. 2.84	0.0823	1.25 vs. 1.62	0.0038
GIL01 vs. CHES02	2.48 vs. 2.35	0.1788	1.25 vs. 1.24	0.9912
JOP01 vs. CHES01	3.64 vs. 2.84	0.0379	2.19 vs. 1.62	0.0013
JOP01 vs. CHES02	3.64 vs. 2.35	< 0.0001	2.19 vs. 1.24	< 0.0001
CHES01 vs. CHES02	2.84 vs. 2.35	0.0587	1.62 vs. 1.24	0.0026

^aAbbreviations: Gly-S, glyphosate-susceptible accession, Huntley, MT; GIL01, Gly-R accession, Gildford, MT; JOP01, Gly-R accession, Joplin, MT; CHES01, Gly-R accession no. 1, Chester, MT; CHES02, Gly-R accession no. 2, Chester, MT; I_{50} is the effective dose (kg ae ha⁻¹) of glyphosate required for 50% control; GR_{50} is the effective dose (kg ae ha⁻¹) of glyphosate required for 50% reduction in shoot dry weight.

and glufosinate, with less control of GIL01 compared with the Gly-S accession. Furthermore, control with bromoxynil + pyrasulfotole (75% control) did not differ from the dicamba and bromoxynil + MCPA treatments but was lower than the diflufenzopyr + dicamba + 2,4-D treatment (85% control). For the JOP01 accession, control

was lower compared with Gly-S or GIL01 accession for all herbicides tested, except dicamba, diflufenzopyr + dicamba + 2,4-D, and paraquat + linuron. Addition of linuron to paraquat improved control, 88% compared with 100% control. Control with dicamba, diflufenzopyr + dicamba + 2,4-D averaged 79%, which did not differ from saflufenacil

Table 3. Percent control and shoot dry weight (percentage of nontreated) of three kochia accessions from Montana 21 d after treatment with various POST herbicides.^{a,b}

Herbicide(s)	Rate(s) kg ae or ai ha ⁻¹	Control ^c			Shoot dry weight ^c		
		Gly-S	GIL01	JOP01	Gly-S	GIL01	JOP01
		%			% of nontreated		
Bromoxynil + MCPA	0.56 + 0.56	100 aA	77 cB	60 fC	6 eC	17 cB	50 aA
Dicamba	0.28	80 cA	80 bcA	78 cdA	29 aA	26 bA	32 bA
Diflufenzopyr + dicamba + 2,4-D ^d	0.02 + 0.07 + 0.28	87 bA	85 bA	80 bcdA	23 bA	25 bA	30 bcA
Bromoxynil + pyrasulfotole ^d	0.24 + 0.03	97 aA	75 cB	67 efB	8 deC	17 cB	24 cdA
Glufosinate ^e	0.59	70 dA	43 dB	45 gB	15 cB	41 aA	43 aA
Paraquat ^d	0.70	100 aA	100 aA	88 bB	6 eB	9 dB	15 efA
Paraquat + linuron ^d	0.70 + 0.84	100 aA	100 aA	100 aA	5 eA	8 dA	10 fA
Carfentrazone-ethyl + 2,4-D ^d	0.03 + 1.66	100 aA	100 aA	72 deB	11 dB	9 dB	29 bcA
Saflufenacil ^{e,f}	0.02	88 bB	100 aA	77 cdC	16 cB	9 dC	23 cdA
Saflufenacil + 2,4-D ^{e,f}	0.02 + 0.28	100 aA	100 aA	78 cdB	5 eB	8 dB	25 bcdA
Bromoxynil + fluroxypyr ^d	0.55 + 0.14	100 aA	99 aA	82 bcB	8 deA	12 dA	18 deA

^a Abbreviations: Gly-S, glyphosate-susceptible accession, Huntley, MT; GIL01, Gly-R accession, Gildford, MT; JOP01, Gly-R accession, Joplin, MT.

^b Herbicide treatments were applied to 8- to 10-cm-tall kochia plants.

^c For percent control or shoot dry weight data, means for a kochia accession within a column followed by similar lowercase letters are not significantly different based on Fisher's protected LSD test at *P* < 0.05; means for an herbicide within a row followed by similar uppercase letters are not significantly different based on Fisher's protected LSD test at *P* < 0.05.

^d Nonionic surfactant (NIS) at 0.25% (v/v) was included.

^e Ammonium sulfate (AMS) at 2% (wt/v) was included.

