CONFIRMATION AND MANAGEMENT OF GLYPHOSATE-RESISTANT KOCHIA (*KOCHIA SCOPARIA*) IN MONTANA

by

Vipan Kumar

A dissertation submitted in partial fulfillment
of the requirements for the degree

of

Doctor of Philosophy

in

Plant Science

MONTANA STATE UNIVERSITY
Bozeman, Montana

April 2015

DEDICATION

This Ph.D. dissertation is dedicated to my father Mr. Mohinder Pal, my mother Mrs. Paramjit Kaur, my brother Mr. Pawan Kumar, my sister Mamta, my beloved wife Mrs. Priyanka, and my nephews and nieces. My father and all family members have always been a motivation for successful completion of my Ph.D program. I am really indebted for their love, affection, patience, encouragement, and support. I am also thankful to the almighty for HIS blessings on me.

ACKNOWLEDGMENTS

First, I would like to thank my graduate advisor Dr. Prashant Jha who spent countless time mentoring me his field of study, management skills, and how to present to a diverse audience. I am really grateful to him for providing me the opportunity to gain knowledge and research experience in weed science discipline. This dissertation would not have been possible without his able guidance and support. I also want to thank him for his timely efforts in making this research worth publishing in high quality journals.

I also express my sincere thanks and regards to my advisory committee for their guidance in successful completion of my Ph.D. program. I would like to extend my appreciation to Dr. William E Dyer, Dr. Kent McVay, Dr. John Martin, and Dr. Andrew Hansen for their help and serving on my graduate committee. I would also like to thank Dr. Philip Westra and his graduate students Darci Giacomini, Christopher Vanhorn and Eric Westra, at Colorado State University, for assisting in molecular experimentation and willingness to share resources at CSU.

I also thank all the faculty and staff members at MSU Southern Agricultural Research Center, near Huntley for their help and support in completing this research. Especially, I like to thank Mr. Nicholas Reichard, Mr. Shane Leland, Mr. Tom Fischer and Mrs. Janna Kransky for their technical assistance in field experiments. I would also like to thank fellow graduate student, Charlemagne Lim and summer interns for their help in greenhouse studies. Lastly, I would like to thank my best friends Gagan, Jasmeet, Sukhbir, and Mandeep for their endless support and encouragement. Thank you all.

TABLE OF CONTENTS

TABLE OF CONTENTS CONTINUED

TABLE OF CONTENTS CONTINUED

LIST OF TABLES

Table Page

LIST OF TABLES CONTINUED

Table Page

LIST OF TABLES CONTINUED

Table Page

LIST OF TABLES CONTINUED

LIST OF FIGURES

Figure Page

LIST OF FIGURES CONTINUED

LIST OF FIGURES CONTINUED

ABSTRACT

Kochia (*Kochia scoparia* L.) is one of the most problematic weeds in cropland and non-cropland areas of the US Great Plains. This research confirms the first report on glyphosate-resistant (GR) *K. scoparia* in Montana, and elucidates the mechanisms of glyphosate and ALS-inhibitor resistance, growth and reproductive fitness of GR *K. scoparia*, and its management. This research also reports the response of *K. scoparia* demographics to glyphosate timings in GR sugar beet and evaluate herbicide options for managing volunteer GR canola in GR sugar beet. Based on whole-plant dose-response experiments, four GR *K. scoparia* accessions (JOP01, GIL01, CHES01, CHES02) had 4.6 to 11-fold levels of resistance to glyphosate compared to a glyphosate-susceptible (GS) accession. Confirmed GR *K. scoparia* accessions (GIL01, JOP01, and CHES01) also exhibited 9.3- to 30-fold resistance to sulfonylurea herbicide (ALS inhibitors). Results from PCR, quantitative PCR, and immunoblotting assays indicated that *EPSPS* gene amplification (~ 4 to 10 copies) and single point mutation at Pro_{197} in *ALS* gene conferred resistance to glyphosate and ALS-inhibitors, respectively. Inbred lines of GR *K. scoparia* (CHES01 and JOP01) with 2- to 14-fold amplification of the *EPSPS* gene did not confer any growth- or fecundity-related fitness cost. From a management standpoint, acetochlor + atrazine, S-metolachlor + atrazine + mesotrione, and sulfentrazone applied PRE and paraquat + atrazine, paraquat + linuron, and paraquat + metribuzin applied POST or in postharvest wheat stubble provided effective (≥ 91%) control of *K. scoparia*. In GR sugar beet, sequential applications of glyphosate (6-leaf fb 10-leaf stage of sugar beet) reduced survival, biomass and seed production of *K. scoparia* plants in cohort 1 and 2; however, an additional application of glyphosate at the canopy closure stage of sugar beet was needed to prevent seed production from the late-emerging *K. scoparia* (cohort 3). Ethofumesate (4,200 g ha^{-1}) PRE followed by sequential POST triflusulfuron methyl (17.5 g ha^{-1}) was the most effective treatment for managing volunteer GR canola in GR sugar beet. Overall, this research demonstrates the adaptability of *K. scoparia* evolving resistance to glyphosate and ALS inhibitors, and suggest the need for adoption of integrated weed management practices.

CHAPTER 1

INTRODUCTION

Kochia Biology

Kochia (*Kochia scoparia* (L.) Schrad), a monoecious summer annual, is one of the most troublesome broadleaf weeds in croplands and non-croplands areas of the US Great Plains. *K. scoparia* is a diploid (2n=18), C_4 plant and belongs to the "Chenopodiaceae" family (Friesen et al. 2009). *K. scoparia* is a highly competitive weed species due to its early germination in the spring, extended period of emergence in the presence or absence of crop, aggressive growth habit, and prolific seed production (>50, 000 seeds per plant) (Christoffoleti et al. 1997; Evetts and Burnside 1972; Stallings et al. 1995). *K. scoparia* seeds are short-lived (1 to 2 yrs) in soil and exhibit low dormancy, with a majority (> 90%) of non-dormant seeds emerge in the spring (Anderson and Nielsen 1996; Schwinghamer and Van Acker 2008; Zorner et al. 1984). *K. scoparia* plants exhibit high tolerance to abiotic stresses such as salt, heat, and drought (Friesen et al. 2009). During late autumn, a mature *K. scoparia* plant breaks off at the soil surface and tumbles with prevailing winds, ensuring long-distance seed dispersal (Baker et al. 2010). Additionally, *K. scoparia* 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). Due to its protogynous flowering and facultative open pollination, *K. scoparia* exhibits high genetic diversity within and among populations (Mengistu and Messersmith 2002). *K. scoparia* is a problem weed in major

cropping systems of the US Great Plains, including 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], where it can reduce crop yields up to 95% (Durgan et al. 1990; Mesbah et al. 1994; Weatherspoon and Schweizer 1969; Wicks et al. 1993, 1994). *K. scoparia* also causes hindrance to mechanical harvest of crops as well as poses a serious challenge to summer fallow (Manthey et al. 1996).

Herbicide-Resistant Kochia

Herbicide-resistant (HR) *K. scoparia* is an increasing concerns for growers in north central and western US as well as Canada (Heap 2015; Preston et al. 2009; Primiani et al. 1990). *K. scoparia* populations resistant to atrazine were first reported in 1976 in Kansas near railroads and in corn fields and subsequently reported in other northwestern states including Montana (Heap 2015). Since 1989, there has been a widespread occurrence of *K. scoparia* biotypes resistant to sulfonylurea herbicides [acetolactate synthase (ALS) inhibitors], predominantly in cereal-based production systems of this region (Beckie et al. 2013; Heap 2015, Primiani et al. 1990). Dicamba (auxinic herbicide)-resistant *K. scoparia* 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 2015; Preston et al. 2009). Glyphosate-resistant (GR) *K. scoparia* was first reported in western Kansas in 2007 under wheat-fallow rotations, and recently being confirmed in nine other states in the US Great Plains, and in three provinces of Canada (Beckie et al. 2013; Heap 2015; Kumar et al. 2014; Waite et al. 2013; Wiersma et al.

2015). Recently, GR *K. scoparia* populations have also been confirmed from GR sugar beet fields in Idaho and Oregon (Jha et al. 2015). In addition to GR crops (corn, soybean, and sugar beet) and wheat-fallow rotation, evolution of GR *K. scoparia* is an increasing challenge in the non-cropland areas of this region.

Glyphosate and Glyphosate-Resistant Weeds

Glyphosate [*N*-(phosphonomethyl) glycine] is a systemic, broad-spectrum, postemergence herbicide and widely used for weed control in croplands and non-croplands worldwide. In plants, glyphosate disrupts the biosynthesis of aromatic amino acids (phenylalanine, tyrosine, and tryptophan) and other aromatic compounds by competitive inhibition of the EPSPS (5-enolpyrovylshikimate-3-phosphate synthase, EC 2.5.1.19) enzyme in the shikimate pathway, ultimately causing growth inhibition and plant death (Duke and Powles 2008; Powles 2010). Glyphosate has been described as once-in-a-century herbicide due to its broad-spectrum efficacy on weeds, low mammalian toxicity, and environmentally benign nature (Duke and Powles 2008). Prior to transgenic GR crops, glyphosate was primarily used to control annual and perennial weed species under orchards, vineyards, and non-agricultural settings.

Since the commercialization of glyphosate-resistant crops (Roundup Ready®) in mid-1990s and adoption of conservation tillage by growers, the glyphosate usage has been rapidly increased over past two decades (Powles 2010; Udeigwe et al. 2015). Due to its high efficacy and relatively low price, glyphosate is widely used to control *K. scoparia* in chemical-fallow, prior to crop planting (burndown), or post-harvest (Donald and Prato

1991; Lloyd et al. 2011; Mickelson et al. 2004). Nevertheless, the increase in selection pressure from repeated use of glyphosate has resulted in evolution of glyphosate resistance in several weed biotypes. To date, glyphosate resistance has been reported in 31 weed species globally (Heap 2015). The rapid evolution and escalading spread of GR weed species is an increasing threat to the sustainability of GR crops and conservation tillage practices.

Glyphosate-Resistant Kochia in Montana

In Montana, wheat -fallow rotation dominates the dryland cropping system, where soil moisture (< 30 cm of average annual precipitation) is often the limiting factor for continuous cropping. The major benefits of summer fallow in this rotation is to prevent soil erosion, soil nutrient depletion, and more importantly, conserve soil moisture from winter precipitation for successful 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 and/or tillage (Fenster and Wicks 1982).

In addition, multiple herbicide applications are required 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). In Montana, each field typically receives three to four applications of glyphosate in the chemical-fallow prior to winter wheat planting. During summer of 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.

Figure 1.1. Glyphosate-resistant *K. scoparia* in wheat-fallow fields of Hill and Liberty Counties in northern Montana during summer and fall 2012.

Mechanisms of Glyphosate Resistance

Currently, the genetic and molecular basis of glyphosate resistance has been elucidated only in few GR weed species. Five major mechanisms of glyphosate resistance has been reported so far, i.e., target-site EPSPS mutations, *EPSPS* gene amplification, reduced translocation, active vacuolar sequestration, and rapid necrosis response (Sammons and Gaines 2014). Only few mutations conferring glyphosate resistance have been reported in GR weeds because glyphosate binds within a highly conserved region of the EPSPS target site (Powles 2010).

Target-site mutations in the *EPSPS* gene conferring resistance to glyphosate has been confirmed in three GR weed species viz. Italian ryegrass (*Lolium multiflorum*), rigid ryegrass (*Lolium rigidum*), and goosegrass (*Eleusine indica*) (Nandula et. al 2010; Sammons and Gaines 2014). The amino acid substitutions at proline-106 residue by alanine, threonine or serine conferred low to moderate level of resistance in these GR weed species (Sammons and Gaines 2014). However, simultaneous double amino acid substitutions ($Thr_{102}Ile$ and $Pro_{106}Ser$) conferring high level of resistance (>180 fold) has recently been reported in GR *E. indica* population from Australia (Yu et al. 2015). This is one of the first field-evolved GR plant species in which double amino acid substitutions have been observed; although these simultaneous double mutations causing insensitive EPSPS have previously been utilized in developing transgenic GR crops (Yu et al. 2015).

Similarly, reduced translocation of glyphosate has been documented as mechanism of resistance in GR weed species such as *L. multiflorum*, *L. rigidum*, horseweed (*Conyza Canadensis*), and hairy fleabane (*Conyza bonariensis*) (Nandula et. al 2010). In these GR weed species, the mobility of glyphosate molecules was restricted to treated leaves as observed in ^{14}C glyphosate absorption and translocation studies (Powles et al. 2010). Recently, vacuolar sequestration of glyphosate in GR *C. Canadensis* has been observed using a ^{31}P nuclear magnetic resonance (NMR) technique (Sammons and Gaines 2014). However, reduced absorption or translocation has not been associated with glyphosate resistance in GR *K. scoparia* in the US Great Plains (Waite et al. 2013; Wiersma et al. 2015).

A rapid necrosis response to glyphosate has also been observed in GR giant ragweed (*Ambrosia trifida*) although an underlying mechanism of resistance is still unknown (Vanhorn and Westra 2014). Furthermore, an increased expression of the target site via EPSPS gene amplification is another mechanism of glyphosate resistance in GR weed species. The *EPSPS* gene amplification is a novel mechanism of glyphosate resistance, first documented in Palmer amaranth (*Amaranthus palmeri* S. Wats.) (Gaines et al. 2010). Increased *EPSPS* gene copies in GR *A. palmeri* was positively correlated with EPSPS protein abundance (Gaines et al. 2010). This resistance mechanism has also been reported in other GR weed species, such as tall waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer] (Lorentz et al. 2014), spiny amaranth (*Amaranthus spinosus*) (Nandula et al. 2014), *L. multiflorum* (Salas et al. 2012) and recently in *K. scoparia* (Wiersma et al. 2015).

Fitness Cost of Glyphosate Resistance

A fitness or resistance cost is defined as reduction in plant fitness in the absence of herbicide selection pressure caused by negative pleiotropic effects of resistance alleles on growth, development, and phenology of resistant plants (Vila-Aiub et al. 2015). Fitness studies provide insights on the population dynamics of herbicide-resistant biotypes, and also help in developing herbicide resistance management strategies (Vila-Aiub et al. 2009; Vila-Aiub et al. 2015). Fitness costs have previously been reported in GR *L. rigidum* and glyphosate-tolerant tall morningglory (*Ipomoea purpurea* (L.) Roth) (Baucom and Mauricio 2004; Pederson et al. 2007). Alteration of the target-site due to

point mutations and reduced translocation of glyphosate conferred resistance in L. rigidum and *I. pupurea* weed species. No fitness cost has been observed in GR *A. palmeri* populations even with massive *EPSPS* gene amplification (Giacomini et al. 2014; Vila-Aiub et al. 2014). However, fitness advantages have been reported in GR wild rice with transgenic over-expression of the native *EPSPS* gene (*Oryza sativa* f. spontanea) (Wang et al. 2013). Interestingly, fitness cost of glyphosate resistance in majority of confirmed GR weed biotypes including GR *K. scoparia* is unknown.

Kochia Management

Herbicides are key tools for weed management programs in modern's agriculture. Growers heavily rely on herbicides (standalone or tank mixtures) for control of *K. scoparia* in cropland and non-croplands settings in the northwestern United States. Annual herbicide rotation and tank-mixtures with multiple modes of action are generally recommended from herbicide resistance management standpoint (Beckie et al. 2006; Norsworthy et al. 2012). Incorporation of soil-applied residual PRE herbicides with effective POST herbicides is also an essential tool for resistance management (Norsworthy et al. 2012). In previous studies, different PRE and POST herbicides have been found to provide variable control of *K. scoparia*. For example, kochia control with flumioxazin (0.28 kg ha^{-1}), pendimethalin (1.4 kg ha^{-1}), or pyroxasulfone (0.418 kg ha^{-1}) applied alone PRE was only 53 to 70% (Lloyd et al. 2011; Stahlman 2010). Among POST herbicides tested by Lloyd et al. (2011), *K. scoparia* control with either fluroxypyr or glyphosate did not exceed 75%, and was as low as 35% with saflufenacil alone.

Although bromoxynil + MCPA, carfentrazone, or paraquat applied POST controlled kochia ≥ 90% under greenhouse conditions (Nandula and Manthey 2002; Tonks and Westra 1997), control as low as 68% was observed with those herbicides under field conditions (Wick et al. 1993).

Under wheat-fallow rotation, *K. scoparia* exhibits an extended period of emergence with multiple cohorts emerging throughout the growing season (Anderson, 1996; Dille et al. 2012). *K. scoparia* cohorts that escape early POST herbicide application (s) or late-emerging cohorts in wheat often remain in vegetative stage at the time of wheat maturity, and hinder mechanical harvest, and cause substantial late-season replenishment of the soil seed bank (Mickelson et al. 2004). Late-season applications of postharvest herbicides are often made at the early bloom stage or initiation of seed set to reduce/prevent late-season weed seed rain and seed bank increase (Bennet and Shaw, 2000; Jha and Norsworthy, 2012; Mickelson et al. 2004). Seed production from late-season weed escapes can favor the evolution of herbicide resistance due to the likelihood of resistant individuals being present in those weed escapes (Bagavathiannan and Norsworthy, 2012). Therefore, late-season herbicide applications in postharvest wheat stubble are crucial to manage GR *K. scoparia* weed escapes and seed bank additions.

Management of Volunteer GR Canola in GR Sugar Beet

Occurrence of volunteer canola (non-HR) is problematic in rotational crops such as corn, soybean, wheat (*Triticum aestivum* L.), dry pea (*Pisum sativum* L.), flax (*Linum usitatissimum* L.), and sunflower (*Helianthus annuus* L.) (Jenks et al. 2006; Rainbolt et

al. 2004). With recent increase in GR canola production in northwestern United States (Devine and Buth 2001), there is an increased concern for the occurrence of GR canola volunteers in GR crops grown in rotation, including GR sugar beet. Herbicides are the most effective tools to control canola volunteers in agronomic crops (Légère et al. 2006; Rainbolt et al. 2004). Alternative herbicides such as 2,4-D or MCPA (synthetic auxins, Group 4) in wheat, metribuzin (photosystem II inhibitor, Group 5) in soybean, and metsulfuron (ALS inhibitor, Group 2) have been found effective in controlling single-HR, multiple-HR, or non-HR canola volunteers (Beckie et al. 2006; Légère et al. 2006; Rainbolt et al. 2004). However, there appears to be no published research in literature on herbicide options to manage volunteer GR canola in GR sugar beet.

Research Directions

After collecting seeds of four putative GR *K. scoparia* accessions during fall 2012 from chemical-fallow fields of Hill and Liberty Counties in northern Montana, One of the objectives of the research presented here was to confirm and determine the levels of glyphosate resistance, and to test the effectiveness of alternative herbicides on those accessions. This was done in chapter 2, where appropriate methods of herbicide resistance screening commonly used in literature such as glyphosate discriminate dose and whole plant dose-response experiments were employed. Efficacy of alternative herbicides (other than glyphosate) labeled for wheat-fallow rotations were also tested on those GR *K. scoparia* accessions under greenhouse conditions. Using a nonlinear 4 parameters log-logistic model for percent control and shoot dry weight reduction with

increasing doses of glyphosate, levels of glyphosate resistance in those GR *K. scoparia* accessions were determined.

In chapter 3, the resistance to ALS-inhibitors was also confirmed in GR *K. scoparia* accessions with whole plant dose-response experiments. Using previously developed molecular techniques (PCR, RT-PCR, and western blot) and protocols, the mechanism of glyphosate resistance was elucidated in GR *K. scoparia* accessions. Chapter 3 also investigated the mechanism of resistance to ALS inhibitors in those multiple herbicide-resistant *K. scoparia* accessions.

Experimentally, chapter 4 was focused on understanding the pleiotropic effects of the EPSPS gene amplification on fitness cost in GR *K. scoparia* accessions. Inbreds from glyphosate-susceptible (SUS) and GR *K. scoparia* accessions were generated following previously established protocols. Intraspecific plant competition studies were conducted to determine the effect of EPSPS gene amplification on fitness of GR *K. scoparia*. The relationship between EPSPS gene copies and glyphosate resistance level was also investigated. Using pairwise correlation analysis between EPSPS copy number and fitness traits and 2 parameters log-logistic nonlinear model for binomial distribution, main objectives of this chapter were addressed.

Chapters 5 and 6 were mainly focused on the management aspects of *K. scoparia* under field conditions. In chapter 5, several preemergence (PRE) and postemergence (POST) herbicides labeled for wheat, barley, corn, sorghum, soybean, and chemical-fallow (standalone, pre-mixtures or tank-mixtures) were tested for *K. scoparia* management. Chapter 6 described the effectiveness of various late-season applied POST

herbicides in wheat stubble on *K. scoparia* control, biomass, fecundity, and progeny fitness.

Chapter 7 of this research was allocated to determine the herbicide options for managing volunteer GR canola in GR sugar beet. PRE and POST herbicides labeled in GR sugar beet with different use rates were investigated on percent control and seed production of volunteer GR canola. The ultimate impact of these herbicide treatments on GR sugar beet yields were also determined.

In chapter 8, the influence of glyphosate application timing (s) on *K. scoparia* demographics were investigated in GR sugar beet. Using well developed methodology in these field experiments, survival of multiple *K. scoparia* cohorts was monitored in response to glyphosate application timing (s) in GR sugar beet. The ultimate impact of various glyphosate timing (s) on fecundity of *K. scoparia* cohorts as well as GR sugar beet yields was also determined.

This research work mainly represents the first confirmation of GR *K. scoparia* accessions in Montana, and provide molecular details on the mechanisms of resistance to glyphosate as well as ALS-inhibitors in those accessions. This research also highlights the absence of any pleiotropic effects of the EPSPS gene amplification on GR *K. scoparia*. Importantly, numerous PRE and POST herbicide options (alternative to glyphosate) for management of GR *K. scoparia* have been investigated in this research. Experimentally, the demographic response of various *K. scoparia* cohorts to different glyphosate timing has also been demonstrated here. In addition, this research provide insights on the availability of herbicide tools for management of volunteer GR canola in

GR sugar beet. Overall, this research work provides useful information to extension education programs for Montana growers, and also lay a foundation for future studies on the biology, ecology, and management of GR *K. scoparia* on regional level.

References

Anderson RL, Nielsen DC (1996) Emergence pattern of five weeds in the central Great Plains. Weed Technol. 10:744–749

Bagavathiannan, M.V., Norsworthy, J.K., 2012. Late-season seed production in arable weed communities: management implications. Weed Sci. 60, 325–334.

Baker DV, Withrow JR, Brown CS, Beck, KG (2010) Tumbling: use of diffuse knapweed (*Centaurea diffusa*) to examine an understudied dispersal mechanism. Invasive Plant Sci Manag 3:301–309

Baucom RS, Mauricio R (2004) Fitness costs and benefits of novel herbicide tolerance in a noxious weed. Proc Natl Acad Sci USA. 101:13386–13390

Beckie, HJ, Swartz GS, Nair HK, Warwick SI, Johnson E (2004) Multiple herbicide–resistant canola can be controlled by alternative herbicides. Weed Sci 52:152–157

Beckie HJ (2006) Herbicide-resistant weeds: Management tactics and practices. Weed Technol 20:793-814

Beckie, HJ, Blackshaw RE, Low R, Hall LM, Sauder CA, Martin S, Brandt EN, Shirriff SW (2013) Glyphosate- and acetolactate synthase inhibitor-resistant kochia (*Kochia scoparia*) in Western Canada. Weed Sci 61:310–318

Bennett, A.C., Shaw, D.R., 2000. Effect of preharvest desiccants on weed seed production and viability. Weed Technol. 14, 530–538.

Christoffoleti PJ, Westra PB, Moore F (1997) Growth analysis of sulfonylurea-resistant and -susceptible kochia (*Kochia scoparia*). Weed Sci 45:691–695

Cranston HJ, Kern AJ, Hackett JL, Miller EK, Maxwell BD, Dyer WE (2001) Dicamba resistance in kochia. Weed Sci 49:164–170

Dille JA, Stahlman PW, Geier PW, Riffel JD, Currie RS, Wilson RG, Sbatella GM, Westra P, Kniss AR, Moechnig MJ, Cole RM (2012) Kochia emergence profiles across the central Great Plains. Proc Weed Sci Soc Am 52:122

Donald WW, Prato T (1991) Profitable, effective herbicides for planting-time weed control in no-till spring wheat (*Triticum aestivum*). Weed Sci 39:83–90

Duke SO, Powles SB (2008) Glyphosate: a once-in-a-century herbicide. Pest Manag Sci 64:319–325

Durgan BR, Dexter AG, Miller SD (1990) Kochia (*Kochia scoparia*) interference in sunflower (*Helianthus annuus*). Weed Technol 4:52–56.

Evetts, LL, Burnside OC (1972) Germination and seedling development of common milkweed and other species. Weed Sci. 20:371–378

Fenster CR, Wicks GA (1982) Fallow systems for winter wheat in western Nebraska. Agron J 74:9–13

Friesen LF, Beckie HJ, Warwick SI, Van Acker RC (2009) The biology of Canadian weeds. 138. *Kochia scoparia* (L.) Schrad. Can J Plant Sci 89:141–167

Gaines TA, Zhang W, Wang D, Bukun B, Chisholm ST, Shaner DL, Nissen SJ, Patzoldt WL, Tranel PJ, Culpepper AS, Grey TL, Webster TM, Vencill WK, Sammons RD, Jiang JM, Preston C, Leach JE, Westra P (2010) Gene amplification confers glyphosate resistance in *Amaranthus palmeri*. Proc Natl Acad Sci 107:1029–1034

Giacomini D, Westra P, Ward SM (2014) Impact of genetic background in fitness cost studies: an example from glyphosate-resistant Palmer amaranth. Weed Sci 62: 29–37

Heap I (2015) The International Survey of Herbicide Resistant Weeds. http://www.weedscience.org. Accessed: Mar 14, 2015

Jenks BM, Willoughby GP, Markle DM (2006) Volunteer canola control in corn, soybean, sunflower, dry pea, and flax. Abstract 24-4 *in* Proceedings of the ASA-CSSA-SSSA International Annual Meetings. US Canola Association. Indianapolis, IN: American Society of Agronomy/Crop Science Society of Agronomy/Soil Science Society of Agronomy

Jha P, Morishita DW, Felix J, Kumar V, Flenniken M (2015) Confirmation of glyphosate-resistant kochia in Idaho and Oregon. Proc Weed Sci Soc Am 104

Jha, P., Norsworthy, J.K., 2012. Influence of late-season herbicide applications on control, fecundity, and progeny fitness of glyphosate-resistant palmer amaranth (*Amaranthus palmeri*) biotypes from Arkansas. Weed Technol. 26, 807–812.

Kumar V, Jha P, Reichard N (2014) Occurrence and characterization of kochia (*Kochia scoparia*) accessions with resistance to glyphosate in Montana. Weed Technol 28:122–130

Légère A, Simard MJ, Johnson E, Stevenson EC, Beckie H, Blackshaw RE (2006) Control of volunteer canola with herbicides; effects of plant growth stage and cold acclimation. Weed Technol 20:485–493

Lenssen AW, Waddell J, Johnson GD, Carlson GR (2007) Diversified cropping systems in semiarid Montana: Nitrogen use during drought. Soil Tillage Res 94:362–375

Lloyd KL, Johnson JM, Gover AE, Sellmer JC (2011) Preemergence and postemergence suppression of kochia on rights-of-way. Weed Technol 25:292–297

Lorentz L, Gaines TA, Nissen SJ, Westra P, Strek HJ, Dehne HW, Ruiz-Santaella JP, Beffa R (2014) Characterization of glyphosate resistance in *Amaranthus tuberculatus* populations. J Agric Food Chem 62:8134–8142

Manthey FA, Hareland GA, Zollinger RK, Huseby DJ (1996) Kochia interference with oat (*Avena sativa*). Weed Technol 10:522–525

Mickelson JA, Bussan AJ, Davis ES, Hulting AG, Dyer WE (2004) Postharvest kochia (*Kochia scoparia*) management with herbicides in small grains. Weed Technol 18:426-431

Mengistu LW, Messersmith CG (2002) Genetic diversity of kochia. Weed Sci 50:498–503

Mesbah, A, Miller SD, Fornstrom KJ, Legg DE (1994) Kochia (*Kochia scoparia*) and green foxtail (*Setaria viridis*) interference in sugar beets (*Beta vulgaris*). Weed Technol 8:754-759

Moyer JR, Roman ES, Lindwall CW, Blackshaw RE (1994) Weed management in conservation tillage systems for wheat production in North and South America. Crop Prot 4:243–259

Nandula VK (Ed) (2010) Glyphosate Resistance in Crops and Weeds. Wiley Inc.

Nandula VK, Manthey FA (2002) Response of kochia (*Kochia scoparia*) inbreds to 2,4-D and dicamba. Weed Technol 16:50-54

Norsworthy, JK, Ward SM, Shaw DR, Llewellyn RS, Nichols RL, Webster TM, Bradley KW, Frisvold G, Powles SB, Burgos NR, Witt WW, Barrett M (2012) Reducing the risks of herbicide resistance: Best management practices and recommendations. Weed Sci 60:31-62

Nandula VK, Wright A, Molin W, Ray J, Bond J, Eubank T (2014) *EPSPS* amplification in glyphosate-resistant spiny amaranth (*Amaranthus spinosus*): a case of gene transfer via interspecific hybridization from glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*). Pest Manag Sci 70:1902-1909

Pederson BP, Neve P, Andreasen C, Powles SB (2007) Ecological fitness of a glyphosate-resistant Lolium rigidum population: growth and seed production along a competition gradient. Basic Appl Ecol 8:258–268

Powles SB (2010) Gene amplification delivers glyphosate-resistant weed evolution. Proc Natl Acad Sci 107:955–956

Primiani MM, Cotterman JC, Saari LL (1990) Resistance of *Kochia scoparia* to sulfonylurea and imidazolinone herbicides. Weed Technol 4:169–172

Preston C, Belles DS, Westra PH, Nissen SJ, Ward SM (2009) Inheritance of resistance to the auxinic herbicide dicamba in kochia (*Kochia scoparia*). Weed Sci 57:43–47

Rainbolt CR, Thill DC, Young FL (2004) Control of volunteer herbicide-resistant wheat and canola. Weed Technol 18:711–718

Salas RA, Dayan FE, Pan Z, Watson SB, Dickson JW, Scott RC, Burgos NR (2012) *EPSPS* gene amplification in glyphosate-resistant Italian ryegrass (*Lolium perenne* ssp. multiflorum) from Arkansas. Pest Manag Sci 68:1223–1230

Sammons DR, Gaines TA (2014) Glyphosate resistance: state of knowledge. Pest Manag Sci 70:1367–1377

Schwinghamer TD, Van Acker RC (2008) Emergence timing and persistence of kochia (*Kochia scoparia*). Weed Sci. 56:37-41

Stahlman PW, Olson BLS, Thompson, CR, Zollinger RK (2010) Pyroxasulfone (KIH-485) for weed control in sunflower. Pages 1-7 *in* Proc of the 1st Australian Summer Grains Conference. Gold Coast, Australia

Stallings GP, Thill DC, Mallory-Smith CA, Shafii B (1995) Pollen-mediated gene flow of sulfonylurea-resistant kochia (*Kochia scoparia*). Weed Sci 43:95–102

Tonks DJ, Westra P (1997) Control of sulfonylurea-resistant kochia (*Kochia scoparia*). Weed Technol 11:270-276

Udeigwe TK, Teboh JM, Eze PN, Stietiya MH, Kumar V, Hendrix J, Mascagni HJ, Ying T, Kandakji T (2015) Implications of leading crop production practices on environmental quality and human health. J Environ Manage 151: 267–279

Van horn CR, Westra P (2014) Investigating the mechanism of resistance in nineteen giant ragweed accessions. Proc Weed Sci Soc Am 92

Vila-Aiub MM, Neve P, Powles SB (2009) Fitness costs associated with evolved herbicide resistance alleles in plants. New Phytol 184: 751–767

Vila-Aiub MM, Goh SS, Gaines TA, Han H, Busi R, Yu Q, Powles SB (2014) No fitness cost of glyphosate resistance endowed by massive EPSPS gene amplification in *Amaranthus palmeri*. Planta. 239:793–801

Vila-Aiub MM, Gundel PE, Preston C (2015) Experimental methods for estimation of plant fitness costs associated with herbicide-resistance genes. Weed Sci SI: 203–216

Waite J, Thompson CR, Peterson DE, Currie RS, Olson BLS, Stahlman PW, Khatib KA (2013) Differential kochia (*Kochia scoparia*) populations response to glyphosate. Weed Sci 61:193–200

Wang W, Xia H, Yang X, Xu T, Si HJ, Cai XX, Wang F, Su J, Snow AA, Lu BR (2013) A novel 5-enolpyruvoylshikimate-3-phosphate (EPSP) synthase transgene for glyphosate resistance stimulates growth and fecundity in weedy rice (Oryza sativa) without herbicide. New Phytol 202: 679–688

Weatherspoon DM, Schweizer EE (1969) Competition between kochia and sugarbeets. Weed Sci 17:464–467

Wicks GA, Martin AR, Mahnken GW (1993) Control of triazine-resistant kochia (*Kochia scoparia*) in conservation tillage corn (*Zea mays*). Weed Sci 41:225–231

Wicks GA, Martin AR, Haack AE, Mahnken GW (1994) Control of triazine-resistant kochia (*Kochia scoparia*) in sorghum (*Sorghum bicolor*). Weed Technol 8:748–753

Wiersma AT, Gaines TA, Preston C, Hamilton JP, Giacomini D, Buell CR, Leach JE, Westra P (2015) Gene amplification of 5-enol-pyruvylshikimate-3-phosphate synthase in glyphosate-resistant *Kochia scoparia*. Planta 241:463–474

Yu Q, Jalaludin A, Han H, Chen M, Sammons RD, Powles SB (2015) Evolution of double amino acid substitution in the EPSP synthase in Eleusine indica conferring high level glyphosate resistance. Plant Physiol. doi: http://dx.doi.org/10.1104/pp.15.00146

Zorner PS, Zimdahl RL, Schweizer EE (1984) Effect of depth and duration of seed burial on kochia (*Kochia scoparia*). Weed Sci. 32:602-607

CHAPTER 2

OCCURRENCE AND CHARACTERIZATION OF KOCHIA (*KOCHIA SCOPARIA*) ACCESSIONS WITH RESISTANCE TO GLYPHOSATE IN MONTANA

Contribution of Authors and Co-Authors

Manuscript in Chapter 2

Author: Vipan Kumar

Contributions: Conceived and implemented the study design. Conducted all greenhouse experiments, reviewed the literature, and transcribed manuscript.

Co-Author: Prashant Jha

Contributions: Advisement and guidance

Co-Author: Nicholas Reichard

Contributions: Assisted in seed collection, experimentation, and data collection

Manuscript Information Page

Vipan Kumar, Prashant Jha, Nicholas Reichard
Weed Technology Journal
Status of Manuscript:
____Prepared for submission to a peer-reviewed journal
____Officially submitted to a peer-review journal
____Accepted by a peer-reviewed journal
__×__Published in a peer-reviewed journal

Published by Allen Press, Inc.
Published in 2014, Volume 28, Issue 1

Abstract

Herbicide-resistant kochia is an increasing concerns for growers in the northwestern US. Four suspected glyphosate-resistant (Gly-R) kochia accessions (referred to as GIL01, JOP01, CHES01, and CHES02) were evaluated that were collected from four different chemical-fallow fields in northern Montana in fall of 2012. 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 level of resistance relative to the Gly-S accession, based on percent control ratings (I_{50} values). Based on shoot dry weight response (GR_{50} values), the four Gly-R kochia accessions exhibited 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 ≥ 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: Glyphosate; *Kochia scoparia* (L.) Schrad.

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

Introduction

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 yrs) in soil and exhibit low dormancy, with a majority (> 90%) of non-dormant seeds that are lying at or near the soil surface following dispersal in fall emerge 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 non-cropland areas of the semi-arid 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 US, 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], where it can reduce crop yields up to 95% (Durgan et al. 1990; Mesbah et al. 1994; Weatherspoon and Schweizer 1969; Wicks et al. 1993, 1994). Also,

kochia 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, 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 and/or tillage (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, prior to crop planting (burndown), or post-harvest (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 prior to winter wheat planting. During summer of 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. Gly-R kochia was first reported in western Kansas in 2007, and

recently being found in six other states in the northwestern US, 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 non-cropland areas of the region.

Furthermore, occurrence of kochia accessions resistant to other herbicide chemistries is a serious concern for growers in north central and western US (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 the 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 of 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 from 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 from each other, and the putative Gly-R accessions were referred to as CHES01 and CHES02. The 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 of 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 media (VermiSoil^{TM}, Vermicrop Organics, 4265 Duluth Ave, Rocklin, CA). Single kochia seedlings were transplanted into 10-cm dia pots containing the same potting media as the germination trays. Experiments were arranged in a randomized complete block design with 8 replications (1 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% (w/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. Following 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 to 100, 0 being no injury/control and 100 being 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 percent of the nontreated control.

Alternative POST Herbicides. Based on the 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 6 replications (one plant per pot) for each herbicide and accession combination, and repeated at least once. Herbicide treatments included: bromoxynil + MCPA (Bronate Advanced™, 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 (Huskie™, Bayer CropScience, Research Triangle Park, NC) at 0.24 + 0.03 kg ai ha^{-1}; glufosinate (Liberty®, Bayer CropScience, Research Triangle Park, NC) 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, Tessenderlo Kerley Inc., Phoenix, AZ) at 0.84 kg ai ha^{-1}; carfentrazone-ethyl + 2,4- D (Rage™ D-Tech, FMC Corp., Philadelphia, PA) at 0.03 + 1.66 kg ai ha^{-1}; saflufenacil (Sharpen®, BASF Corp., 26 Davis Drive, Research Triangle Park, NC) 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 (NIS) at 0.25% (v/v) was added to diflufenzopyr + dicamba + 2, 4-D, bromoxynil + pyrasulfotole, paraquat, paraquat plus 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. Ammonium sulfate (AMS) at 2% (w/v) was added to saflufenacil, saflufenacil + 2, 4-D, and glufosinate treatments. All herbicides were applied at their field-use rates for wheat and/or chemical-fallow. 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 percent 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 (Statistical Analysis Systems®, 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 (% of nontreated) using PROC UNIVARIATE in SAS, which revealed that the distribution of residuals met the criteria for ANOVA. For both the studies, data were pooled across experiment runs based on non-significant experiment run by treatment interaction, and tested for homogeneity of variance.

For the whole-plant dose-response experiments, arcsine-transformation of percent control and shoot dry weight (% of nontreated) data did not improve the results, and 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 percent 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 utilizing *drc* package in *R* software (Knezevic et al. 2007). Based on I_{50} or GR_{50} values, resistance index (referred as R/S ratio) for each kochia accession was calculated by dividing the I_{50} or GR_{50} value of a Gly-R accession by the I_{50} or GR_{50} value of the Gly-S accession. Independent t tests were performed to compare whether the I_{50} or GR_{50} values between accessions were significantly different at $\alpha = 0.05$.

For the alternative POST herbicide experiments, percent control and shoot dry weight (% of nontreated) data were arcsine-transformed before analysis to improve homogeneity of variance and normality of residuals. Non-transformed means are

presented in tables based on the interpretations from 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). While 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. Based on visually assessed percent control, I_{50} 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 2.1; Figure 2.1). Additionally, the I_{90} 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. Based on visually assessed percent control response (I_{50} values), the four Gly-R kochia accessions exhibited R/S ratios of 7.1 to 11.0.

Based on shoot-dry-weight dose response, GR_{50} 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 2.1; Figure 2.2). Furthermore, GR_{90} 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. Based on GR_{50} values, the four Gly-R accessions had R/S ratios of 4.6 to 8.1. This further suggests 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 higher I_{50} or GR_{50} value compared with GIL01, CHES01 and CHES02 accessions (Table 2.2). 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 GR_{50} values or 5.4 to 6.9 based on I_{50} 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, Kansas, had an R/S ratio of 3.7 or 5.8 based on 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 I_{50} values (based on visually assessed percent control) ranging from 470 to 2149 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 2.3). 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, and glufosinate, with less control of GIL01 compared with Gly-S accession. Furthermore, control with bromoxynil + pyrasulfotole (75% control) did not differ from 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 vs. 100% control. Control with dicamba, diflufenzopyr + dicamba + 2,4-D averaged 79%, which did not differ from saflufenacil alone or with 2,4-D, or bromoxynil + fluroxypyr. Unlike Gly-S or GIL01

accession, control of 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 (% of nontreated) 21 DAA. For 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 2.3). 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 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 GIL01 accession, glufosinate was not effective on 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 (BMPs) (Norsworthy et al. 2012). The success of those efforts to control Gly-R kochia will depend on the effectiveness of alternative herbicides. Based on 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.

Acknowledgements

The authors would like to thank Merlin Johnson (crop consultant), Jesse Fulbright (MSU extension agent), and growers at Hill and Liberty County, MT, for contacting us to investigate those suspected resistant kochia accessions and for their valuable assistance in sample collection.

Table 2.1. Regression parameters (Equation 1) for whole-plant dose response based on visual assessment of percent control and shoot dry weight (percent of nontreated) of kochia accessions from Montana treated with glyphosate.

	Regression parameters (±SE)					
Accession[a]	*d*	*C*	*b*	I_{50} or GR_{50}	I_{90} or GR_{90}	R/S[b]
Based on percent 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 wt.						
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 # 1, Chester, MT; CHES02, Gly-R accession # 2, Chester, MT; I_{50} and GR_{50} are effective doses (kg ae ha^{-1}) of glyphosate required for 50% control and shoot dry weight reduction, respectively; I_{90} and GR_{90} 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 I_{50} or GR_{50} of a Gly-R accession to I_{50} or GR_{50} of the Gly-S accession.

Table 2.2. Independent *t* tests of I_{50} (based on visual assessment) and GR_{50} (based on shoot dry weight) values from the whole-plant dose response of kochia accessions from Montana[a]

	Based on visual control		Based on dry weight	
Accessions	I_{50}	P > *F*	GR_{50}	P > *F*
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

[a]Abbreviations: Gly-S, glyphosate-susceptible accession, Huntley, MT; GIL01, Gly-R accession, Gildford, MT; JOP01, Gly-R accession, Joplin, MT; CHES01, Gly-R accession # 1, Chester, MT; CHES02, Gly-R accession # 2, Chester, MT; vs, versus; 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.

Table 2.3. Percent control and shoot dry weight (percent of nontreated) of three kochia accessions from Montana 21 d after treatment with various POST herbicides[a,b].

		Control[f]						Shoot dry weight[f]					
Herbicide(s)	Rate(s)	Gly-S		GIL01		JOP01		Gly-S		GIL01		JOP01	
	kg ae or ai ha^{-1}	%						% of nontreated					
Bromoxynil + MCPA	0.56 + 0.56	100 a	A	77 c	B	60 f	C	6 e	C	17 c	B	50 a	A
Dicamba	0.28	80 c	A	80 bc	A	78 cd	A	29 a	A	26 b	A	32 b	A
Diflufenzopyr + dicamba + 2,4-D[c]	0.02 + 0.07 + 0.28	87 b	A	85 b	A	80 bcd	A	23 b	A	25 b	A	30 bc	A
Bromoxynil + pyrasulfotole[c]	0.24 + 0.03	97 a	A	75 c	B	67 ef	B	8 de	C	17 c	B	24 cd	A
Glufosinate[e]	0.59	70 d	A	43 d	B	45 g	B	15 c	B	41 a	A	43 a	A
Paraquat[c]	0.70	100 a	A	100 a	A	88 b	B	6 e	B	9 d	B	15 ef	A
Paraquat + linuron[c]	0.70 + 0.84	100 a	A	100 a	A	100 a	A	5 e	A	8 d	A	10 f	A
Carfentrazone-ethyl + 2,4-D[c]	0.03 + 1.66	100 a	A	100 a	A	72 de	B	11 d	B	9 d	B	29 bc	A
Saflufenacil[d,e]	0.02	88 b	B	100 a	A	77 cd	C	16 c	B	9 d	C	23 cd	A
Saflufenacil + 2,4-D[d,e]	0.02 + 0.28	100 a	A	100 a	A	78 cd	B	5 e	B	8 d	B	25 bcd	A
Bromoxynil + fluroxypyr[c]	0.55 + 0.14	100 a	A	99 a	A	82 bc	B	8 de	A	12 d	A	18 de	A

[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] Nonionic surfactant (NIS) at 0.25% v/v was included.

[d] Methylated seed oil (MSO) at 1% v/v was included.

[e] Ammonium sulfate (AMS) at 2 % w/v was included.

[f] For percent control or shoot dry weight data, means for a kochia accession within a column followed by similar lower-case 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 upper-case letters are not significantly different based on Fisher's Protected LSD test at $P < 0.05$.

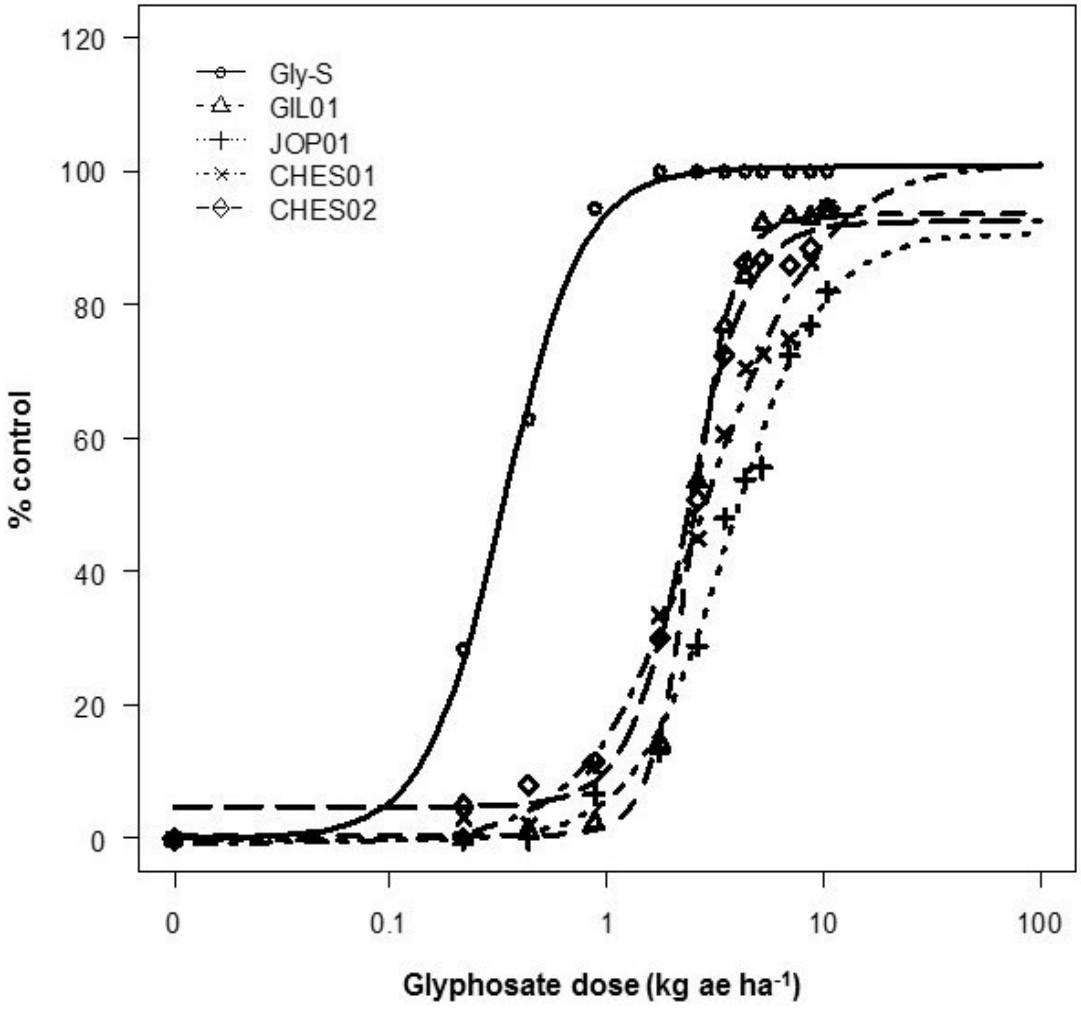

Figure 2.1. Control response based on visual assessment of four Gly-R kochia accessions (GIL01 from Gildford, MT; JOP01 from Joplin, MT; CHES01 and CHES02 from Chester, MT) and a glyphosate-susceptible accession (Gly-S from Huntley, MT) in a whole-plant dose-response experiment.

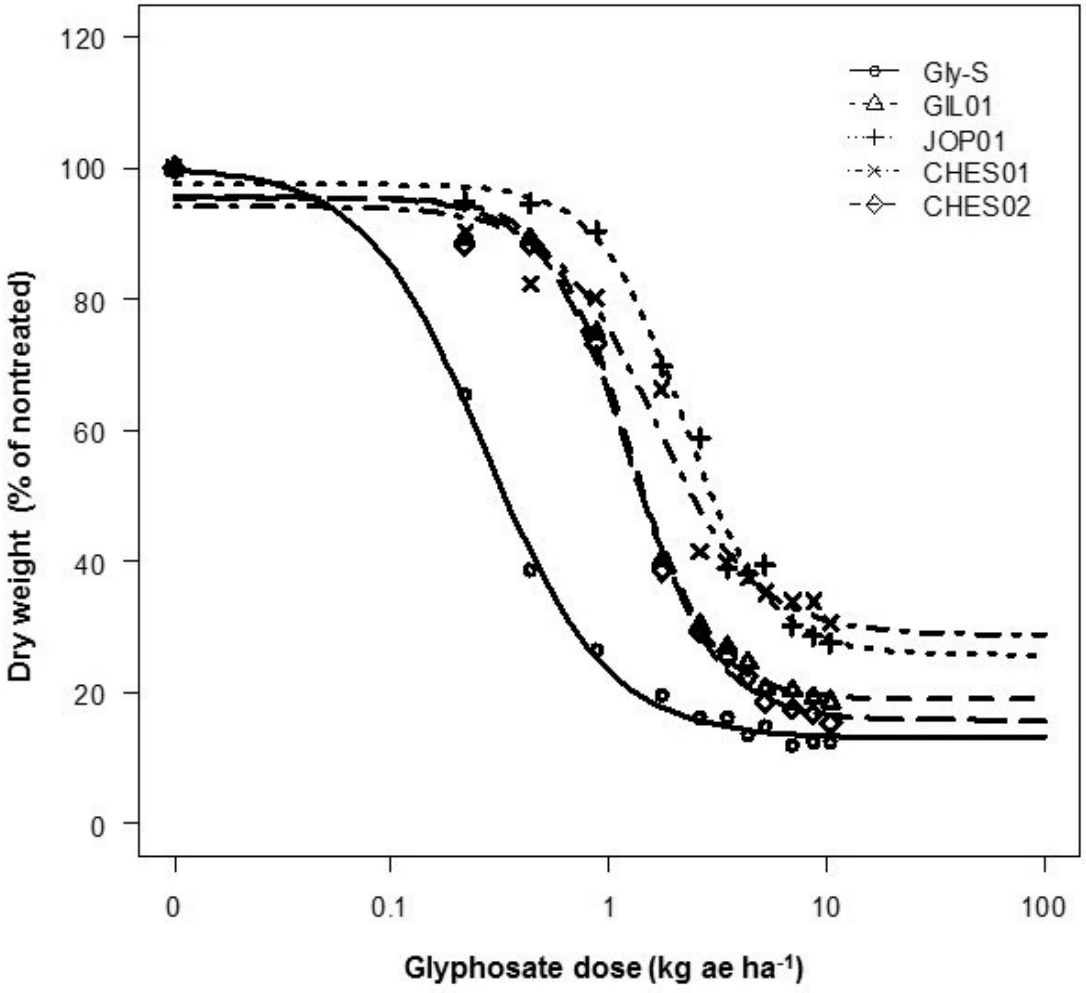

Figure 2.2. Shoot dry weight response of four Gly-R kochia accessions (GIL01 from Gildford, MT; JOP01 from Joplin, MT; CHES01 and CHES02 from Chester, MT) and a glyphosate-susceptible accession (Gly-S from Huntley, MT) in a whole-plant dose-response experiment.