^f Methylated seed oil (MSO) at 1% (v/v) was included.

alone or with 2,4-D or bromoxynil + fluroxypyr. Unlike Gly-S or GIL01 accessions, control of the JOP01 accession with bromoxynil + MCPA, bromoxynil + pyrasulfotole, and carfentrazone + 2,4-D was < 75%. Consistent across the Gly-R accessions, control with glufosinate was poor (< 50%).

Shoot Dry Weight. A significant interaction ($P < 0.0001$) of herbicide treatment by accession was evident on kochia shoot dry weight (percentage of nontreated) 21 DAA. For the majority of herbicides, shoot dry weight response of treated kochia accessions was consistent with the percent control ratings. For the Gly-S accession, bromoxynil + MCPA, bromoxynil + pyrasulfotole, paraquat, paraquat + linuron, saflufenacil + 2,4-D, and bromoxynil + fluroxypyr reduced kochia shoot dry weight by 92 to 95% (Table 3). Shoot dry weight reduction of Gly-S accession 21 DAA of carfentrazone + 2,4-D and saflufenacil was 89 and 84%, respectively. Consistent with percent control, the shoot dry weight of Gly-S plants was slightly lower with diflufenzopyr + dicamba + 2,4-D compared with the dicamba alone treatment. Additionally, shoot dry weight response to POST herbicides did not differ between Gly-S and GIL01 accession, except for bromoxynil + MCPA, bromoxynil + pyrasulfotole, glufosinate, and saflufenacil. Bromoxynil + MCPA or bromoxynil + pyrasulfotole and glufosinate reduced shoot dry weight of the GIL01 kochia accession by 83 and 59%, respectively; nevertheless, those reductions were lower compared with the Gly-S accession. Consistent with poor control, glufosinate-treated plants of GIL01 accession had greater shoot dry weight than all other treatments. For the JOP01 accession, paraquat + linuron treatment had 90% reduction in shoot dry weight and was superior to all other treatments, except paraquat alone (85% dry weight reduction). Saflufenacil + 2,4-D reduced shoot dry weight of JOP01 kochia plants by 75%, which did not differ from saflufenacil alone, dicamba, diflufenzopyr + dicamba + 2,4-D, bromoxynil + pyrasulfotole, carfentrazone + 2,4-D, and bromoxynil + fluroxypyr treatments. Similar to the GIL01 accession, glufosinate was not effective on the JOP01 accession (< 60% dry weight reduction).

This research confirms the occurrence of Gly-R kochia in Montana. This is the first report on evolution of any Gly-R weed species in Montana.

Wind-mediated tumble mechanism of seed dispersal coupled with pollen-mediated gene flow through out-crossing would ensure rapid spread of Gly-R kochia. Growers should make all possible efforts to prevent seed production from Gly-R kochia plants in their production fields. Further spread of resistant alleles will likely be influenced by inheritance pattern and fitness cost associated with glyphosate resistance in those Gly-R kochia accessions, which needs to be investigated. Additionally, the presence of ALS inhibitor- and auxinic-resistant kochia in the northwestern region including Montana, and the recent evidence of glyphosate- and ALS inhibitor-resistant (multiple-resistance) kochia in western Canada is a potential concern for wheat growers (Beckie et al. 2013; Heap 2013). We are currently investigating the physiological, molecular, or genetic basis of glyphosate resistance in the tested Gly-R kochia accessions from Montana.

Management and containment efforts for herbicide-resistant weeds are influenced by grower adoption of best management practices (Norsworthy et al. 2012). The success of those efforts to control Gly-R kochia will depend on the effectiveness of alternative herbicides. On the basis of this research, paraquat alone or with linuron, carfentrazone + 2,4-D, saflufenacil alone or with 2,4-D, and bromoxynil + fluroxypyr provided complete control of the Gly-R GIL01 kochia accession, consistent with the Gly-S accession; however, paraquat + linuron was the only treatment that provided adequate control and shoot dry weight reduction of the Gly-R JOP01 accession. These herbicides can be effectively utilized for control of Gly-R kochia in wheat-fallow rotation; however, the variability in response of those kochia accessions to some of the tested herbicides warrants further investigation on the effect of herbicide rate, tank-mixes, and application timing on control of Gly-R kochia under different environments/field conditions. Differential response of tested kochia accessions to an herbicide also suggests the high genetic and within-accession diversity of kochia (Mengistu and Messersmith 2002), which makes the control efforts even more challenging in the field.

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