Literature Cited

Anderson, R. L. and D. C. Nielsen. 1996. Emergence pattern of five weeds in the central Great Plains. Weed Technol. 10:744–749.

Baker, D. V., J. R. Withrow, C. S. Brown, and K. G. Beck. 2010. Tumbling: Use of diffuse knapweed (*Centaurea diffusa*) to examine an understudied dispersal mechanism. Invasive Plant Sci. Manage. 3:301-309.

Beckie, H. J., R. E. Blackshaw, R. Low, L. M. Hall, C. A. Sauder, S. Martin, E. N. Brandt, and S. W. Shirriff. 2013. Glyphosate- and acetolactate synthase inhibitor-resistant kochia (*Kochia scoparia*) in Western Canada. Weed Sci. 61:310-318.

Christoffoleti P. J., Westra P. B. and Moore F. 1997. Growth analysis of sulfonylurea-resistant and -susceptible kochia (*Kochia scoparia*). Weed Sci. 45:691–695.

Cranston, H. J., A. J. Kern, J. L. Hackett, E. K. Miller, B. D. Maxwell, and W. E. Dyer. 2001. Dicamba resistance in kochia. Weed Sci. 49:164–170.

Donald, W. W. and T. Prato. 1991. Profitable, effective herbicides for planting-time weed control in no-till spring wheat (*Triticum aestivum*). Weed Sci. 39:83–90.

Durgan, B. R., A. G. Dexter, and S. D. Miller. 1990. Kochia (*Kochia scoparia*) interference in sunflower (*Helianthus annuus*). Weed Technol. 4:52–56.

Eberlin, C. V. and Z. A. Fore. 1984. Kochia biology. Weeds Today. 15:5–6.

Evetts, L. L. and O. C. Burnside. 1972. Germination and seedling development of common milkweed and other species. Weed Sci. 20:371–378.

Fenster, C. R. and G. A. Wicks. 1982. Fallow systems for winter wheat in western Nebraska. Agron. J. 74:9-13.

Forcella, F. 1985. Spread of kochia in the northwestern United States. Weeds Today. 16:4–6.

Friesen, L. F., H. J. Beckie, S. I. Warwick, and R. C. Van Acker. 2009. The biology of Canadian weeds. 138. *Kochia scoparia* (L.) Schrad. Can. J. Plant Sci. 89:141–167.

Heap, I. 2013. International Survey of Herbicide Resistant Weeds. www.weedscience.com Accessed: June 09, 2013.

Knezevic, S. Z., J. C. Streibig, and C. Ritz. 2007. Utilizing *R* software package for dose-response studies: The concept and data analysis. Weed Technol. 21: 840-848.

Leeson, J. Y., A. G. Thomas, L. M. Hall, C. A. Brenzil, T. Andrews, K. R. Brown, and R. C. Van Acker. 2005. Prairie Weed Surveys of Cereal, Oilseed and Pulse Crops from the 1970s to the 2000s. Saskatoon, Canada: Agriculture and Agri-Food Canada Weed Survey Series Publ. 05-1. 395p.

Lenssen, A. W., J. Waddell, G. D. Johnson, and G. R. Carlson. 2007. Diversified cropping systems in semiarid Montana: Nitrogen use during drought. Soil Tillage Res. 94:362-375.

Lloyd, K. L., J. M. Johnson, A. E. Gover, and J. C. Sellmer. 2011. Preemergence and postemergence suppression of kochia on rights-of-way. Weed Technol. 25:292–297.

Mengistu, L. W. and C. G. Messersmith. 2002. Genetic diversity of kochia. Weed Sci. 50: 498-503.

Mesbah, A., S. D. Miller, K. J. Fornstrom, and D. E. Legg. 1994. Kochia (*Kochia scoparia*) and green foxtail (*Setaria viridis*) interference in sugar beets (*Beta vulgaris*). Weed Technol. 8:754-759.

Mickelson, J. A., A. J. Bussan, E. S. Davis, A. G. Hulting, and W. E. Dyer. 2004. Postharvest kochia (*Kochia scoparia*) management with herbicides in small grains. Weed Technol. 18:426-431.

Moyer, J. R., E. S. Roman, C. W. Lindwall, and R. E. Blackshaw. 1994. Weed management in conservation tillage systems for wheat production in North and South America. Crop Prot. 4:243–259.

Nandula V. K. and F. A. Manthey. 2002. Response of kochia (*Kochia scoparia*) inbreds to 2,4-D and dicamba. Weed Technol. 16:50-54.

Norsworthy, J. K., S. M. Ward, D. R. Shaw, R. S. Llewellyn, R. L. Nichols, T. M. Webster, K. W. Bradley, G. Frisvold, S. B. Powles, N. R. Burgos, W. W. Witt, and M. Barrett. 2012. Reducing the risks of herbicide resistance: Best management practices and recommendations. Weed Sci. 60:31-62.

Preston, C., D. S. Belles, P. H. Westra, S. J. Nissen, and S. M. Ward. 2009. Inheritance of resistance to the auxinic herbicide dicamba in kochia (*Kochia scoparia*). Weed Sci. 57:43–47.

Primiani, M. M., J. C. Cotterman, and L. L. Saari. 1990. Resistance of *Kochia scoparia* to sulfonylurea and imidazolinone herbicides. Weed Technol. 4:169-172.

Schwinghamer, T. D. and R. C. Van Acker. 2008. Emergence timing and persistence of kochia (*Kochia scoparia*). Weed Sci. 56:37-41.

Seefeldt, S. S., J. E. Jensen, and E. P. Fuerst. 1995. Log-logistic analysis of herbicide dose-response relationships. Weed Technol. 19:218-227.

Stallings, G. P., D. C. Thill, C. A. Mallory-Smith, and B. Shafii. 1995. Pollen-mediated gene flow of sulfonylurea-resistant kochia (*Kochia scoparia*). Weed Sci. 43:95–102.

Tonks, D. J. and P. Westra. 1997. Control of sulfonylurea-resistant kochia (*Kochia scoparia*). Weed Technol. 11:270-276.

Waite, J., C. R. Thompson, D. E. Peterson, R. S. Currie, B. L. S. Olson, P. W. Stahlman, and K. A. Khatib. 2013. Differential kochia (*Kochia scoparia*) populations response to glyphosate. Weed Sci. 61:193-200.

Weatherspoon, D. M. and E. E. Schweizer. 1969. Competition between kochia and sugarbeets. Weed Sci. 17:464–467.

Westra, P., A. Wiersma, J. Leach, and A. Reddy. 2013. Regional whole plant and molecular response of kochia to glyphosate. Proc. Weed Sci. Soc. Am. 53:316.

Wicks, G. A., A. R. Martin, and G. W. Mahnken. 1993. Control of triazine-resistant kochia (*Kochia scoparia*) in conservation tillage corn (*Zea mays*). Weed Sci. 41:225–231.

Wicks, G. A., A. R. Martin, A. E. Haack, and G. W. Mahnken. 1994. Control of triazine-resistant kochia (*Kochia scoparia*) in sorghum (*Sorghum bicolor*). Weed Technol. 8:748–753.

Zorner, P. S., R. L. Zimdahl, and E. E. Schweizer. 1984. Effect of depth and duration of seed burial on kochia (*Kochia scoparia*). Weed Sci. 32:602-607.

CHAPTER 3

MOLECULAR BASIS OF EVOLVED RESISTANCE TO GLYPHOSATE AND ACETOLACTATE SYNTHASE-INHIBITOR HERBICIDES IN KOCHIA (*KOCHIA SCOPARIA*) ACCESSIONS FROM MONTANA

Contribution of Authors and Co-Authors

Manuscript in Chapter 3

Author: Vipan Kumar

Contributions: Conceived and implemented the study design. Conducted experiments, reviewed the literature, and wrote the manuscript.

Co-Author: Prashant Jha

Contributions: Helped conceived the study design, advisement and guidance, review the manuscript drafts

Co-Author: Darci Giacomini

Contributions: Technical assistance in lab experiments, provided field expertise

Co-Author: Eric Westra

Contributions: Technical assistance in lab experiments

Co-Author: Philip Westra

Contributions: Helped conceived the study design and advisement

Manuscript Information Page

Vipan Kumar, Prashant Jha, Darci Giacomini, Eric Westra, Philip Westra
Weed Science Journal
Status of Manuscript:
___Prepared for submission to a peer-reviewed journal
_×_Officially submitted to a peer-review journal
___Accepted by a peer-reviewed journal
___Published in a peer-reviewed journal

Published by Allen Press, Inc.
Submitted February 4, 2015

Abstract

The rapid evolution and spread of glyphosate-resistant (GR) kochia in the Northern Great Plains (NGP) is an increasing threat to GR cropping systems and conservation tillage practices common in this region. GR kochia accessions with 4.6- to 11-fold levels of resistance to glyphosate have recently been reported in Montana, USA. Those GR kochia accessions were also suspected resistant to acetolactate synthase (ALS)-inhibitors i.e. multiple herbicide-resistant (MHR) kochia. In this research, the level of resistance to the ALS-inhibitor herbicides (sulfonylureas) and the molecular mechanisms conferring resistance to glyphosate and ALS-inhibitor herbicides in MHR kochia was investigated. Based on whole-plant dose-response assays, MHR kochia accessions (GIL01, JOP01, and CHES01) were 9.3- to 30-fold more resistant to premixed thifensulfuron methyl + tribenuron methyl + metsulfuron methyl than the susceptible (SUS) accession. In an *in vivo* leaf-disk shikimate assay, MHR plants accumulated less shikimate than the SUS plants at a discriminate dose of 100 μM glyphosate. Sequencing of the conserved region of *EPSPS* revealed no target-site mutation at Thr_{102} or Pro_{106} residue. MHR kochia accessions had increased relative *EPSPS* gene copies (~ 4 to 10) compared with the SUS accession (single copy). Furthermore, MHR kochia accumulated higher EPSPS protein compared with the SUS plants. Resistance to the ALS-inhibitor herbicides was conferred by Pro_{197} amino acid substitution (proline to glutamine). *EPSPS* gene amplification and a single target-site mutation at Pro_{197} in *ALS* gene confer resistance to glyphosate and ALS-inhibitor herbicides, respectively, in MHR kochia

accessions from Montana. This is the first confirmation of occurrence of MHR kochia in Montana.

Nomenclature: Glyphosate; metsulfuron methyl; thifensulfuron methyl; tribenuron methyl; Kochia, *Kochia scoparia* (L.) Schrad.

Keywords: *EPSPS* gene amplification, *ALS* gene mutation, multiple herbicide resistance, glyphosate resistance, ALS-inhibitor resistance.

Short title: Multiple herbicide-resistant kochia

Introduction

Glyphosate [*N*-(phosphonomethyl) glycine] is a systemic, nonselective, postemergence herbicide, widely used for weed control in cropland and non-cropland worldwide. Glyphosate has been described as once-in-a-century herbicide due to its broad-spectrum efficacy on weeds, low mammalian toxicity, and environmentally benign nature (Duke and Powles 2008). Glyphosate inhibits the biosynthesis of aromatic amino acids (phenylalanine, tyrosine, and tryptophan) by inhibiting the EPSPS (5-enolpyrovylshikimate-3-phosphate synthase, EC 2.5.1.19) enzyme in the shikimate pathway in plants, ultimately causing growth inhibition and plant death (Duke and Powles 2008; Powles 2010). Glyphosate usage has increased dramatically after the introduction of glyphosate-resistant (GR) crops in mid-1990s. In addition, this led to an increased adoption of conservation tillage by growers (Powles 2010). The exclusive use of glyphosate for weed control in GR cropping systems has been reported, with multiple applications per growing season (Norsworthy et al. 2007). Nevertheless, the increased selection pressure from glyphosate in GR crops resulted in evolution of glyphosate-resistant weed biotypes. To date, glyphosate resistance has been reported in 31 weed species globally (Heap 2015).

Kochia (*Kochia scoparia* L.), a monoecious C_4 diploid (2n = 18), is among the most problematic summer annual broadleaf weeds in the Northern Great Plains (NGP) of the United States, including Montana (Eberlein 1984; Forcella 1985; Friesen et al. 2009). Kochia possesses unique biological attributes, including early seedling emergence, rapid growth, tolerance to abiotic stresses (heat, salt, drought), prolific seed production (>

50,000 seeds plant^{-1}), low seed dormancy (1 to 2 yr), and long distance seed dispersal by the tumble behavior of the mature plants (Baker et al. 2010; Christoffoleti et al. 1997; Friesen et al. 2009; Schwinghamer and Van Acker 2008). Furthermore, the protogynous nature of kochia flowering enforces a high degree of out-crossing and pollen-mediated gene flow (Stallings et al. 1995; Mengistu and Messersmith 2002). Consequently, there is a high genetic diversity within and among kochia populations (Stallings et al. 1995). All these biological traits make kochia a difficult-to-control weed species, with rapid development and spread of herbicide resistance. Kochia has evolved resistance to several herbicide modes of action, including photosystem II (PS II) inhibitors (atrazine), acetolactate synthase (*ALS*) inhibitors (sulfonylurea and imidazolinone herbicides), and synthetic auxins (dicamba and fluroxypyr) in the NGP (Heap 2015). In 2007, kochia accessions resistant to glyphosate were first detected in Kansas (Waite et al. 2013). Since then, GR kochia has emerged in nine other states in the NGP, including Montana, and in three provinces of Canada (Beckie et al. 2013; Heap 2015; Kumar et al. 2014). The increased occurrence of GR kochia is a potential threat to GR cropping systems such as corn, soybean, and sugar beet, and to no-till wheat-fallow rotation common in this region.

Five major mechanisms of glyphosate resistance have been characterized in weed species so far, e.g. target-site *EPSPS* mutation, *EPSPS* gene amplification, active vacuolar sequestration, reduced translocation, and rapid necrosis response (Sammons and Gaines 2014). *EPSPS* gene amplification is a novel mechanism of glyphosate resistance, first documented in Palmer amaranth (*Amaranthus palmeri* S. Wats.) (Gaines et al. 2010). Increased *EPSPS* gene copies in GR Palmer amaranth was positively correlated with

EPSPS protein abundance (Gaines et al. 2010). This resistance mechanism has also emerged in other GR weed species, such as tall waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer] (Lorentz et al. 2014; Tranel et al. 2011), spiny amaranth (*Amaranthus spinosus*) (Nandula et al. 2014), and Italian ryegrass (*Lolium perenne* ssp. multiflorum) (Salas et al. 2012). Reduced absorption or translocation has not been associated with glyphosate resistance in GR kochia in the US Great Plains (Waite et al. 2013; Wiersma et al. 2014).

ALS-inhibitor-resistant kochia is widely spread across Montana and other NGP states, primarily through the selection of mutations in the *ALS* gene (Heap 2015; Primiani et al. 1990). In particular, three highly conserved amino acids ($Pro_{197,}$ $Asp_{376,}$ and Trp_{574}) in the ALS enzyme are sensitive to alteration by recurrent selection from ALS-inhibitor herbicides in kochia (Beckie et al. 2013; Tranel and Wright 2002; Tranel et al. 2015). An alteration in any of these amino acids confers high level of resistance to sulfonylurea and/or imidazolinone herbicides in kochia (Tranel and Wright 2002; Tranel et al. 2015).

Winter wheat-fallow rotation dominates > 90% of the dryland cropping systems of this region, including Montana. The average precipitation in Montana is < 30 cm, which is often a limiting factor for continuous cropping. The purpose of chemical fallow in wheat-fallow rotation is to prevent soil erosion and to conserve soil moisture for successful establishment of winter wheat planted in the fall (Lenssen et al. 2007). Glyphosate is the most commonly used herbicide to control kochia and other weeds in chemical fallow or in postharvest wheat stubble (Donald and Prato 1991; Kumar and Jha 2015a,b), and a typical chemical-fallow field receives three to four applications of

glyphosate (0.870 kg ae ha^{-1}) before planting of winter wheat in a year. Because of enhanced selection pressure from repeated use of glyphosate, GR kochia accessions with 4.6- to 11-fold levels of resistance to glyphosate have evolved in wheat-fallow fields in northern Montana (Kumar et al. 2014). With historical reports on widespread occurrence of kochia resistant to ALS inhibitors in Montana and other adjoining states (Primiani et al. 1990; Sivakumaran et al. 1993), these confirmed GR kochia biotypes from Montana were also suspected to be resistant to ALS-inhibitors (sulfonylurea) i.e. multiple herbicide-resistant (MHR) kochia. The objectives of this research were (1) to elucidate the mechanism conferring resistance to glyphosate and (2) to characterize the level of resistance to ALS-inhibitor herbicides (sulfonylureas) and determine if target-site mutation(s) in ALS might confer resistance to ALS-inhibitors in MHR kochia accessions from Montana.

Materials and Methods

Plant Material. Three GR kochia accessions (GIL01, JOP01, and CHES01) collected in fall of 2012 from chemical-fallow fields (wheat-fallow rotation) in Hill and Liberty Counties of northern Montana, USA, were investigated. The glyphosate-susceptible (SUS) kochia accession was collected from a field used for long-term organic trials near Huntley, Montana. Preliminary greenhouse dose-response experiments indicated that the SUS population was susceptible to glyphosate and ALS-inhibitor herbicides (data not shown). Plants from the dose-response study (Kumar et al. 2014) that survived at 1.68 kg ae ha^{-1} were transplanted into 5-L plastic pots containing a commercial potting mix

(VermiSoil™, Vermicrop Organics, 4265 Duluth Avenue, Rocklin, CA) in a greenhouse at the Montana State University, Southern Agricultural Research Center (MSU-SARC) near Huntley, MT. The greenhouse was maintained at 25/23 ± 3 C day/night temperatures and 16/8 h day/night photoperiods supplemented with metal halide lamps (400 μmol m^{-2} s^{-1}). Kochia plants were watered once daily to avoid moisture stress, and fertilized (Miracle-Gro water-soluble fertilizer [24-8-16], Scotts Miracle-Gro Products Inc., 14111ScottslawnRoad, Marysville, OH) once a week to maintain good growth. Kochia plants (60 to 70 cm tall) were covered with pollen bags (DelStar Technologies, Inc., 601 Industrial drive, Middletown, DE 19709) to avoid cross-pollination, and allowed to produce F1 seed. Two pollen bags (each of 50.8 × 45.7 cm size) were glued together lengthwise to fully cover each kochia plant. The F1 generation was selfed to obtain the F2 seed. Screening of F2 plants of the GR populations indicated that > 95% individuals survived the 1.68 kg ha^{-1} rate of glyphosate (data not shown). Similarly, F2 seeds of the SUS accession was obtained by selfing a group (to prevent inbreeding depression) of F1 plants under pollen isolation conditions as described previously, in a separate greenhouse. The F2 seeds of SUS and GR kochia accessions were used for subsequent experiments.

Shikimate Accumulation. Three plants per kochia accession (JOP01, GIL01, CHES01, and SUS) were tested for shikimate accumulation using an *in vivo* leaf-disk assay (Shaner et al. 2005). Three technical replicates (5-mm diam leaf disks) from each young kochia plant (8- to 10-cm tall) per accession were sampled, and the assay was repeated in time. Based on preliminary experiments, 100 μM glyphosate (discriminating dose) was chosen to differentiate the shikimate accumulation between GR and SUS kochia plants. The

excised leaf disks were placed into the wells of a 96-well microtiter plate containing 7.7 mM ammonium phosphate and glyphosate (molecular grade) at doses of 0, 100, or 1000 μM. The samples were incubated in light for 16 h at room temperature, frozen (- 20 C) and thawed (60 C), and subjected to the extraction procedure described by Shaner et al. (2005) and Wiersma et al. (2015). Shikimate levels were recorded at 380 nm on a 96-well plate reader (BioTek™ Synergy™ 2 Multi-Mode Microplate Reader, Winooski, VT). A shikimate standard curve was developed to quantify shikimate accumulation (ng shikimate μL^{-1}) in the experimental samples (Shaner et al. 2005). Experiments were conducted in a completely randomized design. Data were subjected to ANOVA using PROC MIXED in SAS 9.2 (SAS Institute, Inc., SAS Campus Dr., Cary, NC 27513). Means were separated using Fisher's Protected LSD test at $P < 0.05$. The interaction of experimental run with accession, glyphosate dose, or accession by glyphosate dose was nonsignificant; therefore, data were pooled over experimental runs.

Sequencing of *EPSPS* Thr$_{102}$ and Pro$_{106}$ codons. Three plants from each kochia accession were sampled, and the experiment was repeated in time. A 100-mg sample of young leaf tissue was flash frozen with liquid nitrogen and ground to fine powder. The genomic DNA (gDNA) extraction was performed using the Qiagen DNeasy Plant mini kit. The DNA quality and concentration was determined using a Bio-Rad SmartSpect™ Plus Spectrophotometer. The conserved region of *EPSPS* gene encompassing Pro$_{106}$ and Thr$_{102}$ codons were amplified by polymerase chain reaction (PCR). The primer set previously designed for Palmer amaranth *EPSPS* sequence (200-bp) were utilized (Gaines et al. 2010); the forward and reverse primer sequences were 5'-

ATGTTGGACGCTCTCAGAACT-3' and 5'-TGAATTTCCTCCA GCAACGGC-3', respectively. Each PCR reaction contained 10µL of GoTaq® Green Master Mix (containing *Taq*DNA polymerase, dNTPs, $MgCl_2$, and reaction buffers), 1 µL each of forward and reverse primer (5µM), and 5 µL of gDNA (2 ng ul^{-1}). The thermo-cycle conditions for PCR were: initial denaturation at 98 C for 1 min, followed by 30 cycles of denaturation at 98 C for 15 sec, primer annealing at 50 C for 30 sec, and product extension at 72 C for 45 sec. A final extension cycle of 10 min at 72 C was included, and the reaction was terminated and held at 4 C. PCR products were separated on 1% agarose gel stained with ethidium bromide, and isolated from the gel using the GENECLEAN® II Kit (MP Biochemical™) by following manufacturer's protocol. Sanger sequencing of purified PCR fragments was performed using an ABI™ 3130×L Genetic Analyzer. CLC genomics workbench software was utilized to analyze the sequence reads, and the *EPSPS* sequence of kochia samples was aligned to a reference glyphosate-susceptible Palmer amaranth *EPSPS* sequence to determine substitutions at Pro_{106} or Thr_{102} codon.

***EPSPS* Genomic Copy Number.** Real-time quantitative PCR (qPCR) experiments on genomic DNA were performed to determine relative *EPSPS:ALS* genomic copy number in the kochia accessions tested. Three plants per accession were sampled. The *ALS* gene was chosen as a reference gene because of the stability of *ALS* gene expression across kochia populations (Wiersma et al. 2015). For all samples, the *ALS*plant1:*ALS*plant2 relative gene copy number was equal to 1, also reported by Wiersma et al. (2015). The gDNA extracted for *EPSPS* gene sequencing was used for determining the gene copy number because of its high quality (260/280 ratio of ≥ 1.8). Primer sequences specific to

kochia *EPSPS* and *ALS* gene were similar to those previously reported (Wiersma et al. 2015). The primer set for *EPSPS* gene (*EPSPF1*: 5'-GGC CAA AAGGGCAATCGTGG AG-3' and *EPSPR1*: 5'-CATTGCCGTTCCCGCGTTTCC-3') produced approximately 102-bp product, and the *ALS* primer set (*ALS*F1: 5'-ATGCAGA CAATGTTGGATAC-3' and *ALS*R1: 5'-TCAACCATCG ATA CGAACAT-3') amplified 159-bp PCR products. Each qPCR reaction contained 2 µl of gDNA (2 ng ul^{-1}) template, 1X Perfecta SYBR Green Supermix, 250 nM each of forward and reverse primer (Giacomini et al. 2014). Each qPCR reaction was performed with a final reaction volume of 12.5 µl on a 96-well PCR plate. The qPCR thermo profiles were: 95 C for 15 min, 40 cycles of 95 C for 30 s, and 60 C for 1 min, followed by melt-curve analysis. Negative controls containing 10 µl of Mastermix, 250 nM each of forward and reverse primer, and 2.5 µl of HPLC water with no gDNA template were also included. Standard curves for each primer pair were developed using a 10-fold serial dilution of the genomic DNA, and primer efficiency was 102% for *EPSPS* and 101% for *ALS*. Threshold cycles (C_t) were recorded by the Bio-Rad CFX96™ Real Time System C 1000 Touch™ thermal cycler. The qPCR reaction efficiency was 102% (R^2 of 0.99 and a slope of -3.215). There was a linear relationship between C_t values and log DNA concentration, indicating that the C_t values were a reliable estimate of the relative *EPSPS* gene copy number. The relative *EPSPS* gene copy number was calculated as $2^{-\Delta Ct}$, where $\Delta C_T = C_t$, *EPSPS* – C_t, *ALS* (Gaines et al. 2010). Each sample was run in triplicate to calculate the mean and standard error of the increase in relative *EPSPS* copy number. Correlation analysis between relative *EPSPS* copy

number and shikimate accumulation or glyphosate resistance level (I_{50} or GR_{50} values) was performed using PROC CORR in SAS 9.2.

EPSPS Protein Abundance. To further confirm the *EPSPS* gene amplification in the GR kochia accessions, the relative abundance of the EPSPS protein was determined using a western blot. A protein assay was conducted by following a previously established protocol (Bolt et al. 1997) with slight modification. Three plants per kochia accession were sampled, and the experiment was repeated. Young leaf tissue (100 mg) was ground to fine powder in liquid nitrogen. Total protein were extracted using 1:4 ratio of leaf tissue to laemmli buffer (10% β-mercaptoethanol, 60% SDS (10% w/v), 20% glycerol, 10% H_2O). Protein samples were then boiled for 5 min and centrifuged for 5 min at 16, 900 x *g*. Total protein in 15 µL aliquots were loaded in each lane and resolved by 10% (w v^{-1}) sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) at 25 mA per gel for 1 h. Gel was stained with Ponceau S before the membrane transfer to visually confirm that each lane had the same amount of protein loaded. Protein of each sample was transferred to nitrocellulose membrane (Osmonics Nitrobind nitrocellulose transfer membranes, GE Water and Process Technologies, 4636 Somerton Road, Feasterville-Trevose, PA 19053) at 300 mA for approximately 1 h. The nitrocellulose membrane was blocked for 12 h at 4 C in TBST (20 mM Tris base [pH 7.5], 0.5 M NaCl, 0.05% Tween-20 (Sigma-Aldrich Co. LLC., 3050 Spruce Street, St. Louis, MO 63103) containing 5% milk powder. Membrane was incubated at 20 C for 1 h with primary EPSPS antibody (Monsanto Co., St. Louis, MO) that was diluted 1:2000 in TBST milk solution. Membrane was rinsed three times in TBST, and incubated at 20 C for 1 h with secondary

antibody (goat: rabbit) conjugated with horseradish peroxidase (ImmunoPure Antibody; Thermo Fischer) using a dilution of 1:5000 in TBST milk solution. Membrane was again rinsed three times in TBST milk solution, and an activator 24 solution (SuperSignal West Pico Chemiluminescent Substrate from Thermo Fischer) was applied to the membrane to activate chemiluminescence. The protein bands (signals) were detected using a Bio-Rad ChemiDoc™ XRS imager. One susceptible and two confirmed GR and Palmer amaranth accession from Georgia (Gaines et al. 2010), and a confirmed GR kochia accession from Colorado (Wiersma et al. 2015) were included for comparison (positive controls). The selected positive controls of the GR weeds were known to have high EPSPS protein abundance (Gaines et al. 2010; Wiersma et al. 2015), and were used for qualitative assessment of EPSPS protein in the GR vs. SUS kochia accessions tested.

Whole-Plant Dose-Response for ALS-Inhibitor Herbicide. Whole-plant dose-response experiments were conducted in the greenhouse at the MSU-SARC near Huntley, MT, to quantify the level of resistance to premixed thifensulfuron methyl (27.30% by wt) + tribenuron methyl (13.60% by wt) + metsulfuron methyl (10.90% by wt) (Ally® Extra SG, DuPont Company, Wilmington, DE 19898) in the GR kochia accessions (GIL01, JOP01, and CHES01) referred to as MHR, compared to the SUS accession. The experiment was conducted under similar growth conditions as previously described. Single kochia seedlings were transplanted into 10-cm-diam pots containing the same potting mix. Experiments were arranged in a randomized complete block design with eight replications (one plant per pot), and repeated two times. Actively growing 8- to 10-cm-tall kochia plants were sprayed with the premixed ALS-inhibitor herbicide at doses of

0, 0.0025, 0.0045, 0.009, 0.018, 0.036, 0.072, 0.144, 0.288, and 0.648 kg ai ha^{-1}. Methylated seed oil (MSO) at 1% (v/v) was included. Herbicide applications were made inside a stationary cabinet spray chamber 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 (as previously described). Kochia plants were harvested at the soil level at 3 weeks after treatment (WAT) and dried at 60 C for 3 d to determine shoot dry weight (g $plant^{-1}$).

Shoot dry weight data from the whole-plant dose-response experiments were subjected to ANOVA using PROC MIXED procedure in SAS (Version 9.2, SAS Institute, Cary, NC 27513). Residual analyses were performed using PROC UNIVARIATE in SAS. Data were combined across experimental runs due to non-significant interaction of experimental run by treatment. Pooled data for each kochia accession were regressed over doses of premixed thifensulfuron methyl + tribenuron methyl + metsulfuron methyl herbicide 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 shoot dry weight (g $plant^{-1}$), C is the lower limit, D is the upper limit, B is the slope, E is the herbicide dose required for 50% reduction in shoot dry weight referred to as GR_{50}, and X is the herbicide dose. A lack-of-fit test showed the accuracy of the nonlinear model (P = 0.516). Parameter estimates, standard errors, and GR_{90} values (dose of the herbicide required for 90% reduction in shoot dry weight) of the dose

response curves were determined using the *drc* package in *R* software (Knezevic et al. 2007). Resistance index (referred to as R/S ratio) for each kochia accession was estimated by dividing the GR_{50} value of a resistant accession by the GR_{50} value of the SUS accession.

***ALS* Gene Sequencing.** The gDNA was extracted from three kochia plants per accession using the Qiagen DNeasy Plant mini kit, and the experiment was repeated. The quality and concentration of gDNA for each sample was determined using a Bio-Rad SmartSpect™ Plus Spectrophotometer. From previously published research (Beckie et al. 2013; Warwick et al. 2008), a primer set (*ALSF1*: 5'-ATGGCGTCTACTGTGCAAA TCCC-3' and *ALSR1*: 5'-AACTT GTTCTTCCA TCACCTTC G-3') was designed to amplify almost the entire *ALS* gene (1800-bp). Each PCR reaction contained 8.75 µL of iTaq™ DNA Polymerase Master Mix (containing iTaq™ DNA polymerase, dNTPs, $MgCl_2$, and reaction buffers), 0.5 µL each of forward and reverse primer (25µM), and 2.5 µL of DNA template (30 ng ul^{-1}). PCR was performed using a Bio-Rad T100™ thermal cycler under the following conditions: initial denaturation at 95 C for 3 min, 30 cycles of 30 s at 95 C, 30 s at 66 C, and 90 s at 72 C followed by 10 min at 72 C. The reaction was terminated and held at 4 C. The PCR fragment was purified using QIAquick PCR purification kit following the manufacturer's protocol. The Sanger sequencing of purified PCR fragments was performed. Samples were sequenced with the same primer set used for PCR amplification, and another internal primer set (*ALSF2*: 5'-GAC GGATGCTTTT CAGGAG-3' and *ALSR2*: 5'-TTGGCGAGGGTTTCTGTAC-3'). The sequence reads of experimental samples were analyzed using the MultAlin software, and aligned to a

reference *ALS* gene from SUS kochia. The aligned nucleotides sequences were analyzed for any mutation at the single nucleotide position. The derived amino acid positions were based on the amino acid sequence of the *ALS* gene in *Arabidopsis* (Sathasivan et al. 1990).

Results and Discussion

Shikimate Accumulation. No difference in shikimate accumulation was observed between the nontreated (0 μM glyphosate) GR and SUS kochia accessions (Table 3.1). However, at a discriminate dose of 100 μM glyphosate, the SUS kochia plants accumulated 19.83 ng of shikimate μl^{-1}, which was 6- to 17-fold higher than the shikimate accumulated by GR plants of JOP01, CHES01, and GIL01 accessions. No differences in shikimate accumulation was observed among kochia accessions at 1000 μM glyphosate (data not shown). Similarly, GR plants of *Amaranthus* species and Italian ryegrass accumulated less shikimate compared with the susceptible plants at a glyphosate dose of 100 μM, and levels of shikimate did not differ between susceptible and GR plants at 500 and 1000 μM glyphosate (Gaines et al. 2010; Nandula et al. 2008, 2012). Shikimate, a dephosphorylated substrate of the EPSPS enzyme, accumulates in plants when treated with glyphosate (Singh and Shaner 1998). This assay can reliably be used to confirm resistance to glyphosate, also reported for several other GR weed species (Gaines et al. 2010; Nandula et al. 2014; Shaner et al. 2005).

Sequencing of *EPSPS* Thr_{102} and Pro_{106} codons. In general, target-site mutation in GR weed species involve amino acid substitution at Pro_{106} position (proline to serine,

threonine, alanine, or leucine) (Sammons and Gaines 2014). However, a field-evolved population of GR goosegrass [*Eleusine indica* (L.) Gaertn.] has recently been identified in Australia with a double mutation at Thr_{102} codon (threonine to isoleucine) and Pro_{106} codon (proline to serine); which conferred a high level of glyphosate resistance in the GR goosegrass population (Yu et al. 2015). There was no point mutation at Pro_{106} residue of the derived amino acid sequences in any of the tested GR kochia accessions from Montana (Table 3.2). Furthermore, there was no mutation observed at the Thr_{102} codon in the 200-bp *EPSPS* sequence of GR kochia (Table 3.2). Sequence alignment of tested individuals with reference *EPSPS* sequence of Palmer amaranth exhibited similar consensus at Pro_{106} and Thr_{102} (Table 3.2). Therefore, amino acid substitution at Pro_{106} or Thr_{102} codon of *EPSPS* can be ruled out as a possible mechanism of glyphosate resistance in the GR kochia populations tested in this research. Although mutation at the Thr_{102} position was not investigated, there was a lack of Pro_{106} mutation in the *EPSPS* gene in GR kochia populations from Kansas and Colorado (Wiersma et al. 2015). A lack of target-site mutation exists in GR populations of other weed species, such as Palmer amaranth and spiny amaranth (Gaines et al. 2010; Nandula et al. 2014).

***EPSPS* Genomic Copy Number.** Results from the qPCR analysis revealed that plants of JOP01 accession had approximately 6 to 8, GIL01 had 4 to 9, and CHES01 had 6 to 10 relative copies of *EPSPS* gene (Figure 3.1). The relative *EPSPS* copy number varied among plants within a GR accession, which may be expected in kochia, a cross-pollinated species with high level of genetic diversity (Mengistu and Messersmith 2002, Stallings et al. 1995). In contrast, all SUS kochia plants had only 1 relative *EPSPS* copy

number. GR kochia accessions from Kansas and Colorado had 3 to 8 relative *EPSPS:ALS* genomic copy numbers (Wiersma et al. 2015). *EPSPS* gene amplification (increased copy number) is a novel mechanism of resistance to glyphosate, also reported in other GR weed species. For instance, GR Palmer amaranth had high relative *EPSPS* gene copies of 40 to 100 in populations from Georgia (Gaines et al. 2010), 20 to 60 in populations from North Carolina (Chandi et al. 2012), and 33 to 59 in populations from Mississippi (Ribeiro et al. 2014). GR spiny amaranth (*Amaranthus spinosus* L.) from Mississippi had 26 to 37 copies, and Italian ryegrass from Arkansas had 15 to 25 relative copies of *EPSPS* gene (Nandula et al. 2014; Salas et al. 2012). However, lower folds of *EPSPS* gene amplification (2- to 10-fold) has been reported in GR Palmer amaranth accessions from New Mexico (Mohseni-Moghadam et al. 2013). The range of relative *EPSPS* gene copies (4 to 10) observed in GR kochia accessions from Montana was comparable to the range (3 to 8 relative *EPSPS* copies) reported recently in GR kochia accessions from Kansas and Colorado (Wiersma et al. 2015), and also to that observed in GR tall waterhemp (2 to 8 copies) (Lorentz et al. 2014).

Correlation of *EPSPS* Gene Copy Number with Glyphosate Resistance. The *EPSPS* gene copy number of SUS and GR kochia plants was correlated with the shikimate accumulation at the discriminating dose of glyphosate (Figure 3.2). The SUS plants with one relative *EPSPS* copy had higher shikimate accumulation compared with the GR kochia plants with 4 to 10 relative *EPSPS* copies. These results further support the hypothesis of overproduction of the EPSPS enzyme from increased copies of the *EPSPS*

gene produced by GR kochia plants; thus, allowing them to tolerate lethal doses (> 1.26 kg ae ha^{-1}) of glyphosate.

The I_{50} and GR_{50} values (Figure 3.3) were obtained from our previously reported whole-plant dose response curves of the selected GR and SUS accessions (Kumar et al. 2014). A strong positive linear relationship was observed between I_{50} and *EPSPS* gene copy number ($r = 0.92$) (Figure 3.3a), and between GR_{50} and *EPSPS* gene copy number ($r = 0.86$) (Figure 3.3b). This further suggests that 4- to 10-fold increased *EPSPS* gene copy numbers resulted in 4.6- to 11-fold levels of resistance [based on resistance index (R/S ratio)] to glyphosate in selected GR kochia accessions from Montana. It is likely that increase in selection pressure from glyphosate may select GR kochia individuals with higher copies of the *EPSPS* gene, conferring higher levels of glyphosate resistance.

EPSP*S* Protein Abundance. A representative image of EPSPS protein abundance of the tested kochia accessions (SUS vs. GIL01, JOP01, and CHES01) is presented in Figure 3.4. The protein assay with polyclonal anti-EPSPS antibody using western blot indicated that the GR kochia accessions from Montana had higher EPSPS protein abundance compared with the SUS kochia accession. To elaborate, the three GR kochia accessions (lanes 2, 3, and 4, respectively) with 4 to 10 relative copies of the EPSPS gene showed intense EPSPS protein signal, and the signal saturated at a molecular weight of 48 kDa (Figure 3.4). No signal was detected in lane 1 containing the SUS kochia accession. A comparable signal of the EPSPS protein extract was observed in a GR kochia accession (GR-CO) from Colorado (lane 5), with 8 relative *EPSPS:ALS* gene copies. Similarly, a highly intense EPSPS signal (saturated the film) was observed in lanes 7 and 8 containing

GR Palmer amaranth (GR1 and GR2, with approximately 50 and 100 relative copies of the *EPSPS* gene, respectively) protein extracts, whereas no signal was detected at 48 kDa in lane 6 containing glyphosate-susceptible (SUS-P) Palmer amaranth (1 relative copy of *EPSPS:ALS* gene) protein extract. There is generally a positive correlation between thicker EPSPS protein bands and higher *EPSPS* gene copy numbers (Gaines et al. 2010; Wiersma et al. 2015). The qualitative protein assay indicated that GR kochia accessions from Montana with increased *EPSPS* gene copies accumulated higher EPSPS protein.

Whole-Plant Dose-Response for ALS-Inhibitor Herbicide. On the basis of shoot dry weight, GR_{50} values for JOP01, CHES01, and GIL01 MHR kochia accessions were 0.090, 0.028, and 0.087 kg ha^{-1}, respectively, compared with the GR_{50} value of 0.003 kg ha^{-1} for the SUS accession (Table 3.3). The R/S ratios (resistance index) indicate that the MHR kochia accessions were 9.3- to 30-fold more resistant to the ALS-inhibitor herbicide premix (thifensulfuron methyl + tribenuron methyl + metsulfuron methyl) compared with the SUS population. Also, the GR_{90} values of MHR kochia accessions were 159 to 281 times the field-use rate (0.018 kg ai ha^{-1}) of the herbicide (Table 3.3). This is expected because of continuous use of sulfonylurea herbicides for > 8 yrs in dryland wheat-fallow fields in northern Montana from where the MHR kochia accessions were collected. Furthermore, the MHR kochia accessions showed differential response to the ALS-inhibitor herbicide, and the hierarchy of resistance was: JOP01 = GIL01 > CHES01 based on pairwise t-test of the GR_{50} values (data not shown). Kochia accessions from Kansas reported by Saari et al. (1990) had 12- to 28-fold levels of resistance to sulfonylurea herbicides. Similarly, Primiani et al. (1990) reported 8- to 30-fold levels of

resistance to chlorsulfuron, metsulfuron and sulfometuron herbicides in kochia accessions collected from wheat fields in Kansas. With continued use of sulfonylurea herbicides, there is a widespread occurrence of ALS-inhibitor-resistant kochia in the cereal productions regions of the US Great Plains and in Canada (Beckie et al. 2013; Heap 2015).

***ALS* Gene Sequencing.** There was a point mutation causing amino acid substitution at Pro_{197} residue in the *ALS* gene sequence (1800 bp) of MHR kochia accessions (JOP01, CHES01, and GIL01). Compared to the SUS accession with CCG codon at Pro_{197}, there was a change from C<u>C</u>G to C<u>A</u>G codon in all MHR kochia plants (Table 3.4). This point mutation resulted in an amino acid substitution from Pro_{197} to Gln_{197}, which conferred high level of resistance (up to 30-folds) to the ALS-inhibitor sulfonylurea herbicide tested (Table 3.3). Although multiple amino acid substitutions (Pro_{197}, Asp_{376}, and Trp_{574} sites) have been reported in ALS-inhibitor-resistant kochia populations (Beckie et al. 2013; Tranel et al. 2015), we did not observe any amino acid substitution at Asp_{376} or Trp_{574} position of the *ALS* gene (almost the entire gene was sequenced) in the tested kochia populations. Among all previously known mutations in *ALS* gene, substitution of proline at position 197 with threonine, arginine, leucine, glutamine, serine, or alanine is known to confer high level of resistance to sulfonylurea herbicides in kochia (Beckie et al. 2013; Tranel and Wright 2002; Tranel et al. 2015).

In conclusion, this research serves as a first report on the molecular mechanisms of resistance to glyphosate and ALS-inhibitor herbicides in MHR kochia from Montana. There was no mutation found at the Pro_{106} codon of the *EPSPS* gene, but the MHR

kochia accessions had 4 to 10-fold amplification of the *EPSPS* gene compared with a single relative *EPSPS* genomic copy in the SUS accession. MHR kochia plants also accumulated higher EPSPS protein compared with the SUS plants. Substitution of glutamine for proline at position-197 of the *ALS* gene conferred 9.3- to 30-fold levels of resistance to the ALS-inhibitor herbicide in MHR kochia.

Evolution of glyphosate- and ALS-inhibitor MHR kochia in wheat-fallow fields would be a potential concern for growers in the NGP. Wind-mediated tumble mechanism of seed dispersal coupled with pollen-mediated gene flow through out-crossing would ensure rapid spread of MHR kochia. In separate studies, we evaluated the effectiveness of various alternative PRE and POST herbicides to be used for kochia control in wheat, corn/grain sorghum, soybean, chemical-fallow, and postharvest wheat stubble (Kumar et al. 2014; Kumar and Jha 2015a,b). Growers should adopt those effective alternative modes of action herbicides and prevent seed production from MHR kochia plants in their production farms. Reduced absorption/translocation and metabolism were ruled out as mechanisms of glyphosate resistance in kochia accessions from Kansas (Waite et al. 2013); however, the possibility of multiple mechanisms of resistance to glyphosate in the selected GR kochia accessions needs to be considered. We are investigating the inheritance pattern of amplified *EPSPS* gene copies and fitness cost (if any) associated with increase in relative *EPSPS* gene copies in GR kochia in the presence and absence of glyphosate selection pressure. A random field survey was conducted in 2013 and 2014 to collect kochia populations (approximately 200) from cropland and noncropland areas across northcentral Montana. The populations are under investigation for possible

multiple resistance to three different herbicide groups (glyphosate, ALS inhibitors, and dicamba) and for determining the frequency and geographical distribution of MHR kochia in Montana. These key research questions will provide insights into the strategies to prevent further spread of MHR kochia in this region.

Acknowledgements

The authors thank Montana Wheat and Barley Committee for funding this research. We gratefully acknowledge Merlin Johnson, Shane Leland, and growers from Hill and Liberty Counties of Montana for their valuable assistance in kochia seed collection. We are grateful to Dr. Brian Scheffler and Ms. Fanny Liu at the Genomics and Bioinformatics Research Unit, USDA-ARS, MS, for their help in the gene sequencing work, and Dr. Aruna V. Varanasi and Christopher Vanhorn for their technical assistance.

Table 3.1. Shikimate accumulation in glyphosate-susceptible and glyphosate-resistant kochia accessions from MT.

	Shikimate accumulation[a]			
Accession[b]	0 µM glyphosate[c]		100 µM glyphosate	
	ng μL^{-1}			
SUS	2.06	a	19.83	a
JOP01	2.21	a	1.91	b
CHES01	1.79	a	3.30	b
GIL01	1.20	a	1.15	b

[a] Leaf disks (5-mm diam) were excised from young (8- to 10-cm tall) actively growing kochia plants, and accumulation of shikimate was determined by *in vivo* assay.
[b] Abbreviations: SUS, susceptible kochia accession, Huntley, MT; GIL01, glyphosate-resistant (GR) kochia accession from Gildford, MT; JOP01, GR kochia accession from Joplin, MT; CHES01, GR kochia accession from Chester, MT.
[c] Means within the column followed by the same letters are not significantly different based on Fisher's Protected LSD test at $P < 0.05$.

Table 3.2. Nucleotide bases and derived amino acid sequence in the conserved region of *EPSPS* gene from glyphosate-susceptible and glyphosate-resistant kochia accessions from MT. The bold nucleotide codons encoded Thr_{102} and Pro_{106} residues.[a]

Reference *EPSPS* gene sequence of Palmer amaranth	GGA Gly	**ACG** Thr	GCA Ala	ATG Met	CGC Arg	**CCA** Pro	TTG Leu	ACA Thr	GCT Ala	GCA Ala	GTT Val
Amino acid positions	101	102	103	104	105	106	107	108	109	110	111
SUS1	GGA	**ACG**	GCA	ATG	CGC	**CCA**	TTG	ACA	GCT	GCA	GTT
SUS2	GGA	**ACG**	GCA	ATG	CGC	**CCA**	TTG	ACA	GCT	GCA	GTT
SUS3	GGA	**ACG**	GCA	ATG	CGC	**CCA**	TTG	ACA	GCT	GCA	GTT
JOP011	GGA	**ACG**	GCA	ATG	CGC	**CCA**	TTG	ACA	GCT	GCA	GTT
JOP012	GGA	**ACG**	GCA	ATG	CGC	**CCA**	TTG	ACA	GCT	GCA	GTT
JOP013	GGA	**ACG**	GCA	ATG	CGC	**CCA**	TTG	ACA	GCT	GCA	GTT
GIL011	GGA	**ACG**	GCA	ATG	CGC	**CCA**	TTG	ACA	GCT	GCA	GTT
GIL012	GGA	**ACG**	GCA	ATG	CGC	**CCA**	TTG	ACA	GCT	GCA	GTT
GIL013	GGA	**ACG**	GCA	ATG	CGC	**CCA**	TTG	ACA	GCT	GCA	GTT
CHES011	GGA	**ACG**	GCA	ATG	CGC	**CCA**	TTG	ACA	GCT	GCA	GTT
CHES012	GGA	**ACG**	GCA	ATG	CGC	**CCA**	TTG	ACA	GCT	GCA	GTT
CHES013	GGA	**ACG**	GCA	ATG	CGC	**CCA**	TTG	ACA	GCT	GCA	GTT

[a] Abbreviations: SUS1, SUS2, and SUS3, three kochia plants from glyphosate-susceptible (SUS) accession; JOP011, JOP012, and JOP013, three kochia plants from glyphosate-resistant (GR) JOP01 accession; GIL011, GIL012, and GIL013, three kochia plants from GR GIL01 accession; CHES011, CHES012, and CHES013, three kochia plants from GR CHES01 accession.

Table 3.3. Regression parameters (Equation 1) for whole-plant dose-response based on shoot dry weight (g plant^{-1}) of multiple herbicide-resistant (MHR) and susceptible (SUS) kochia accessions from Montana treated with thifensulfuron methyl + tribenuron methyl + metsulfuron methyl.

	Regression parameters (±SE)					
Accession[a]	*d*	*c*	*b*	GR_{50}[b]	R/S[c]	GR_{90}[b]
SUS	4.24 (0.06)	0.14 (0.02)	1.8 (0.09)	0.003 (0.0001)	—	0.01 (0.007)
JOP01	4.17 (0.05)	0.56 (0.04)	0.5 (0.06)	0.090 (0.004)	30	5.06 (0.46)
CHES01	3.57 (0.06)	0.89 (0.03)	0.4 (0.07)	0.028 (0.001)	9.3	2.86 (0.35)
GIL01	3.57 (0.06)	0.73 (0.03)	0.6 (0.08)	0.087 (0.004)	29	3.75 (0.39)

[a] Abbreviations: SUS, susceptible kochia accession, Huntley, MT; GIL01, multiple herbicide-resistant (MHR) kochia accession from Gildford, MT; JOP01, MHR kochia accession from Joplin, MT; CHES01, MHR kochia accession from Chester, MT.

[b] GR_{50} is the effective dose (kg ai ha^{-1}) of thifensulfuron methyl + tribenuron methyl + metsulfuron methyl required for 50% shoot dry weight reduction; GR_{90} is the effective dose (kg ai ha^{-1}) of thifensulfuron methyl + tribenuron methyl + metsulfuron methyl required for 90% shoot dry weight reduction.

[c] R/S is calculated as a ratio of GR_{50} of a MHR accession to GR_{50} of the SUS kochia accession.

Table 3.4. Nucleotide bases and derived amino acid sequences of a fragment of *ALS* gene from susceptible and multiple herbicide-resistant kochia accessions from MT, showing a single nucleotide mutation (bold and underlined codon) at Pro_{197} residue.[a]

Reference *ALS* gene sequence of kochia[b]	ACG	GGG	CAG	GTG	**CCG**	CGG	CGA	ATG	ATT	GGG	ACG
Amino acid	Thr	Gly	Gln	Val	**Pro**	Arg	Arg	Met	Ile	Gly	Thr
positions	193	194	195	196	197	198	199	200	201	202	203
SUS1	ACG	GGG	CAG	GTG	**CCG**	CGG	CGA	ATG	ATT	GGG	ACG
SUS2	ACG	GGG	CAG	GTG	**CCG**	CGG	CGA	ATG	ATT	GGG	ACG
SUS3	ACG	GGG	CAG	GTG	**CCG**	CGG	CGA	ATG	ATT	GGG	ACG
JOP011	ACG	GGG	CAG	GTG	**CAG**	CGG	CGA	ATG	ATT	GGG	ACG
JOP012	ACG	GGG	CAG	GTG	**CAG**	CGG	CGA	ATG	ATT	GGG	ACG
JOP013	ACG	GGG	CAG	GTG	**CAG**	CGG	CGA	ATG	ATT	GGG	ACG
GIL011	ACG	GGG	CAG	GTG	**CAG**	CGG	CGA	ATG	ATT	GGG	ACG
GIL012	ACG	GGG	CAG	GTG	**CAG**	CGG	CGA	ATG	ATT	GGG	ACG
GIL013	ACG	GGG	CAG	GTG	**CAG**	CGG	CGA	ATG	ATT	GGG	ACG
CHES011	ACG	GGG	CAG	GTG	**CAG**	CGG	CGA	ATG	ATT	GGG	ACG
CHES012	ACG	GGG	CAG	GTG	**CAG**	CGG	CGA	ATG	ATT	GGG	ACG
CHES013	ACG	GGG	CAG	GTG	**CAG**	CGG	CGA	ATG	ATT	GGG	ACG

[a] Almost the entire *ALS* gene was sequenced (1800 bp).

[b]Abbreviations: SUS1, SUS2, and SUS3, three kochia plants from susceptible (SUS) accession; JOP011, JOP012, and JOP013, three kochia plants from multiple herbicide-resistant (MHR) JOP01 accession; GIL011, GIL012, and GIL013, three kochia plants from MHR GIL01 accession; CHES011, CHES012, and CHES013, three kochia plants from MHR CHES01 accession.

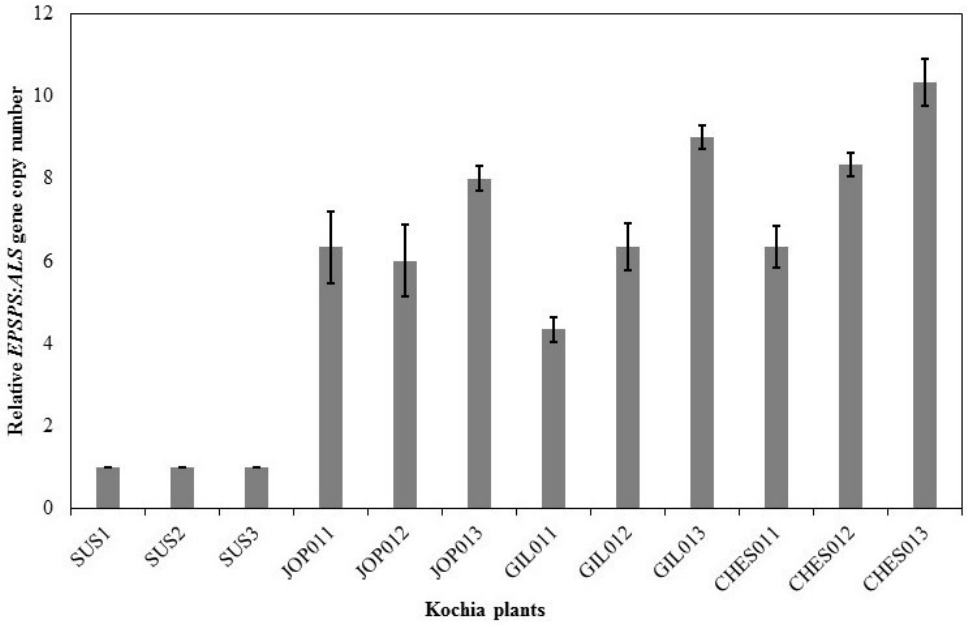

Figure 3.1. Relative *EPSPS* genomic copy number in glyphosate-resistant (JOP01, GIL01, and CHES01) and susceptible (SUS) kochia accessions from MT. Vertical bars represent standard error of mean (n = 6).

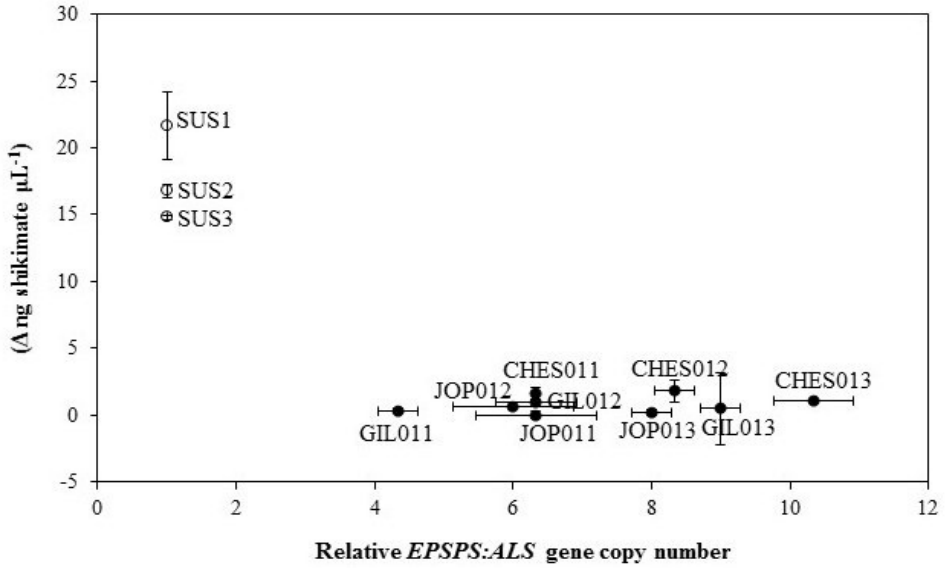

Figure 3.2. Correlation of relative *EPSPS* genomic copy number vs. shikimate accumulation in glyphosate-resistant (JOP01, GIL01, and CHES01) and susceptible (SUS) kochia accessions from MT. Shikimate accumulation was measured after incubation of samples in 100 µM glyphosate in an *in vivo* leaf-disk assay. The Δ ng shikimate μL^{-1} was calculated as the difference in shikimate accumulation between treated and untreated plants. Error bars indicate standard error.

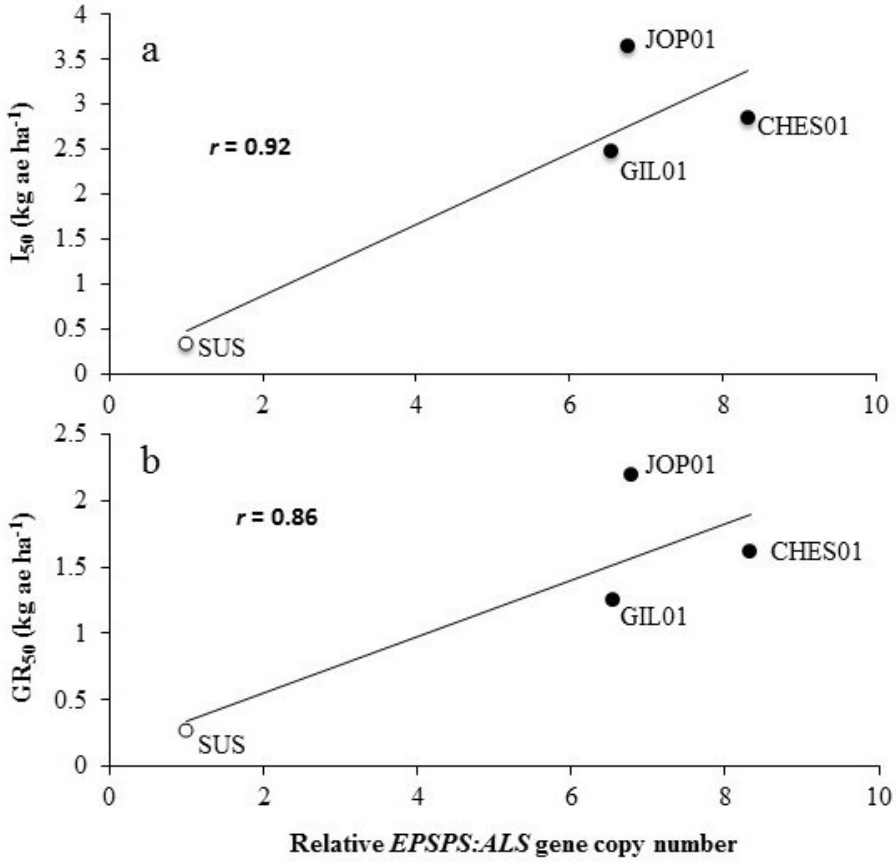

Figure 3.3. (a) Correlation of relative *EPSPS* genomic copy number with the amount of glyphosate needed for 50% control (I_{50} value) of glyphosate-resistant (JOP01, GIL01, and CHES01) and susceptible (SUS) accessions from MT. (b) Correlation of relative *EPSPS* genomic copy number with the amount of glyphosate needed for 50% shoot dry weight reduction (GR_{50} value) of glyphosate-resistant (JOP01, GIL01, and CHES01) and susceptible (SUS) kochia accessions from MT. The I_{50} and GR_{50} values were obtained from the glyphosate dose-response study on the selected accessions (Kumar et al. 2014).

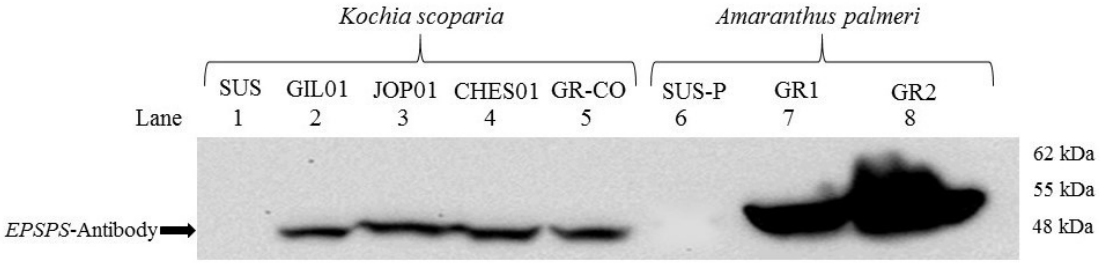

Figure 3.4. A representative image of nitrocellulose membrance showing EPSPS protein bands probed with anti-EPSPS polyclonal antibody in a western blot. Lane 1, glyphosate-susceptible kochia accession (SUS) from MT; lanes 2 to 4, glyphosate-resistant kochia accessions (GIL01, JOP01, and CHES01, respectively) from MT; lane 5, GR kochia accession (GR-CO) from Colorado (8 copies of *EPSPS*); lane 6, glyphosate-susceptible (SUS-P) Palmer amaranth accession from Georgia; lane 7 to 8, glyphosate-resistant Palmer amaranth accessions (GR1 and GR2 with ~50 and 100 copies of *EPSPS*, respectively) from Georgia. A total of three plants per kochia accession were sampled in each experimental run.

Literature Cited

Baker DV, Withrow JR, Brown CS, Beck, KG (2010) Tumbling: use of diffuse knapweed (*Centaurea diffusa*) to examine an understudied dispersal mechanism. Invasive Plant Sci Manag 3:301–309

Beckie, HJ, Blackshaw RE, Low R, Hall LM, Sauder CA, Martin S, Brandt EN, Shirriff SW (2013) Glyphosate- and acetolactate synthase inhibitor-resistant kochia (*Kochia scoparia*) in Western Canada. Weed Sci 61:310–318

Bolt MW, Mahoney PA (1997) High-efficiency blotting of proteins of diverse sizes following sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Anal Biochem 247:185–192

Chandi A, Milla-Lewis SR, Giacomini D, Westra P, Preston C, Jordan DL, York AC, Burton JD, Whitaker JR (2012) Inheritance of evolved glyphosate resistance in a North Carolina Palmer amaranth (*Amaranthus palmeri*) biotype. Int J Agron doi:10.1155/2012/176108

Christoffoleti PJ, Westra PB, Moore F (1997) Growth analysis of sulfonylurea-resistant and -susceptible kochia (*Kochia scoparia*). Weed Sci 45:691–695

Donald WW, Prato T (1991) Profitable, effective herbicides for planting-time weed control in no-till spring wheat (*Triticum aestivum*). Weed Sci 39:83–90

Duke SO, Powles SB (2008) Glyphosate: a once-in-a-century herbicide. Pest Manag Sci 64:319–325

Eberlein CV, Fore ZA (1984) Kochia biology. Weeds Today 15:5–6

Forcella F (1985) Spread of kochia in the northwestern United States. Weeds Today 16:4–6

Friesen LF, Beckie HJ, Warwick SI, Van Acker RC (2009) The biology of Canadian weeds. 138. *Kochia scoparia* (L.) Schrad. Can J Plant Sci 89:141–167

Gaines TA, Zhang W, Wang D, Bukun B, Chisholm ST, Shaner DL, Nissen SJ, Patzoldt WL, Tranel PJ, Culpepper AS, Grey TL, Webster TM, Vencill WK, Sammons RD, Jiang JM, Preston C, Leach JE, Westra P (2010) Gene amplification confers glyphosate resistance in *Amaranthus palmeri*. Proc Natl Acad Sci 107:1029–1034

Giacomini D, Westra P, Ward SM (2014) Impact of genetic background in fitness cost studies: An example from glyphosate-resistant Palmer amaranth. Weed Sci 62:29–37

Heap I (2015) The International Survey of Herbicide Resistant Weeds. http://www.weedscience.org. Accessed: January 14, 2015

Knezevic SZ, Streibig JC, Ritz C (2007) Utilizing R software package for dose-response studies: the concept and data analysis. Weed Technol 21:840–848

Kumar V, Jha P, Reichard N (2014) Occurrence and characterization of kochia (*Kochia scoparia*) accessions with resistance to glyphosate in Montana. Weed Technol 28:122–130

Kumar V, Jha P (2015a) Effective preemergence and postemergence herbicide programs for kochia control. Weed Technol 29:24–34

Kumar V, Jha P (2015b) Influence of herbicides applied postharvest in wheat stubble on control, fecundity, and progeny fitness of *Kochia scoparia* in the US Great Plains. Crop Prot 71:144–149

Lenssen AW, Waddell J, Johnson GD, Carlson GR (2007) Diversified cropping systems in semiarid Montana: Nitrogen use during drought. Soil Tillage Res 94:362–375

Lorentz L, Gaines TA, Nissen SJ, Westra P, Strek HJ, Dehne HW, Ruiz-Santaella JP, Beffa R (2014) Characterization of glyphosate resistance in *Amaranthus tuberculatus* populations. J Agric Food Chem 62:8134–8142

Mengistu LW, Messersmith CG (2002) Genetic diversity of kochia. Weed Sci 50:498–503

Mohseni-Moghadam M, Schroeder J, Ashigh J (2013) Mechanism of resistance and inheritance in glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) populations from New Mexico, USA. Weed Sci 61:517–525

Nandula VK, Reddy KN, Poston DH, Rimando AM, Duke SO (2008) Glyphosate tolerance mechanism in Italian ryegrass (*Lolium multiflorum*) from Mississippi. Weed Sci 56:344–349

Nandula VK, Reddy KN, Koger CH, Poston DH, Rimando AM, Duke SO, Bond JA, Ribeiro DN (2012) Multiple resistance to glyphosate and pyrithiobac in Palmer amaranth (*Amaranthus palmeri*) from Mississippi and response to flumiclorac. Weed Sci 60:179–188

Nandula VK, Wright A, Molin W, Ray J, Bond J, Eubank T (2014) *EPSPS* amplification in glyphosate-resistant spiny amaranth (*Amaranthus spinosus*): a case of gene transfer

via interspecific hybridization from glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*). Pest Manag Sci 70:1902-1909

Norsworthy JK, Smith KL, Scott RC, Gbur EE (2007) Consultant perspectives on weed management needs in Arkansas cotton. Weed Technol 21:825-831

Primiani MM, Cotterman JC, Saari LL (1990) Resistance of *Kochia scoparia* to sulfonylurea and imidazolinone herbicides. Weed Technol 4:169–172

Powles SB (2010) Gene amplification delivers glyphosate-resistant weed evolution. Proc Natl Acad Sci 107:955–956

Ribeiro DN, Pan Z, Duke SO, Nandula VK, Baldwin BS, Shaw DR, Dayan FE (2014) Involvement of facultative apomixis in inheritance of *EPSPS* gene amplification in glyphosate-resistant *Amaranthus palmeri*. Planta 239:199–212

Saari LL, Cotterman JC, Primiani MM (1990) Mechanism of sulfonylurea herbicide resistance in the broadleaf weed, *Kochia scoparia.* Plant Physiol 93:55–61

Salas RA, Dayan FE, Pan Z, Watson SB, Dickson JW, Scott RC, Burgos NR (2012) *EPSPS* gene amplification in glyphosate-resistant Italian ryegrass (*Lolium perenne* ssp. multiflorum) from Arkansas. Pest Manag Sci 68:1223–1230

Sammons DR, Gaines TA (2014) Glyphosate resistance: state of knowledge. Pest Manag Sci 70:1367–1377

Sathasivan K, Haughn GW, Murai N (1990) Nucleotide sequence of a mutant acetolactate synthase gene from imidazolinone-resistant *Arabidopsis thaliana* var. Columbia. Nucleic Acids Res 18:2188

Schwinghamer TD, Van Acker RC (2008) Emergence timing and persistence of kochia (*Kochia Scoparia*). Weed Sci 56:37–41

Seefeldt SS, Jensen JE, Fuerst EP (1995) Log-logistic analysis of herbicide dose-response relationships. Weed Technol 9:218–227

Shaner DL, Nadler-Hassar T, Henry WB, Koger CH (2005) A rapid *in vivo* shikimate accumulation assay with excised leaf discs. Weed Sci 53, 769–774

Singh BJ, Shaner DL (1998) Rapid determination of glyphosate injury to plants and identification of glyphosate-resistant plants. Weed Technol 12:527–530

Sivakumaran K, Mulugeta D, Fay PK, Dyer WE (1993) Differential herbicide response among sulfonylurea-resistant *Kochia scoparia* L. accessions. Weed Sci 41:159–

165Stallings GP, Thill DC, Mallory-Smith CA, Shafii B (1995) Pollen-mediated gene flow of sulfonylurea-resistant kochia (*Kochia scoparia*). Weed Sci 43:95–102

Stallings GP, Thill DC, Mallory-Smith CA, Shafii B (1995) Pollen-mediated gene flow of sulfonylurea-resistant kochia (*Kochia scoparia*). Weed Sci 43:95–102

Tranel PJ, Wright TR (2002) Resistance of weeds to ALS-inhibiting herbicides: what have we learned? Weed Sci 50:700–712

Tranel PJ, Riggins CW, Bell MS, Hager AG (2011) Herbicide resistance in *Amaranthus tuberculatus*: a call for new options. Pest Manag Sci 59:5808–5812

Tranel PJ, Wright TR, Heap IM (2015) Mutations in herbicide-resistant weeds to ALS inhibitors. http://www.weedscience.org. Accessed: January 7, 2015

Waite J, Thompson CR, Peterson DE, Currie RS, Olson BLS, Stahlman PW, Khatib KA (2013) Differential kochia (*Kochia scoparia*) populations response to glyphosate. Weed Sci 61:193–200

Warwick SI, Xu R, Sauder C, Beckie HJ (2008) Acetolactate synthase target-site mutations and single nucleotide polymorphism genotyping in ALS-resistant kochia (*Kochia scoparia*). Weed Sci 56:797–806

Wiersma AT, Gaines TA, Preston C, Hamilton JP, Giacomini D, Buell CR, Leach JE, Westra P (2015) Gene amplification of 5-enol-pyruvylshikimate-3-phosphate synthase in glyphosate-resistant *Kochia scoparia*. Planta 241:463–474

Yu Q, Jalaludin A, Han H, Chen M, Sammons RD, Powles SB (2015) Evolution of double amino acid substitution in the EPSP synthase in *Eleusine indica* conferring high level glyphosate resistance. Plant Physiol doi: http://dx.doi.org/10.1104/pp.15.00146

CHAPTER 4

IMPACT OF *EPSPS* GENE AMPLIFICATION AND INTRASPECIFIC COMPETITION ON FITNESS OF GLYPHOSATE-RESISTAN *KOCHIA SCOPARIA* INBREDS

Contribution of Authors and Co-Authors

Manuscript in Chapter 4

Author: Vipan Kumar

Contributions: Conceived and implemented the study design. Conducted experiments, reviewed the literature, and wrote the manuscript.

Co-Author: Prashant Jha

Contributions: Helped conceived the study design, advisement and guidance, reviewed the manuscript drafts

Manuscript Information Page

Vipan Kumar, Prashant Jha
PLoS One Journal
Status of Manuscript:
_×_Prepared for submission to a peer-reviewed journal
___Officially submitted to a peer-review journal
___Accepted by a peer-reviewed journal
___Published in a peer-reviewed journal

Published by Public Library of Science
To be Submitted 2015

Abstract

Glyphosate-resistant (GR) kochia (*Kochia scoparia* L.) is an increasing threat to the no-till wheat-fallow and GR cropping systems in the US Great Plains. It has previously been reported that amplification of the 5-enolpyruvylshikimate-3-phosphate synthase (*EPSPS*) gene confers glyphosate resistance in GR *K. scoparia* in Montana. It is hypothesized that the *EPSPS* gene amplification could potentially impact the growth and reproductive fitness of GR *K. scoparia*. Greenhouse experiments were conducted to investigate the effects of the *EPSPS* gene amplification on fitness traits and level of glyphosate resistance in GR *K. scoparia*. Inbred lines (developed after three generations of recurrent group selection under pollen isolation conditions) of GR (CHES01 and JOP01) and glyphosate-susceptible (SUS) *K. scoparia* were grown under intraspecific competition at 1, 85, and 170 plants m^{-2}. Fitness traits of GR and SUS *K. scoparia* plants including plant height, width, primary branches, shoot dry weight, 1000 seed weight, reproductive effort, progeny seed germination and viability, and radical length of germinating seedlings were assessed across competition gradient. Survival of GR *K. scoparia* plants when exposed to glyphosate at 870 (field use rate) and 4350 g ae ha^{-1} (5-times the field use rate) was also evaluated at 21 DAT. Results indicated no significant differences in growth and fitness of GR vs. SUS *K. scoparia* on the basis of plant height, width, primary branches, total leaf area, shoot biomass, reproductive effort, progeny 1000-seed weight, seed viability, seed germination, and radicle length. Furthermore, increasing the intraspecific competition (from 1 to 170 plant m^{-2}) caused significant reduction in all growth and fecundity-related traits tested. GR *K. scoparia* with

approximately 5 to 14 *EPSPS* gene copies survived glyphosate at 4350 g ha^{-1}, whereas plants with 2 to 4 *EPSPS* gene copies failed to survive this high use rate. No fitness cost conferred by *EPSPS* gene amplification and the additive effect of the gene amplification on glyphosate-resistance level indicate that GR *K. scoparia* with high *EPSPS* gene copies will most likely persist in field populations, irrespective of glyphosate selection pressure. Growers should target GR *K. scoparia* seed bank with alternative effective modes of action herbicides, and integrate non-chemical control tactics, including crop rotation and tillage.

Keywords: 5-enolpyruvylshikimate-3-phosphate synthase (*EPSPS*), gene amplification, fitness cost, herbicide resistance, intraspecific competition, glyphosate, kochia (*Kochia scoparia* (L.) Schrad.)

Introduction

Kochia scoparia (L.) Schrad. (kochia) is a monoecious, diploid (2n = 18) weed species, prevalent across the northern and central Great Plains of the North America [1,2,3]. It is one of the most problematic, summer annual broadleaf weeds in cropland and non-cropland areas of this region. *K. scoparia* exhibits low seed dormancy, early seedling emergence, and rapid growth, and is tolerant to heat, salt, and drought stresses [3-6]. A single plant can produce > 100,000 seeds in the absence of crop competition, and manifests a unique mechanism of seed dispersal through tumbling [3, 4]. During late autumn, a matured plant break off at the base of the stem and tumbles across the landscape with the prevailing wind, dispersing seeds over a long distance [3, 4]. Because of protogynous flowering, *K. scoparia* exhibits a high degree of outcrossing or pollen-mediated gene flow, which contributes to high genetic diversity within and among populations [7, 8]. *K. scoparia* populations resistant to several herbicide site of action, including photosystem II (PS II) inhibitors (atrazine), acetolactate synthase (ALS) inhibitors (sulfonylurea and imidazolinone), synthetic auxins (dicamba and fluroxypyr), and recently to glyphosate have been reported in the US Great Plains [9-12]. Resistance to glyphosate was first reported in 2007 from wheat fields in western Kansas [10, 13]. Since then, GR *K. scoparia* has been confirmed in nine US states, including Montana [10, 11], and in three Canadian provinces [10, 14].

Glyphosate resistance in weed species is generally endowed by evolution of various target-site or non-target-site mechanisms [15, 16]. Among all mechanisms, amplification of the *EPSPS* gene is a novel way of acquiring glyphosate resistance; first

discovered in GR Palmer amaranth (*Amaranthus palmeri* S. Wats.) in Georgia [17]. In addition, the *EPSPS* gene amplification has also been reported in four other GR weed species, including tall waterhemp (*Amaranthus tuberculatus*) [18], spiny amaranth (*Amaranthus spinosus*) [19], Italian ryegrass (*Lolium perenne* ssp. multiflorum) [20], and recently in *K. scoparia* [21]. A massive amplification of the *EPSPS* gene in *A. palmeri* was likely mediated by transposable genetic elements, and amplified copies were dispersed throughout the genome [22]. However, the *EPSPS* gene amplification in GR *K. scoparia* is likely to be mediated by unequal crossing over during meiosis, and the segregation of the amplified gene copies follow a single locus inheritance [23].

Evolutionary theory predicts that the herbicide resistance may confer fitness disadvantages or fitness cost in herbicide-resistant (HR) weed biotypes in the absence of herbicide selection pressure [24]. Fitness of a particular phenotype refers to the successful production of offspring relative to other phenotypes in a population, as described by Vila-Aiub et al. [25]. Fitness cost is defined as the reduction of fitness caused by negative pleiotropic effects of a resistance allele on growth, development, phenology, and fecundity of a HR weed biotype [25]. Fitness cost conferred by glyphosate resistance has been reported to vary depending on the mechanism of resistance and the weed species. For example, fitness cost has been observed in glyphosate-tolerant tall morningglory [*Ipomoea purpurea* (L.) Roth]; where a decline in the frequency of resistant individuals was observed in the absence of glyphosate [26]. Fitness cost has also been reported in GR rigid ryegrass (*Lolium rigidum* Gaudin) in which a reduced translocation conferred resistance to glyphosate [27]. However,

Pederson et al. [28] reported that GR and glyphosate-susceptible (GS) individuals had similar competitiveness and biomass accumulation, when grown in competition with wheat. Similarly, no apparent fitness costs (based on biomass or seed production) have been observed in glyphosate-tolerant common lambsquarters (*Chenopodium album* L.) and GR horseweed [*Conyza canadensis* (L.)] biotypes from Indiana [29, 30]. Furthermore, the massive amplification of the *EPSPS* gene does not endow any fitness cost in GR *A. palmeri* accessions from Georgia [31, 32]. However, fitness advantages (48 to 125% more seeds plant^{-1}, greater photosynthetic rates, and higher seed germination percentages) associated with transgenic over-expression of the native *EPSPS* gene have been reported in GR wild rice (*Oryza sativa* f. spontanea) [33]. In addition, a biotype of GR horseweed [*Conyza canadensis* (L.)] from California has also been reported to accumulate double amount of dry biomass compared with a susceptible biotype, when grown either in isolation or in competition with grapevine (*Vitis vinifera* L.) [34].

Recently, we reported *K. scoparia* accessions with resistance to glyphosate (4.6- to 11-fold) from chemical fallow fields (wheat-fallow rotation) in the northern Montana [11]. Amplification of the *EPSPS* gene (~ 4 to 10 copies) conferred resistance to glyphosate in those accessions [35]. It is hypothesized that the *EPSPS* gene amplification could potentially confer fitness cost in GR *K. scoparia* due to (1) metabolic energy incurred for production of additional EPSPS enzyme, and (2) functional disruption of other genes from insertion of amplified copies of the *EPSPS* gene, as also hypothesized in GR *A. palmeri* [31]. The investigations on the fitness cost endowed by *EPSPS* gene amplification in GR *K. scoparia* are particularly important because evaluation of fitness

traits in GR *K. scoparia* will help in 1) predicting further spread of glyphosate resistance in this region and 2) developing effective resistance management strategies. There is currently no published report on fitness of GR *K. scoparia*, and the *K. scoparia* accessions with *EPSPS* gene amplification would provide the best genetic resource to fill this knowledge gap.

The main objectives of this research were (1) to investigate the pleiotropic effects of the *EPSPS* gene amplification on the fitness (growth and fecundity traits) of GR vs. glyphosate-susceptible (SUS) *K. scoparia* under different levels of intraspecific competition, and (2) to determine the effect of *EPSPS* gene amplification on glyphosate resistance level in GR *K. scoparia.*

Materials and Methods

Plant Materials. Seeds of two putative GR *K. scoparia* accessions (designated as JOP01; 48° 57′ N, 110° 84′ W and CHES01; 48° 56′ N, 110° 92′ W) were collected in late autumn 2012 from two different chemical fallow fields (under wheat-fallow rotation) in the Liberty County, Montana, USA [11]. Those sampled fields typically received three to four applications of glyphosate (≥ 870 g ae ha^{-1} per application) during the chemical fallow period preceding winter wheat in a year, over > 5 years. The SUS *K. scoparia* accession was collected from an organic wheat field (48° 58′ N, 110° 88′ W) within a 2- to 3-km radius of the two fields in Liberty County, where the GR accessions were collected. Seeds of putative GR and SUS *K. scoparia* accessions were sown on germination flats (53 × 35 × 10 cm) containing a commercial potting mix (VermiSoil™,

Vermicrop Organics, 4265 Duluth Avenue, Rocklin, CA) in a greenhouse at the Montana State University, Southern Agricultural Research Center (MSU-SARC) near Huntley, MT. The greenhouse was maintained at 25/23 ± 3 ˚C day/night temperature and 16 h photoperiod, supplemented with metal halide lamps (400 μmol m^{-2} s^{-1}). *K. scoparia* plants (8 to 10 cm tall) from CHES01 and JOP01 accessions (~150 plants per accession) were treated with a discriminating dose of glyphosate at 1740 g ha^{-1} (twice the field-use rate of 870 g ha^{-1}) (Roundup PowerMax™, Monsanto Company, St Louis, MO). Glyphosate was applied using a stationary cabinet spray chamber 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. The treated *K. scoparia* plants were returned to the greenhouse and survival was recorded 21 d after glyphosate treatment (DAT). *K. scoparia* plants from the CHES01 and JOP01 accessions that survived the glyphosate treatment and produced new growth at 21 DAT were classified as GR plants. In similar experiments, *K. scoparia* plants from the SUS accession treated with 435 g ha^{-1} of glyphosate (half the field-use rate) had > 98% control at 21 DAT, confirming the susceptibility of SUS accession to glyphosate (data not shown).

Inbred Lines of GR and SUS *K. scoparia.* Inbred lines (CHES01, JOP01, and SUS) were developed from groups of phenotypically uniform plants of GR and SUS accessions by recurrent selection under pollen isolation, rather than from a single seed to prevent any inbreeding depression, as previously reported for dicamba-resistant *K. scoparia* [9]. To elaborate the procedure, plants of CHES01 or JOP01 accession (20 phenotypically uniform plants per accession) that survived (<10% injury) 1740 g ha^{-1} of glyphosate were

transplanted into 10-L plastic pots containing the same potting mix described previously, and were covered with pollination bags (DelStar Technologies, Inc., 601 Industrial Drive, Middletown, DE) before flowering. Seeds were collected and bulked separately for each accession. Seeds obtained from the selfed GR plants were further subjected to two generations of recurrent group selection (20 plants per generation) by spraying with the same dose of glyphosate. Similarly, seeds of the SUS *K. scoparia* were obtained by selfing a group of phenotypically uniform plants for three generations under pollen isolation conditions. None of the SUS plants tested in each generation survived glyphosate applied at 435 g ha^{-1}. Those inbred lines of GR (CHES01 and JOP01) and SUS *K. scoparia* obtained after three generations of recurrent group selection were used for subsequent experiments. A similar approach to study fitness using recurrent group selection has previously been reported in multiple herbicide-resistant *Avena fatua* (wild oat), ALS inhibitor-resistant *K. scoparia*, and auxinic-herbicide resistant *K. scoparia* [36, 37, 38].

Fitness Study. Greenhouse experiments were conducted to investigate the fitness of GR *K. scoparia* inbred lines (with increased copies of the *EPSPS* gene, Fig. 4.1) relative to SUS inbred (with single *EPSPS* gene copy) under an intraspecific competition gradient, and repeated in time. The study was conducted in a completely randomized design with a factorial arrangement of treatments: three *K. scoparia* inbred lines (CHES01, JOP01, and SUS), three levels of plant density, and ten replicates, with a total of 90 experimental units (20-L plastic pots, 26 cm diameter × 38 cm deep). Density treatments included 1, 4, and 8 *K. scoparia* plants pot^{-1} (intraspecific competition), which corresponded to 1, 85

and 170 plants m^{-2} under field conditions. Seeds of each GR and SUS *K. scoparia* inbred lines were separately sown on germination flats containing a commercial potting mix described previously. During pot filling, 5 g L^{-1} of slow release fertilizer (Osmocote® Vegetable and Bedding; 14-14-14 The Scotts Company LLC, Marysville, OH) was added to the potting mix. *K. scoparia* seedlings (2- to 4-cm tall) were transplanted into 20-L plastic pots filled with the same potting mix. Plants of each *K. scoparia* inbred grown under intraspecific competition (4 and 8 plants pot^{-1}) were evenly spaced around the circumference (2 to 4 cm away from the edge) of the pot at the time of transplanting. Greenhouse was maintained at similar temperature and photoperiod regimes as described previously. All plants were watered daily (90 to 100% of the field capacity) 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. Growth measurements including plant height (distance between base and terminal leaf), plant width (maximum plant canopy diameter), and number of primary branches $plant^{-1}$ were recorded at the maximum vegetative stage (at the first visible sign of flower initiation). Plants were then harvested at the soil level, and leaves were manually separated from the branches and stem tissues. The total leaf area $plant^{-1}$ was determined using a leaf area meter (LI-COR 3000, LII-COR, Inc., Lincoln, NE). The aboveground vegetative biomass (leaves, stems/branches) was oven dried at 60 °C for 5 d, and shoot biomass $plant^{-1}$ was determined.

For fecundity assessments, out of ten replicated *K. scoparia* plants, five plants per treatment were individually covered with pollination bags to prevent cross pollination,

and to measure the fecundity parameters at maturity. *K. scoparia* plants covered with pollination bags were manually harvested at maturity (plant fully brown in appearance), and placed in paper bags. Seeds were manually separated from the inflorescence and air dried at room temperature for two weeks; the coarse debris was removed using a 2-mm mesh size sieve, and the small debris was removed with an air-propelled column blower (Seedburo Equipment Co., 2293 S. Mt. Prospect Road, Des Plaines, IL 60018). Shoot dry weight of the aboveground vegetative tissue (stem, branches, and necrotic leaves) was determined as described previously. The weight of 1000 seeds and total seed weight plant^{-1} were determined to estimate the seed production per plant. The reproductive effort of each *K. scoparia* plant was calculated as the ratio of total seed weight to shoot dry weight.

Seed Viability and Seedling Vigor. Laboratory experiments were conducted in a completely randomized design with four replications (50 seeds per petri dish), and repeated in time. A subsample of 200 seeds per treatment was randomly selected for each inbred line from the plants grown in the fitness study. Seeds were placed between two layers of filter papers (Whatman®, Grade 2, Sigma-Aldrich Inc., St. Louis, MO 63178, USA) in 10-cm diameter petri dishes (Sigma-Aldrich) moistened with 10 ml double-distilled water. Because light is not needed for seed germination of *K. scoparia* [39], petri dishes were wrapped in aluminum foil, and placed in the dark in an incubator (VMR International, Sheldon Manufacturing Inc., Cornelius, OR 97113, USA). The incubator was set to a constant temperature of 24 °C, which is considered optimum for germination of *K. scoparia* seeds [40]. Radical lengths of germinating seedlings were recorded after

24 h of incubation. The amount of germinated seeds was counted daily until 15 d after incubation. The non-germinated seeds were tested for viability using a 1% w/v tetrazolium chloride solution [41]. Seeds were considered germinated when the radicals emerged and the tip of the radicle was uncoiled [42]. Seed viability was estimated as the percentage of total seeds that germinated plus those tested positive in the tetrazolium chloride test.

***EPSPS* Gene Copy Number and Glyphosate Resistance Level.** Greenhouse and laboratory experiments were conducted at the MSU-SARC near Huntley, MT to determine the effect of *EPSPS* gene amplification on glyphosate resistance level in GR *K. scoparia*, and repeated in time. The GR and SUS *K. scoparia* plants from the fitness experiments were used to determine relative *EPSPS* genomic copy number by using quantitative real-time PCR (qPCR) as per the previously established protocol [17, 21]. From each actively growing young GR and SUS *K. scoparia* plant (8- to 10-cm tall), 100 mg of leaf tissue was sampled and the genomic DNA was extracted using a DNeasy plant mini kit (Qiagen) following the manufacturer's protocol. The DNA concentration from each sample was determined with a SmartSpec plus spectrophotometer (Bio-Rad Company), and the quality was ensured using 1% agarose gel electrophoresis. Only high quality (260/280 ratio of $\geq$ 1.8) DNA samples were utilized in the *EPSPS* copy number determination. The *ALS* gene was selected as a reference gene due to its stability across kochia populations [21]. Primer sequences used for the *EPSPS* and *ALS* genes of *K. scoparia* have been previously reported [21]. The primer set for the *EPSPS* gene (EPSPF1: 5'-GGCCAAAAGGG CAATC GTGGAG-3' and EPSPR1: 5'-

CATTGCCGTTCCCG CGTTTCC-3') produced approximately 102-bp product, and the *ALS* primer set (ALSF1: 5'-ATGCAGA CAATGT TGGATAC-3' and ALSR1: 5'-TCAACCATCG ATA CGAACAT-3') amplified 159-bp PCR products. For each primer pair, standard curves were developed using a 10-fold serial dilution of the high quality DNA, and primer efficiency was estimated to be 101% for *EPSPS* and 100% for *ALS*. The qPCR reaction contained 2.5 µl of DNA (2 ng ul^{-1}) template, 1X Perfecta SYBR green supermix, 250 nM of each of forward and reverse primers. From the standard curves on dilution series, the reaction efficiency for qPCR assay was estimated to be 101% (R^2 of 0.99 and a slope of -3.291). Each qPCR reaction was performed in triplicates, with a final reaction volume of 20 µl on a Bio-Rad 96-well PCR plate. The qPCR assay was performed on CFX Connect Real-Time PCR detection system (Bio-Rad Company) under following conditions: 98 °C for 2 min, 40 cycles of 98 °C for 5 s, and 60 °C for 30 s, followed by melt-curve analysis. A negative control consisting of 250 nM of each primer, SYBR green supermix, and deionized water with no DNA template was included. The *EPSPS* genomic copy number relative to *ALS* gene was quantified by ΔC_T method ($\Delta C_T = C_T$, *ALS* – C_T, *EPSPS*) [17, 21]. The relative increase in the *EPSPS* gene copy number was calculated as $2^{\Delta CT}$ [17].

After determining the *EPSPS* gene copy number, two set of clones from the GR and SUS *K. scoparia* plant used in the fitness experiments were prepared by precisely cutting two 5-cm long sections from the lateral branches of each plant, when the plants attained 80 cm in height. After removing leaves from the base, those branches were re-cut at an angle to enhance the uptake of rooting hormone. Cuttings were dipped into

commercial root hormone [RootBoost (Indole-3-butyric acid 0.1%) -Rooting Hormone, TechPac LLC. 2030 Powers Ferry Road, Ste. 370 Atlanta, GA 30339] and planted in plastic pots (6 × 6 × 10 cm) containing the same potting mix as previously described. A total of 150 clones per *K. scoparia* inbred line (SUS, CHES01, or JOP01) were prepared, and subdivided into two different sets. One set of actively growing 8- to 10-cm-tall *K. scoparia* clones were treated with 870 g ha^{-1} of glyphosate. The second set of clones were treated with glyphosate at 4350 g ha^{-1}. Glyphosate treatments were applied using a stationary cabinet spray chamber as described previously. Ammonium sulfate (AMS) at 2% (w/v) was included with all glyphosate treatments. After the glyphosate treatment, clones were returned back to the greenhouse, watered daily to avoid moisture stress, and fertilized. Survival scores on a scale of 0 to 1 (0 = dead, 1 = live) were assigned depending on whether those *K. scoparia* clones survived 870 or 4350 g ha^{-1} of glyphosate at 21 DAT.

Statistical Analyses. All data were subjected to ANOVA using PROC MIXED in SAS (SAS Institute Inc., Cary, NC) to test the significance of experimental run, *K. scoparia* inbred line, and treatment (plant density in the fitness study or glyphosate dose in resistance level experiment) and their interactions. Data were checked to test normality of residuals and homogeneity of variance using PROC UNIVARIATE and PROC GLM in SAS (Statistical Analysis Systems®, version 9.2, SAS Institute Inc., SAS Campus Drive, Cary, NC 27513-2414). Based on the nonsignificant ($P > 0.05$) experimental run by treatment interaction, data were pooled across runs for both the studies. A Pearson's correlation analysis was performed to test the relationship between the *EPSPS* gene copy

number and fitness-related traits in GR and SUS *K. scoparia*, and the Pearson's correlation coefficient (r) was estimated using a simple cor.test in *R* software (R core team, R foundation for statistical computing). To assess the effects of the *EPSPS* gene amplification on level of glyphosate resistance, the survival scores (binary response) of CHES01 and JOP01 *K. scoparia* clones treated with glyphosate were regressed against the *EPSPS* gene copy number using a two-parameter log-logistic model,

$$Y = \frac{1}{1+\exp[B\ (\log(X)-\log(E)]} \qquad (1)$$

where Y refers to survival (0 or 1) of GR *K. scoparia* clone, B is the slope, E is the *EPSPS* genomic copy number required for 50% probability of survival at a glyphosate dose, and X is the *EPSPS* genomic copy number.

Results

Fitness Study

Vegetative Parameters The pleiotropic effects of the *EPSPS* gene amplification on fitness of GR *K. scoparia* were assessed across an intraspecific competition gradient. Results from ANOVA indicated that the vegetative growth parameters tested did not differ between the *K. scoparia* inbred lines (GR or SUS) ($P = 0.1425$); however, those parameters were influenced by the main effect of *K. scoparia* density ($P < 0.001$). Increasing the density from 1 to 170 plants m^{-2} reduced the vegetative growth of plants, indicating the effect of intraspecific competition. Averaged across inbred lines, increasing the density from 1 to 170 plants m^{-2} reduced plant height by 19%, plant width by 73%, and number of primary branches by 36% (Table 4.1). Total leaf area of *K. scoparia* plants

grown under intraspecific competition with densities of 85 and 170 plants m^{-2} were 88 and 95%, respectively, less compared with the plants grown in the absence of competition. Similarly, increasing the intraspecific competition from 1 to 170 plants m^{-2} reduced shoot biomass accumulation per plant by 83%, irrespective of GR or SUS plants (Table 4.1).

Reproductive Parameters. Similar to vegetative growth parameters, the reproductive fitness attributes were also influenced by the *K. scoparia* density only ($P < 0.001$). Averaged across the three inbred lines tested, plants grown under 85 and 170 plants m^{-2} had 32 and 55% less reproductive effort, respectively, compared with the plants grown in the absence of competition (1 plant m^{-2}) (Table 4.2). Similarly, an increase in density from 1 to 85 plants m^{-2} caused a significant reduction in 1000 seed weight; however, no further decline was observed at a density of 170 plants m^{-2} (Table 4.2). Seed weight is an indicator of seedling vigor [43].

Unlike other reproductive traits, seed viability was not influenced by *K. scoparia* density, and ranged from 98 to 99% across the GR and SUS inbred lines (Table 4.2). In the germination assay, progeny seedlings produced by GR or SUS plants grown under intraspecific competition level of 85 and 170 plants m^{-2} had 30 and 33% less radical lengths, respectively, compared with the seedlings from the plants grown in the absence of competition (1 plant m^{-2}).

Relationship between *EPSPS* Genomic Copy Number and Fitness. Averaged across the intraspecific competition gradient (1 to 170 plants m^{-2}), the *EPSPS* genomic copy

number ranged from 2 to 14 among individuals of CHES01 and JOP01 inbred lines tested (Fig. 4.1). In contrast, the SUS individuals had a single copy of the *EPSPS* gene.

The quantitative differences in fitness traits (vegetative and reproductive) of GR vs. SUS *K. scoparia* plants in relation to the *EPSPS* gene copy number were investigated by pairwise correlation analysis. The *EPSPS* gene copy number did not exhibit any significant correlation with plant height (P = 0.337), plant width (P = 0.418), primary branches (P = 0.457), total leaf area (P = 0.284), and shoot biomass (P = 0.319) (Fig. 4.2). Similarly, there was no significant correlation of the *EPSPS* genomic copy number with reproductive effort (P = 0.370) and 1000 seed weight (P = 0.592) of GR and SUS *K. scoparia* plants. Plants of GR *K. scoparia* (CHES01 and JOP01) with 10 to 14 copies of the *EPSPS* gene had similar growth and reproductive characteristics, as observed in GR plants with 2 to 5 *EPSPS* gene copy number or SUS plants with a single copy of the gene (Fig. 4.2).

***EPSPS* Gene Copy Number and Glyphosate Resistance Level.** Survival of clones derived from GR (CHES01 and JOP01 with 2 to 14-fold amplification of the *EPSPS* gene) and SUS (with a single copy of the *EPSPS* gene) *K. scoparia* inbred lines was determined by spraying with 870 or 4350 g ha^{-1} of glyphosate. None of the SUS *K. scoparia* clones survived (100% visual control at 21 DAT) glyphosate applied at 870 or 4356 g ha^{-1}; whereas, all clones of CHES01 and JOP01 survived (<15% visual control) the field-use rate (870 g ha^{-1}) of glyphosate at 21 DAT (Fig. 4.3). Compared to complete death of SUS *K. scoparia* with a single *EPSPS* gene copy, the response of GR CHES01 and JOP01 clones to the high rate of glyphosate (4350 g ha^{-1}) varied with *EPSPS*

genomic copy number. Some individuals of GR CHES01 and JOP01 *K. scoparia* were alive (≤ 65% control) and some were dead (≥ 95% control), when exposed to five times the field-use rate of glyphosate (4350 g ha^{-1}) at 21 DAT (Fig. 4.3 and 4.4). The Pearson's correlation analysis revealed a significant ($P < 0.0001$) positive relationship ($r = 0.72$) between the *EPSPS* genomic copy number and survival of GR CHES01 and JOP01 clones treated with 4350 g ha^{-1} of glyphosate. Survival of the glyphosate-treated GR *K. scoparia* clones (CHES01 and JOP01) increased with increase in the *EPSPS* gene copy numbers (Fig. 4.4). On the basis of the fitted log-logistic model (equation 1), *K. scoparia* clones of GR CHES01 inbred with approximately 4 *EPSPS* gene copies (95% CI: 1-6) had 5% chances of survival at the glyphosate dose of 4350 g ha^{-1}. The survival probability increased to 95% when the clones of GR CHES01 inbred had approximately 5 *EPSPS* gene copies (95% CI: 2-7) (Fig. 4.4). For GR JOP01 *K. scoparia* clones, approximately 3 (95% CI: 2-5) and 6 (95% CI: 4-8) copies of the *EPSPS* gene were needed to exhibit 5 and 95% survival probability to glyphosate applied at the 4350 g ha^{-1} rate.

Discussion and Conclusions

The amplification of the *EPSPS* gene concomitant with a high level of the EPSPS enzyme production endows glyphosate resistance in GR *K. scoparia* accessions from Montana [35], similar to that reported in GR *K. scoparia* from Kansas and Colorado, USA [21, 23]. Overproduction of the EPSPS enzyme enables GR plants to survive lethal doses of glyphosate without affecting the functionality of the shikimate pathway [16, 17].

Although not studied in GR *K. scoparia* accessions from MT, a recent study on GR *K. scoparia* from Kansas indicated a tandem configuration of the amplified copies of the *EPSPS* gene arranged about 40 to 70 kb apart, possibly mediated by unequal crossing-over during meiosis [23]. It was demonstrated that amplified copies of the *EPSPS* gene in GR *K. scoparia* exhibited a single-locus inheritance, and did not affect other enzymes involved in the shikimate pathway [21, 23]. Resource-based allocation theory dictates that acquiring such herbicide defensive trait by expressing high levels of a target-site enzyme could be associated with pleiotropic effects on the fitness traits of an HR biotype [24]. In GR *K. scoparia* with constitutive high levels of the EPSPS enzyme through amplification of the *EPSPS* gene, we hypothesized that the pleiotropic effects may arise from metabolic cost (due to extra use of cellular machinery in overproduction of the enzyme) and functional disruption of other genes by extra copies of the *EPSPS* gene in GR *K. scoparia* genome.

This research investigated if there is any pleiotropic effect of the *EPSPS* gene amplification mechanism of glyphosate resistance on growth and reproductive fitness of GR *K. scoparia.* The *EPSPS* genomic copy number varied from 2- to 14-fold among individuals of GR *K. scoparia* tested. GR *K. scoparia* accessions from Kansas and Colorado had relatively 3 to 9 *EPSPS* genomic copy numbers [21]. Results from our research indicated that the *EPSPS* gene amplification did not confer any vegetative or reproductive fitness penalty or benefits in GR *K. scoparia* in the absence of glyphosate. The *EPSPS* gene amplification had an additive effect on glyphosate resistance level in GR *K. scoparia*. From a management standpoint, glyphosate resistance level in *K.*

scoparia populations are most likely to increase with increase in glyphosate selection pressure.

Giacomini et al. [31] and Vila-Aiub et al. [32] also reported no fitness cost associated with amplification of the *EPSPS* gene in GR *A. palmeri.* However, our results are contrary to those previously reported by Wang et al. [33], where a fitness advantage in growth, seed production, and seed germination was observed with transgenic over-expression of the native *EPSPS* gene in GR wild rice. A fitness cost associated with the amplification of the resistant genes has also been observed in other organisms like insects, bacteria, and yeast [44, 45, 46]. These discrepancies further support the previously established hypothesis that fitness cost associated with a resistance trait is not universal, and it varies with species, underlying resistance mechanism, genetic background, interrelating biotic and abiotic stresses, and environmental conditions [24, 25].

Prior to initiating any fitness study on an HR weed biotype, control of genetic background and understanding the biochemical or genetic mechanism of resistance are important factors which should be considered [24, 25]. *K. scoparia* is a monoecious species, with numerous small inconspicuous flowers present in the leaf axil of the whole plant, and the protogynous nature of flowering (stigma receptive before anthers dehisce) does not allow individual flowers to self-pollinate easily [3, 7]; therefore, creation of isogenic lines of GR *K. scoparia* is both time consuming and labor intensive. In the present research, we employed an alternative approach of recurrent group selection to generate the GR and SUS *K. scoparia* inbred lines from field-collected accessions (in

close proximity) to assess the fitness traits, an established method utilized by several other researchers conducting fitness studies in HR weed species [36-38]. It is important to note that this study utilized two GR *K. scoparia* inbred lines to assess the pleiotropic effects of the glyphosate resistance on fitness traits. To further confirm the results reported in this study, inheritance in the *EPSPS* gene copy number of GR *K. scoparia* should be assessed over multiple generations in the absence of glyphosate selection pressure. Future research should also focus on the growth and reproductive fitness of GR *K. scoparia* in the presence of crop competition. Other fitness traits such as seed germination dynamics, seedling emergence pattern, and fecundity should also be assessed under varying levels of glyphosate selection pressure [47]. In these contexts, results obtained from the current research will serve as a foundation for designing future studies on population dynamics and demographics of GR vs. SUS *K. scoparia* under field conditions.

To best of our knowledge, this is first report on the effect of *EPSPS* gene amplification on growth and reproductive fitness of GR *K. scoparia*. In the absence of any fitness penalty, and with an additive effect of the *EPSPS* gene amplification on glyphosate resistance level, GR *K. scoparia* individuals with high *EPSPS* gene copies will most likely persist in field populations under continuous glyphosate selection pressure. Furthermore, wind-mediated tumble mechanism of seed dispersal coupled with pollen-mediated gene flow would ensure rapid spread of glyphosate resistance in *K. scoparia* populations in the Great Plains of North America. Growers should make all

possible efforts to prevent seed production from GR *K. scoparia* plants in their production farms.

Acknowledgments

We greatly appreciate Mr. M. Johnson, and growers from Liberty County of Montana for their valuable assistance in kochia seed collection. We also thank Ms. Anjani, Mr. S. Misra, and Mr. C. A. Lim for their assistance in greenhouse and laboratory experiments.

Table 4.1. Effect of increasing intraspecific competition on growth traits averaged over glyphosate-resistant (CHES01 and JOP01) and glyphosate-susceptible (SUS) *Kochia scoparia* inbred lines tested [a-c]

Density (plants m^{-2})	Plant height (cm)	Plant width (cm)	Primary branches (# $plant^{-1}$)	Leaf area (cm^2 $plant^{-1}$)	Shoot biomass (g $plant^{-1}$)
1	196.6 (2.1) A	143.3 (1.2) A	55 (1.1) A	11750.0 (201.1) A	150.5 (0.9) A
85	167.1 (1.0) B	56.3 (1.3) B	44 (0.6) B	1437.4 (172.7) B	31.8 (0.6) B
170	160.1 (0.5) C	39.5 (1.5) C	35 (0.4) C	562.2 (66.1) C	25.2 (0.5) C

[a]Values in parenthesis represent standard error of the mean.

[b] Means within a column with similar letters are not different based on Fisher's Protected LSD test at $P < 0.05$

[c] Data were pooled from two replicated experiments (n = 110).

Table 4.2. Effect of increasing intraspecific competition on reproductive effort, 1000 seed weight, seed viability, and radical length of germinating seedlings averaged over glyphosate-resistant (CHES01 and JOP01) and glyphosate-susceptible (SUS) *Kochia scoparia* inbred lines [a, b]

Density (plants m^{-2})	Reproductive effort [c]	1000 seed weight (g)	Seed viability [d] (%)	Radical length (cm) [e]
1	0.038 (0.002) A	0.686 (0.03) A	98 (0.93) A	1.90 (0.03) A
85	0.026 (0.001) B	0.501 (0.02) B	99 (0.84) A	1.32 (0.01) B
170	0.017 (0.001) C	0.465 (0.01) B	99 (0.81) A	1.27 (0.02) B

[a] Values in parenthesis represent standard error of the mean.
[b] Means within a column with similar letters are not different based on Fisher's protected LSD test at $P < 0.05$. Data were pooled from two replicated experiments (n = 110)
[c] Estimated as ratio of seed weight to shoot dry weight per plant.
[d] Seed viability was determined as the percentage of total seeds that germinated plus those tested positive in tetrazolium chloride test.
[e] Radical length was recorded at 24 h after incubation of GR and SUS *K. scoparia* seeds in the germination assay.

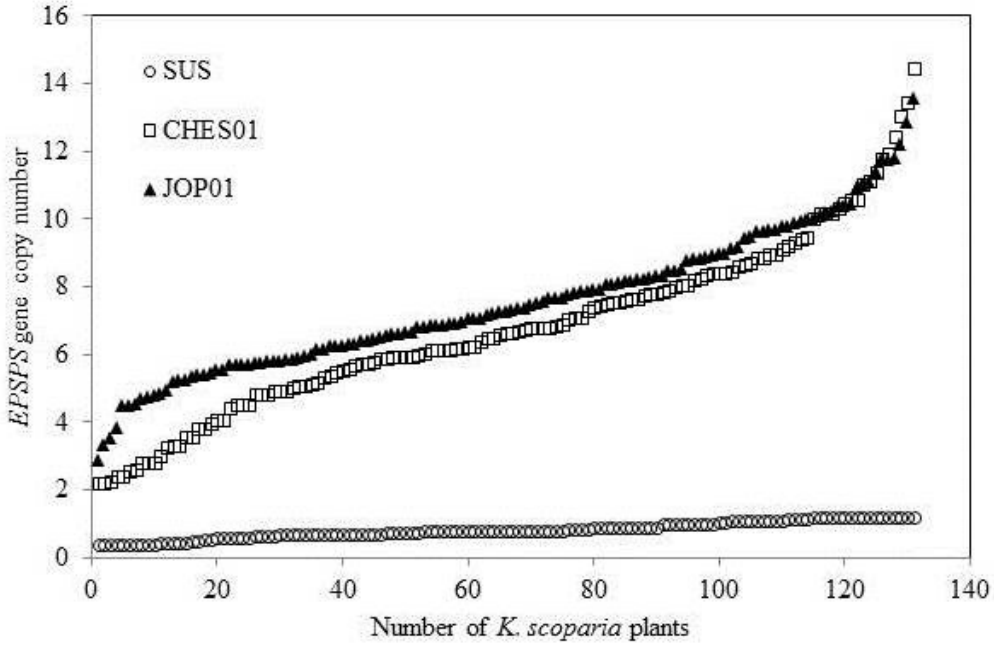

Figure 4.1. Variation of the *EPSPS* gene copy number in inbred lines of GR and SUS *K. scoparia*. CHES01 and JOP01 are glyphosate-resistant *K. scoparia* inbred lines and SUS is the glyphosate-susceptible inbred line.

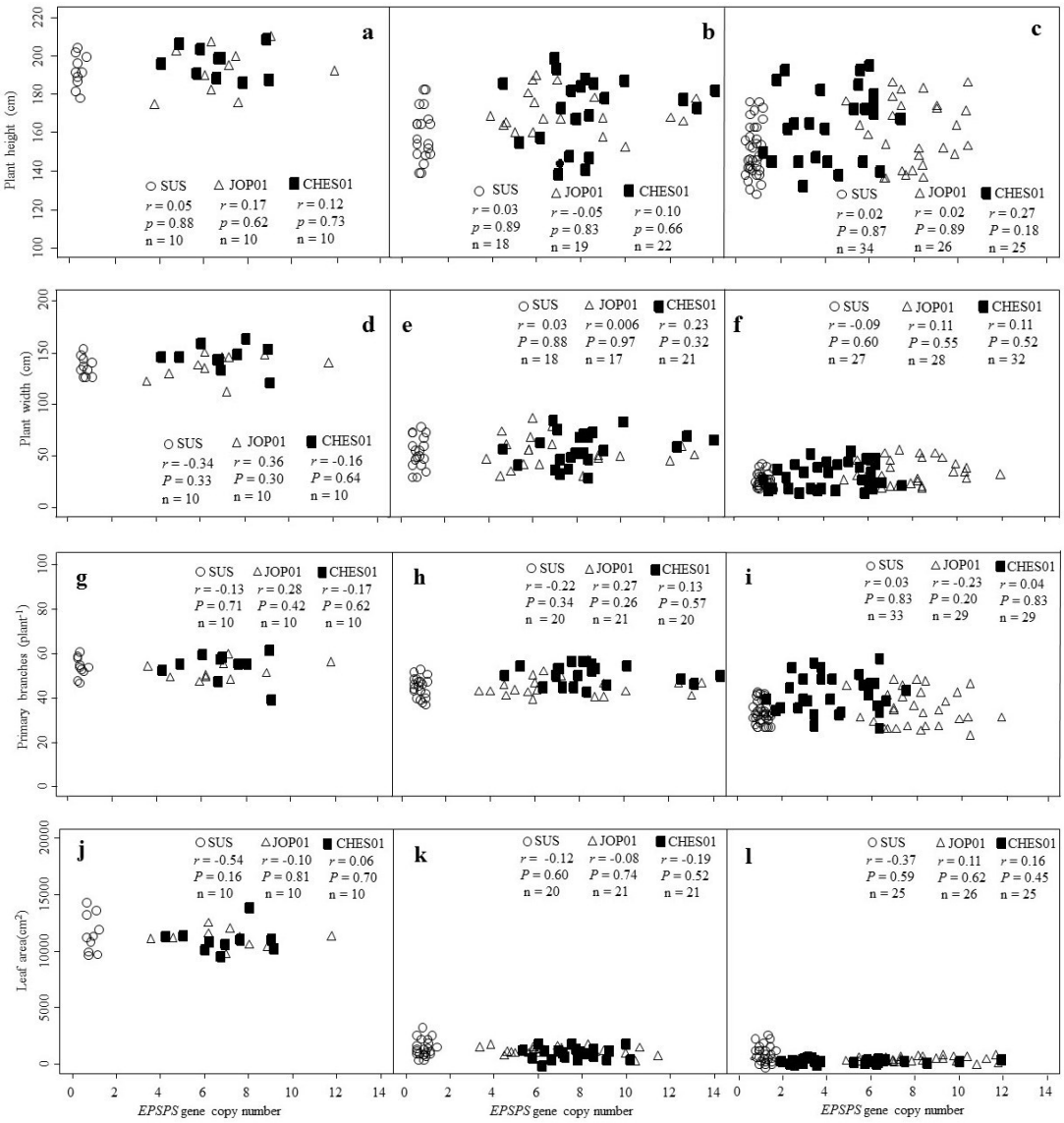

Figure 4.2. Relationship of fitness traits with *EPSPS* gene copy number in GR (CHES01 and JOP01) vs. SUS *K. scoparia* inbred lines grown under densities of 1 (a, d, g, j, m, p, s), 85 (b, e, h, k, n, q, t), and 170 (c, f, i, l, o, r, u) plants m^{-2}. CHES01 and JOP01 are glyphosate-resistant *K. scoparia* inbred lines and SUS is a glyphosate-susceptible inbred (Continued...)

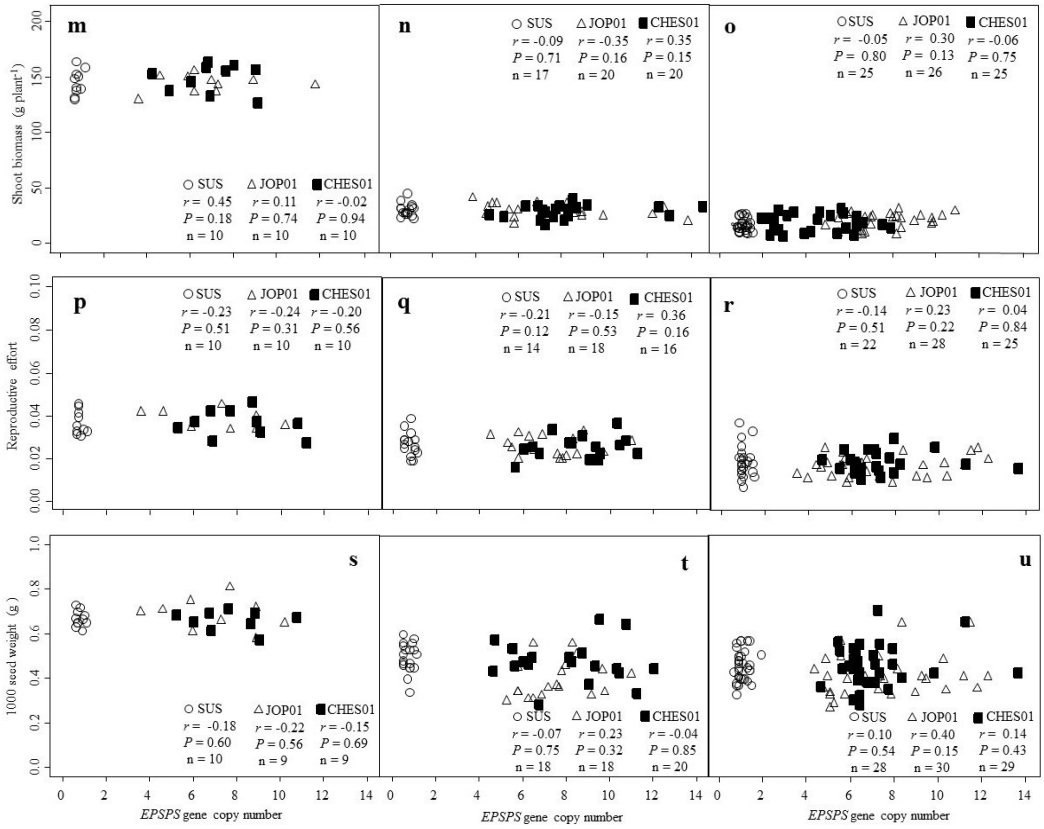

Figure 4.2. Relationship of fitness traits with *EPSPS* gene copy number in GR (CHES01 and JOP01) vs. SUS *K. scoparia* inbred lines grown under densities of 1 (a, d, g, j, m, p, s), 85 (b, e, h, k, n, q, t), and 170 (c, f, i, l, o, r, u) plants m^{-2}. CHES01 and JOP01 are glyphosate-resistant *K. scoparia* inbred lines and SUS is a glyphosate-susceptible inbred.

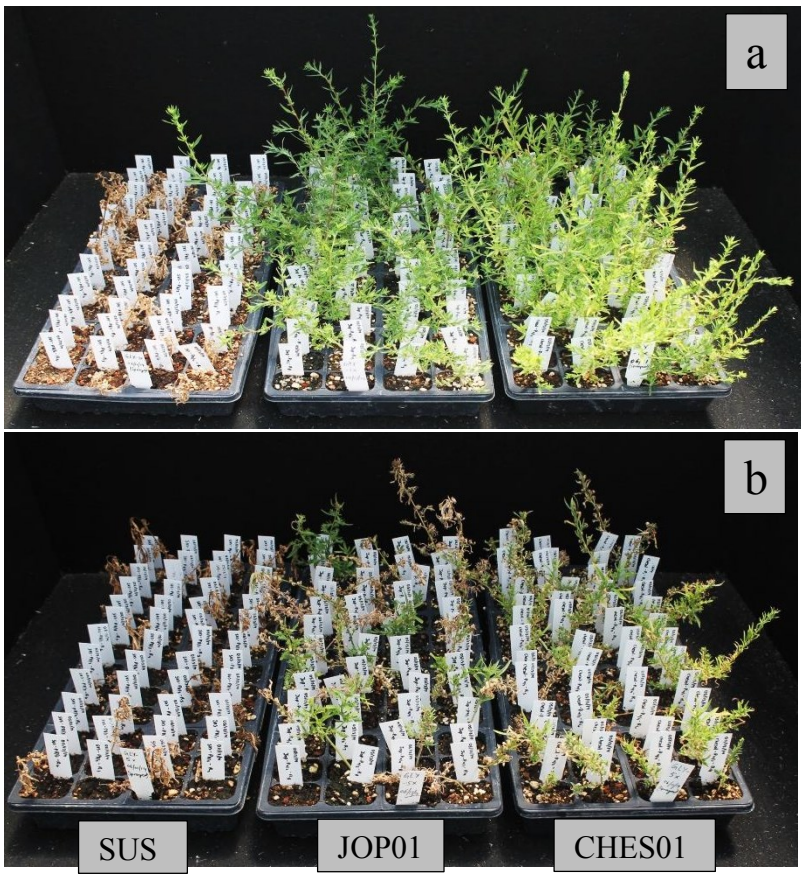

Figure 4.3. Response of SUS, JOP01, and CHES01 *K. scoparia* clones (derived from inbred lines) to (a) 870 g ha^{-1} and (b) 4350 g ha^{-1} of glyphosate at 21 d after treatment. CHES01 and JOP01 are glyphosate-resistant *K. scoparia* inbred lines and SUS is a susceptible inbred.

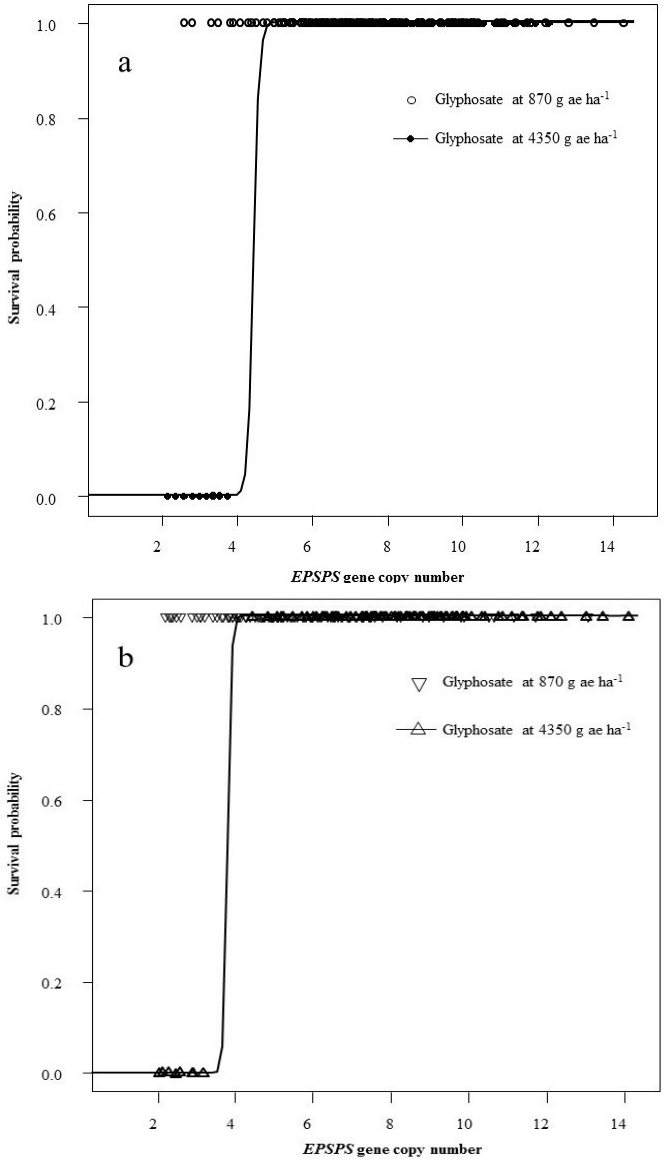

Figure 4.4. Relationship of glyphosate resistance level to *EPSPS* gene copy number of (a) CHES01 and (b) JOP01 GR *K. scoparia* inbred lines treated with 870 or 4350 g ae ha^{-1} of glyphosate.

References

(1) Eberlein CV, Fore ZA. Kochia biology. Weeds Today. 1984; 15: 5–6.

(2) Forcella F. Spread of kochia in the northwestern United States. Weeds Today. 1985; 16: 4–6.

(3) Friesen LF, Beckie HJ, Warwick SI, Van Acker RC. The biology of Canadian weeds. 138. *Kochia scoparia* (L.) Schrad. Can J Plant Sci. 2009; 89: 141–167.

(4) Baker DV, Withrow JR, Brown CS, Beck KG. Tumbling: Use of diffuse knapweed (*Centaurea diffusa*) to examine an understudied dispersal mechanism. Invasive Plant Sci Manag. 2010; 3: 301–309.

(5) Christoffoleti PJ, Westra PB, Moore F. Growth analysis of sulfonylurea-resistant and -susceptible kochia (*Kochia scoparia*). Weed Sci. 1997; 45: 691–695.

(6) Schwinghamer TD, Van Acker RC. Emergence timing and persistence of kochia (*Kochia Scoparia*). Weed Sci. 2008; 56: 37–41.

(7) Mengistu, LW, Messersmith CG. Genetic diversity of kochia. Weed Sci. 2002; 50: 498–503.

(8) Stallings GP, Thill DC, Mallory-Smith CA, Shafii B. Pollen-mediated gene flow of sulfonylurea-resistant kochia (*Kochia scoparia*). Weed Sci. 1995; 43: 95–102.

(9) Cranston, HJ, Kern AJ, Hackett JL, Miller EK, Maxwell BD, Dyer WE, et al. Dicamba resistance in kochia. Weed Sci. 2001; 49: 164–170.

(10) Heap IM. International survey of herbicide resistant weeds. Weed Science Society of America website. Available: http://www.weedscience.org. Accessed 2015 Jan 15.

(11) Kumar V, Jha P, Reichard N. Occurrence and characterization of kochia (*Kochia scoparia*) accessions with resistance to glyphosate in Montana. Weed Technol. 2014; 28: 122–130.

(12) Primiani, MM, Cotterman JC, Saari LL. Resistance of *Kochia scoparia* to sulfonylurea and imidazolinone herbicides. Weed Technol. 1990; 4: 169–172.

(13) Waite J, Thompson CR, Peterson DE, Currie RS, Olson BLS, Stahlman PW, Khatib KA, et al. Differential kochia (*Kochia scoparia*) populations response to glyphosate. Weed Sci. 2013; 61:193–200.

(14) Beckie HJ, Blackshaw RE, Low R, Hall LM, Sauder CA, Martin S, Brandt EN, Shirriff SW, et al. Glyphosate- and acetolactate synthase inhibitor-resistant kochia (*Kochia scoparia*) in western Canada. Weed Sci. 2013; 61: 310–318.

(15) Powles SB, Yu Q. Evolution in action: plants resistant to herbicides. Annu Rev Plant Biol. 2010; 61: 317–347.

(16) Sammons DR, Gaines TA. Glyphosate resistance: state of knowledge. Pest Manag Sci. 2014; 70: 1367–1377.

(17) Gaines TA, Zhang W, Wang D, Bukun B, Chisholm ST, Shaner DL, Nissen SJ, Patzoldt WL, Tranel PJ, Culpepper AS, Grey TL, Webster TM, Vencill WK, Sammons RD, Jiang J, Preston C, Leach JE, Westra P, et al. Gene amplification confers glyphosate resistance in Amaranthus palmeri. Proc Natl Acad Sci USA. 2010; 107: 1029–1034.

(18) Lorentz L, Gaines TA, Nissen SJ, Westra P, Strek H, Dehne HW, Ruiz-Santaella JP, Beffa R, et al. Characterization of glyphosate resistance in *Amaranthus tuberculatus* populations. J Agric Food Chem. 2014; 62: 8134–8142.

(19) Nandula VK, Wright AA, Bond JA, Ray JD, Eubank TW, Molin WT, et al. EPSPS amplification in glyphosate-resistant spiny amaranth (*Amaranthus spinosus*): a case of gene transfer via interspecific hybridization from glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*). Pest Manag Sci. 2014; 70: 1902–1909.

(20) Salas RA, Dayan FE, Pan Z, Watson SB, Dickson JW, Scott RC, Burgos NR, et al. EPSPS gene amplification in glyphosate-resistant Italian ryegrass (Lolium perenne ssp. multiflorum) from Arkansas. Pest Manag Sci. 2012; 68: 1223–1230.

(21) Wiersma AT, Gaines TA, Preston C, Hamilton JP, Giacomini D, Buell CR, Leach JE, Westra P, et al. Gene amplification of 5-enol-pyruvylshikimate-3-phosphate synthase in glyphosate-resistant *Kochia scoparia.* Planta. 2015; 241: 463–474.

(22) Gaines TA, Wright AA, Molin WM, Lorentz L, Riggins CW, Tranel PJ, Beffa R, Westra P, Powles SB, et al. Identification of genetic elements associated with EPSPS gene amplification. PLoS One.2013:doi:101371/journalpone0065819

(23) Jugulam M, Niehues K, Godar AS, Koo D-H, Danilova T, Friebe B, Sehgal S, Varanasi VK, Wiersma A, Westra P, Stahlman PW, Gill BS, et al. Tandem amplification of a chromosomal segment harboring EPSPS locus confers glyphosate resistance in Kochia scoparia. Plant Physiol. 2014. doi:10.1104/pp.1114.242826

(24) Vila-Aiub MM, Neve P, Powles SB. Fitness costs associated with evolved herbicide resistance alleles in plants. New Phytol. 2009; 184: 751–767.

(25) Vila-Aiub MM, Gundel PE, Preston C. Experimental methods for estimation of plant fitness costs associated with herbicide-resistance genes. Weed Sci. 2015; SI: 203–216.

(26) Baucom RS, Mauricio R. Fitness costs and benefits of novel herbicide tolerance in a noxious weed. Proc Natl Acad Sci USA. 2004; 101: 13386–13390.

(27) Preston C, Wakelin AM. Resistance to glyphosate from altered herbicide translocation patterns. Pest Manage Sci. 2008; 64: 372-376.

(28) Pederson BP, Neve P, Andreasen C, Powles SB. Ecological fitness of a glyphosate-resistant Lolium rigidum population: growth and seed production along a competition gradient. Basic Appl Ecol. 2007; 8: 258–268.

(29) Westhoven AM, Kruger GR, Gerber CK, Stachler JM, Loux MM, Johnson WG. Characterization of selected common lambsquarters (*Chenopodium album*) biotypes with tolerance to glyphosate. Weed Sci. 2008; 56:685–691.

(30) Davis VM, Gibson KD, Bauman TT, Weller SC, Johnson WG. Influence of weed management practices and crop rotation on glyphosate-resistant horseweed (*Conyza canadensis*) population dynamics and crop yield—years III and IV. Weed Sci. 2009; 57: 417–426.

(31) Giacomini D, Westra P, Ward SM. Impact of genetic background in fitness cost studies: an example from glyphosate-resistant Palmer amaranth. Weed Sci. 2014; 62: 29–37.

(32) Vila-Aiub MM, Goh SS, Gaines TA, Han H, Busi R, Yu Q, Powles SB, et al. No fitness cost of glyphosate resistance endowed by massive EPSPS gene amplification in *Amaranthus palmeri*. Planta. 2014; 239: 793–801.

(33) Wang W, Xia H, Yang X, Xu T, Si HJ, Cai XX, Wang F, Su J, Snow AA, Lu BR, et al. A novel 5-enolpyruvoylshikimate-3-phosphate (EPSP) synthase transgene for glyphosate resistance stimulates growth and fecundity in weedy rice (Oryza sativa) without herbicide. New Phytol. 2013; 202: 679–688. doi:10.1111/nph.12428

(34) Alcorta M, Fidelibus MW, Steenwerth KL, Shrestha A. Competitive effects of glyphosate-resistant and glyphosate-susceptible horseweed (*Conyza canadensis*) on young grapevines (*Vitis vinifera*). Weed Sci. 2011; 59:489–494.

(35) Kumar V, Jha P, Westra P, Westra E, Giacomini D, Vanhorn C. EPSPS gene amplification confers glyphosate resistance in kochia (*Kochia scoparia*). Proc. Weed Sci Soc Am. 2014.381.Available: http://wssaabstracts.com/public/22/abstract-381.html.

(36) Légère A, Stevenson FC, Beckie HJ, Warwick SI, Johnson EN, Hrynewich B, Lozinski C. Growth characterization of kochia (*Kochia scoparia*) with substitutions at Pro^{197} or Trp^{574} conferring resistance to acetolactate synthase–inhibiting herbicides. Weed Sci. 2013; 61:267–276.

(37) Lehnhoff EA, Keith BK, Dyer WE, Menalled FD. Impact of biotic and abiotic stresses on the competitive ability of multiple herbicide resistant wild oats (*Avena fatua*). PLoS ONE. 2013; 8 (5):e64478. doi: 10.1371/journal.pone.0064478

(38) Menalled FD, Smith RG. Competitiveness of herbicide-resistant and herbicide-susceptible kochia (*Kochia scoparia* [L.] Schrad.) under contrasting management practices. Weed Biol Manag. 2007; 7:115–119.

(39) Everitt JH, Alaniz MA, Lee JB. Seed germination characteristics of *Kochia scoparia*. J. Range Manage. 1983; 36: 646−648.

(40) Al-Ahmadi MJ, Kafi M. Cardinal temperatures for germination of *Kochia scoparia* (L.). J. Arid Environ. 2007; 68: 308−314.

(41) Sbatella GM, Wilson RG. Isoxaflutole shifts kochia (*Kochia scoparia*) populations in continuous corn. Weed Technol. 2010; 24: 392−396.

(42) Young JA, Evans RA, Stevens R, Everett RL. Germination of *Kochia prostrata* seed. Agron. J. 1981; 73: 957–961.

(43) Steadman KJ, Eaton DM, Plummer JA, Ferris DJ, Powles SB. Late-season non-selective herbicide applications reduce Lolium rigidum seed numbers, seed viability, and seedling fitness. Aust J Agric Res. 2006; 57:133–141.

(44) Field LM, Foster SP. Amplified esterase genes and their relationship with other insecticide resistance mechanisms in English field populations of the aphid, Myzus persicae (Sulzer). Pest Manag Sci. 2002; 58: 889–894.

(45) Stoebel DM, Dean AM, Dykhuizen DE. The cost of expression of Escherichia coli lac operon proteins is in the process, not in the products. Genetics. 2008; 178: 1653–1660.

(46) Wagner A. Energy constraints on the evolution of gene expression. Mol Biol Evol. 2005; 22: 1365–1374.

(47) Délye C, Menchari Y, Michel S, Cadet É, Corre VL. A new insight into arable weed adaptive evolution: mutations endowing herbicide resistance also affect germination dynamics and seedling emergence. Ann of Bot. 2013; 111: 481–691.

CHAPTER 5

EFFECTIVE PREEMERGENCE AND POSTEMERGENCE HERBICIDE PROGRAMS FOR KOCHIA CONTROL

Contribution of Authors and Co-Authors

Manuscript in Chapter 5

Author: Vipan Kumar

Contributions: Conceived and implemented the study design. Conducted experiments, reviewed the literature, and wrote the manuscript.

Co-Author: Prashant Jha

Contributions: Advisement, provided field expertise and feedback on manuscript drafts

Manuscript Information Page

Vipan Kumar, Prashant Jha
Weed Technology Journal
Status of Manuscript:
___Prepared for submission to a peer-reviewed journal
___Officially submitted to a peer-review journal
___Accepted by a peer-reviewed journal
_×_Published in a peer-reviewed journal

Published by Allen Press, Inc.
Published in 2015, volume 29, Issue 1

Abstract

Field experiments were conducted in 2011 through 2013 at the MSU Southern Agricultural Research Center near Huntley, MT, to evaluate the effectiveness of various PRE and POST herbicide programs for kochia control in the absence of a crop. PRE herbicides labeled for corn, grain sorghum, soybean, wheat/barley, and/or in chemical-fallow were applied at recommended field-use rates. Acetochlor + atrazine, S-metolachlor + atrazine + mesotrione, and sulfentrazone applied PRE provided ≥ 91% control of kochia at 12 WAT. Metribuzin, metribuzin + linuron, and pyroxasulfone + atrazine PRE provided 82% control at 12 WAT. PRE control with acetochlor + flumetsulam + clopyralid, pyroxasulfone alone, and saflufenacil + 2, 4-D was ≤ 23% at 12 WAT. Paraquat + atrazine, paraquat + linuron, and paraquat + metribuzin controlled ≥ 98% kochia at 5 WAT. POST control with bromoxynil + fluroxypyr, paraquat, tembotrione + atrazine, and topramezone + atrazine treatments averaged 84% at 5 WAT, and did not differ from glyphosate. Control with POST-applied bromoxynil + pyrasulfotole, dicamba, diflufenzopyr + dicamba + 2, 4-D, saflufenacil, saflufenacil + 2, 4-D, saflufenacil + linuron was 67 to 78% at 5 WAT. Due to presence of ALS-inhibitor-resistant kochia at the test site, cloransulam-methyl was not a viable option for kochia control. In a separate greenhouse study, kochia populations showed differential response to the POST herbicides (labeled for corn or soybean) tested. Tembotrione + atrazine, topramezone + atrazine, lactofen, or fomesafen effectively controlled the Gly-R kochia populations tested. Growers should utilize these effective PRE- or POST-applied herbicide premixes or tank mixtures (multiple modes of action) to control herbicide-

resistant kochia populations in the field. PRE herbicides with 8 wk of soil-residual activity on kochia would be acceptable if crop competition were present; however, a follow-up herbicide application may be needed to obtain season-long kochia control in the absence of crop competition.

Nomenclature: Glyphosate; dicamba; diflufenzopyr; fluroxypyr; glufosinate; linuron; 2,4-D; MCPA; paraquat; fomesafen; lactofen; thifensulfuron; tribenuron; flumetsulam; clopyralid; cloransulam-methyl; tembotrione; topramezone; carfentrazone-ethyl; bromoxynil; pyrasulfotole; pyroxasulfone; sulfentrazone; atrazine; acetochlor; metolachlor; flumioxazin; saflufenacil; isoxaflutole; mesotrione; kochia, *Kochia scoparia* (L.) Schrad.

Key words: PRE, preemergence; POST, postemergenc; glyphosate-resistant kochia, herbicide efficacy, residual control, herbicide resistance management.

Introduction

Kochia is one of the most troublesome summer annual broadleaf weeds in croplands and non-croplands across the Great Plains of North America (Eberlein and Fore 1984; Forcella 1985; Wicks et al. 1994). The invasiveness of kochia is attributed to its unique biological characteristics including early seedling emergence, C_4 photosynthesis, rapid growth rates, heat and salt tolerance, prolific seed production (>50,000 seeds plant^{-1}), and long-distance seed dispersal by tumbling (Baker et al. 2010; Christoffoleti et al. 1997; Friesen et al. 2009; Schwinghamer and Van Acker 2008). Furthermore, the protogynous nature of kochia flowering enforces a high degree of out-crossing and pollen-mediated gene flow (Mengistu and Messersmith 2002; Stallings et al. 1995). Consequently, the presence of high genetic diversity within and among kochia populations has also been reported (Mengistu and Messersmith 2002).

Kochia causes season-long interference and yield reductions in agronomic crops of the Great Plains including wheat (*Triticum aestivum* L.), corn (*Zea mays* L.), sorghum (*Sorghum bicolor* L.), sugar beet (*Beta vulgaris* L.), sunflower (*Helianthus annus* L.), and soybean (*Glycine max* L. Merr). Season-long interference of kochia can reduce soybean, sorghum, wheat, corn, and sugar beet yields by 30, 38, 58, 40, and 95%, respectively (Dahl 1982; Durgan et al. 1990; Waite et al. 2013; Weatherspoon 1971; Wicks et al. 1993, 1994).

Growers in the northern and central Great Plains are facing ever-increasing challenges in controlling kochia due to the widespread occurrence of kochia populations resistant to one or more herbicide chemistries (Heap 2014). Kochia resistance to

herbicide modes of action including group 5 (atrazine), group 2 (sulfonylurea and imidazolinone herbicides), group 4 (dicamba and fluroxypyr), and more recently to group 9 (glyphosate) has been reported in this region (Cranston et al. 2001; Heap 2014; Kumar et al. 2014; Preston et al. 2009; Primiani et al. 1990). Glyphosate-resistant (Gly-R) kochia has been reported in seven US states, and in Alberta, Canada (Beckie et al. 2013; Heap 2014). The escalating spread of Gly-R kochia is a potential threat to Gly-R cropping systems and to the no-till chemical fallow wheat rotation common in the region. Occurrence of Gly-R kochia populations with 4.6- to 11-fold levels of resistance to glyphosate has been recently confirmed in chemical-fallow (chemical-fallow and wheat rotation) fields of Hill, Liberty, and Toole Counties of Montana (Kumar et al. 2014).

Weed control programs aimed at managing herbicide-resistant weeds recommend the use of annual herbicide rotation and tank-mixtures with multiple modes of action (Beckie et al. 2006; Norsworthy et al. 2012). Diversifying herbicide programs with the use of soil-applied residual PRE herbicides in conjunction with effective POST herbicides is critical for management of herbicide-resistant weeds (Norsworthy et al. 2012; Stephenson et al. 2013). Previous researchers have reported variable levels of kochia control with PRE or POST herbicides. In addition, use of a single mode of action herbicide often results in inconsistent control of kochia. For instance, kochia control with flumioxazin (0.28 kg ha^{-1}), pendimethalin (1.4 kg ha^{-1}), or pyroxasulfone (0.418 kg ha^{-1}) applied alone PRE was only 53 to 70% (Lloyd et al. 2011; Stahlman 2010). Among POST herbicides tested by Lloyd et al. (2011), control with either fluroxypyr or glyphosate did not exceed 75%, and was as low as 35% with saflufenacil alone.

Although bromoxynil + MCPA, carfentrazone, or paraquat applied POST controlled kochia ≥ 90% under greenhouse conditions (Nandula and Manthey 2002; Tonks and Westra 1997), control as low as 68% was observed with those herbicides under field conditions (Wick et al. 1993). This might be due to potential regrowth of the treated plants or lack of residual activity of the herbicide on cohort(s) that emerged after the POST application, which warrants the need to investigate effective PRE- or POST-applied herbicide combinations (multiple modes of action) for full-season kochia control.

The ever-increasing frequency of herbicide-resistant kochia is a major threat to the Great Plains cropping systems. Although alternative (non-glyphosate) herbicide options for Gly-R kochia control in wheat-fallow rotation has been recently documented (Kumar et al. 2014), there is no published research on alternative herbicides for effective control of Gly-R kochia in Gly-R corn or soybean in the Great Plains. The objectives of this research were to determine effective PRE and POST herbicide programs for kochia control in the field, and to test the effectiveness of alternative (non-glyphosate) POST herbicides labeled in corn or soybean for control of Gly-R kochia populations.

Materials and Methods

PRE Herbicide Programs. Field experiments were initiated at the Montana State University (MSU) Southern Agricultural Research Center (SARC) near Huntley, MT in 2011, and repeated in 2012 and 2013, to test the effectiveness of soil-applied PRE herbicides labeled for corn, grain sorghum, soybean, wheat/barley, and/or chemical-fallow for kochia control. Experiments were conducted in a fallow situation for long-term

evaluation of herbicide efficacy. The soil type at the study location was a Fort Collins clay loam (Fine-loamy, mixed, superactive, mesic Aridic Haplustalfs) with 2.8% organic matter and a pH of 7.8. Monthly mean air temperatures and total precipitation during the study periods are shown in Table 5.1. The experimental site was in a minimum of five year no-till dryland wheat-fallow rotation prior to the initiation of the study. Each year, the experimental site was moved to avoid any residual activity from herbicides applied the previous year. Historically, each experimental site had received two or three applications of glyphosate during the summer-fallow period, and fluroxypyr and bromoxynil plus MCPA in the wheat crop for weed control. Furthermore, the sites selected had no history of kochia infestation. During fall of each year, fully-matured kochia seeds were collected from a naturally infested area at the MSU-SARC research farm. Seeds were broadcasted in the fall to obtain uniform kochia densities in plots the following spring. PRE herbicide programs included in the study and their application date each year are presented in Tables 5.2 and 5.3. A nontreated control was included for comparison. PRE herbicide treatments were applied at their recommended field-use rates with a CO_2-pressurized backpack sprayer equipped with flat-fan nozzles (TeeJet 8001XR, Spraying Systems Co., P.O. Box 7900, Wheaton, IL), calibrated to deliver 94 L ha^{-1} of spray solution at 276 kPa

Experiments were conducted in a randomized complete block design with four replications, and plot size of 2 × 4.5 m. Kochia densities were recorded within 1-m^2 quadrats placed at the center of a nontreated plot (Table 5.3). Percent control of kochia from PRE herbicides was visually rated at 6, 8, 10, and 12 wk after treatment (WAT) on a

scale of 0 to 100%, (0 being no control and 100 being complete control). Visual assessments for percent control were based on emergence and general suppression/stunting of kochia seedlings in treated compared with nontreated plots.

POST Herbicide Programs. Separate field experiments were conducted at the MSU-SARC near Huntley, MT, in 2011, 2012, and 2013, to evaluate the effectiveness of various POST herbicide programs for kochia control. The soil and environmental conditions were similar to those described in the PRE herbicide trials. POST herbicide experiments were also conducted in a crop-free situation. In the fall of each year, the experimental site was uniformly infested with kochia seeds as described previously, and was kept free from other weed species throughout the growing season by hand weeding. POST herbicides labeled in wheat/barley, corn/grain sorghum, soybean, or chemical-fallow were selected. A nontreated control was included for comparison. POST herbicides were applied at their recommended field-use rates when kochia plants were 8- to 10-cm tall (Tables 5.3 and 5.4) in the field, using a CO2-pressurized backpack sprayer described above.

The experimental design was a randomized complete block with four replications. Kochia densities were recorded within 1-m^2 quadrats placed at the center of each plot (2 × 4.5 m) 1 d prior to the POST herbicide application (Table 5.3). Kochia control was recorded at 1, 3, and 5 WAT on a scale of 0 to 100% (0 being no control and 100 being complete control). Visual assessments for percent control were based on general chlorosis, stunting, or necrosis of treated plants.

Gly-R Kochia Control. Experiments were conducted in a greenhouse at the MSU-SARC near Huntley, MT, during spring 2013, to determine the response of Gly-R kochia populations to alternative POST herbicides (labeled in corn or soybean). The effectiveness of various POST herbicides was tested on four confirmed Gly-R kochia populations from MT including GIL01, JOP01, CHES01, and CHES02 (as described in Kumar et al. 2014) in comparison to a known glyphosate-susceptible (Gly-S) population. The Gly-R GIL01 and JOP01 populations were collected in fall 2012 from two different fields within 5 km of each other in Hill County, MT; Gly-R CHES01 and CHES02 were collected from two separate fields within 3 km of each other near Chester, Liberty County, MT. The glyphosate-susceptible (Gly-S) population was collected in fall 2012 from a field near Huntley, MT, used for long-term organic trials. Seeds of Gly-R and Gly-S populations were sown separately on the surface of 53 by 35 by 10 cm flats filled with a commercial potting media (VermiSoil^{TM}, Vermicrop Organics, 4265 Duluth Ave, Rocklin, CA). Single kochia seedlings were transplanted into 10-cm-diam pots containing the same potting medium as the germination flats. Experiments were arranged in a randomized complete block design with eight replications [one plant per pot] for each population and herbicide combination, and the experiment was repeated. The greenhouse was maintained at 26/23 ± 3 C day/night temperatures with a 16/8 h day/night photoperiods, and the supplemental photoperiod was obtained with metal halide lamps (400 μmol m^{-2} s^{-1}).

Alternative herbicide programs included in the study and their application rates are summarized in Table 5.5. All herbicides were applied at their recommended field-use

rates to 8- to 10-cm-tall kochia plants using a cabinet spray chamber (Research Track Sprayer, De Vries Manufacturing, RR 1 Box 184, Hollandale, MN) equipped with a flat-fan nozzle tip (TeeJet 8001 XR, 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., 14111 Scottslawn Road, Marysville, OH] weekly to maintain good growth.

Percent control of kochia was visually assessed at 1, 2, and 3 WAT using the rating scale described for the field experiments. At 3 WAT, kochia plants were harvested at the soil level, and shoot dry weights were determined after oven-drying the samples at 60 C for 3 d. For each kochia population, shoot dry weight data were expressed as percentage of the nontreated control.

Statistical Analyses. All data from field and greenhouse experiments were subjected to ANOVA using the PROC MIXED procedure in SAS (Statistical Analysis Systems®, version 9.2, SAS Institute Inc., SAS Campus Drive, Cary, NC 27513).Variances were divided into random effects (year or run, replication nested within a year or run, and interactions involving either of these two variables) and fixed effects (herbicide treatment for the field studies or herbicide treatment, population, and their interaction for the greenhouse study). Data on percent control and shoot dry weight (% of nontreated) were arcsine-square-root-transformed before analysis to improve the normality of residuals and homogeneity of variance. Non-transformed means are presented in tables based on the interpretation from the transformed data. Control data from nontreated plots were not

included in the analyses. Means were separated using Fisher's Protected LSD test at P <0.05.

Results and Discussion

The majority of the kochia emerged between May 10 and May 18 each year. Mean air temperatures during the month of May were 11, 13, and 14 C, in 2011, 2012, and 2013, respectively (Table 5.1). There was sufficient soil moisture from the major precipitation events (totaling ≥76 mm) that occurred between April 25 and May 10 each year to activate the PRE herbicides.

PRE Herbicide Programs. Kochia densities in PRE herbicide experiments ranged from 54 to 78 plants m^{-2} over the three years (Table 5.3). Kochia control with acetochlor + atrazine, dicamba + pendimethalin, isoxaflutole, metribuzin, metribuzin + linuron, pyroxasulfone + atrazine, S-metolachlor + atrazine + mesotrione, and sulfentrazone treatments applied PRE was 93 to 100% at 8 WAT (Table 5.6). Flumioxazin + pyroxasulfone and dicamba alone or with 2,4-D PRE provided 84 to 90% control 8 WAT (Table 5.6). PRE control with flumioxazin + pyroxasulfone (90%) was superior to flumioxazin alone (66%) or pyroxasulfone alone (61%) 8 WAT.

Consistent with the previous evaluation dates (8 and 10 WAT), control at 12 WAT with acetochlor + atrazine, acetochlor + atrazine + mesotrione, and sulfentrazone was >90% (Table 5.6). In previous research, poor kochia control (<50%) has been reported with acetochlor (1.960 kg ha^{-1}) or metolachlor (1.070 kg ha^{-1}) applied PRE in sunflower and corn (Jha et al. 2012; Reddy et al. 2012). Although atrazine alone

treatment was not included in this study, consistent control of kochia (up to 12 WAT) obtained with acetochlor + atrazine was possibly due to the residual activity of atrazine on kochia. However, widespread occurrence of triazine-resistant kochia in the US Great Plains (Heap 2014) limits the use of atrazine as a stand-alone PRE herbicide in corn. Control with metribuzin, metribuzin + linuron, and pyroxasulfone + atrazine was 80 to 83% 12 WAT.

Control with flumioxazin + pyroxasulfone and isoxaflutole declined from 92 to 70% at 12 WAT. Also, residual activity of dicamba, dicamba + 2, 4-D, dicamba + pendimethalin, and flumioxazin declined up to 50% by 12 WAT (Table 5.6). A shift in kochia population to late-emerging cohorts was a mechanism to escape residual activity of isoxaflutole applied PRE in corn over 8 yr (Sbatella and Wilson 2010). Our results further suggest that kochia has an extended period of emergence (Dille et al. 2012). Although sequential herbicide applications may be desirable to obtain season-long kochia control in fallow with some of the PRE herbicides tested, treatments providing high residual control for 8 wk (Table 5.6) may still be acceptable in the presence of a crop that will compete with the late emerging kochia cohort(s). Performance of acetochlor + flumetsulam + clopyralid, pyroxasulfone alone, or saflufenacil + 2, 4-D was poor, with PRE control as low as 14% at 12 WAT.

POST Herbicide Programs. Kochia densities at the experimental sites ranged from 79 to 108 plants m^{-2} (Table 5.3). Paraquat + atrazine provided 100% control at all evaluation dates, and was comparable to paraquat + linuron and paraquat + metribuzin treatments

(Table 5.7). The results indicate a combined soil and foliar activity of these effective herbicide mixtures on kochia.

POST control 1 WAT with bromoxynil + fluroxypyr, bromoxynil + pyrasulfotole, glyphosate, saflufenacil + linuron, and tembotrione + atrazine treatments was 92 to 95% (Table 5.7). Control with bromoxynil + MCPA, carfentrazone-ethyl + 2, 4-D, glufosinate, lactofen, and saflufenacil was 87 to 90% 1 WAT. Control with dicamba alone, diflufenzopyr + dicamba + 2, 4-D, and topramezone + atrazine was 82% at 1 WAT. Fomesafen and S-metolachlor + mesotrione applied POST provided 62 to 77% control of kochia 1 WAT. Control with cloransulam-methyl and thifensulfuron + tribenuron [Group 2, acetolactate synthase (ALS) inhibitor herbicides)] + 2,4 D was 35 and 57%, respectively, which was due to the presence of ALS-inhibitor-resistant kochia at the experimental sites (data not shown).

In general, kochia control declined by 3 or 5 WAT compared to 1 WAT with majority of the POST herbicides tested (Table 5.7), except paraquat + atrazine, paraquat + linuron, or paraquat + metribuzin treatments. The observed decline in control was partially due to recovery of treated plants from the herbicide injury and/or survival of late-emerging (July) kochia plants that escaped the POST herbicide application in the absence of a residual tank-mix partner.

In a greenhouse study, Wicks et al. (1993) also reported >95% control of 7.5- to 10-cm tall kochia plants treated with paraquat (0.3 kg ha^{-1}) 15 DAT. Although carfentrazone or fluroxypyr were not tested alone in our study, >88% kochia control was reported with carfentrazone (0.02 kg ha^{-1}) or fluroxypyr (0.14 kg ha^{-1}) in a greenhouse

study on dicamba-resistant kochia inbreds (Nandula and Manthey 2002). Although a dicamba rate of 0.28 kg ha^{-1} was not evaluated previously, kochia control ranged from 70 to 76% with dicamba applied at 0.14 kg ha^{-1} (Nandula and Manthey 2002; Tonks and Westra 1997). Consistent with our results, Lloyd et al (2011) reported 68 to 77% kochia control 4 WAT with dicamba + diflufenzopyr POST at similar rates. Knezevic et al. (2009) also observed <90% control of 10-cm tall kochia plants treated with glyphosate (1.059 kg ha^{-1}) in Gly-R soybean. Compared to our results, others reported greater kochia control (75 to 90%) with bromoxynil + MCPA applied even at lower rates (0.2 to 0.3 kg ha^{-1}) (Tonks and Westra 1997; Wolf et al. 2000), and control with glufosinate in our study was lower (52% with 0.593 kg ha^{-1} glufosinate) compared to that obtained (77% with 0.42 kg ha^{-1} glufosinate) in a study conducted by Eberlein et al. (1993).

Alternative POST Herbicides for Gly-R Kochia Control. *Visual Assessment of Percent Control*. The interaction of population by treatment was significant ($P < 0.0005$), indicating that kochia populations showed differential response to POST herbicides (labeled for corn/soybean) evaluated in the study. The differential response of the populations might be attributed to the high genetic diversity of kochia (Kumar et al. 2014; Mengistu and Messersmith 2002).

For the Gly-S kochia population tested, cloransulam-methyl, lactofen, and tembotrione + atrazine provided 91 to 94% control 3 WAT (Table 5.8). Control with fomesafen (87%) did not differ from control with lactofen (91%). The addition of atrazine to tembotrione or topramezone improved kochia control by 38 and 73%,

respectively, compared with tembotrione or topramezone alone 3 WAT. Control of the Gly-S population with S-metolachlor + mesotrione was 78% 3 WAT.

Besides modest differences in response to herbicides across populations, control of Gly-R kochia was 71 to 91% at 3 WAT with lactofen, fomesafen, tembotrione + atrazine, or topramezone + atrazine (Table 5.8). Control of the four Gly-R kochia populations with S-metolachlor + mesotrione was 64 to 77%. Gly-R kochia control with cloransulam-methyl, tembotrione alone, or topramezone alone was poor (≤ 41 %). The lower control of the Gly-R populations compared with the Gly-S population observed with cloransulam-methyl was because the Gly-R populations were also resistant to the ALS-inhibitor herbicide (data not shown).

Shoot Dry Weight Response. The interaction of population by treatment was significant ($P < 0.0001$). In general, shoot dry weight reductions of treated kochia populations were consistent with the control ratings for the herbicides tested. For the Gly-S population, cloransulam-methyl and tembotrione + atrazine reduced kochia shoot dry weight by 89 to 92% 3 WAT followed by fomesafen, lactofen, and topramezone + atrazine treatments (82% average reduction in shoot dry weight) (Table 5.8). For the Gly-R populations, lactofen, fomesafen, tembotrione + atrazine, or topramezone + atrazine were the best treatments tested, and reduced shoot dry weight up to 82% 3 WAT. S-metolachlor + mesotrione reduced shoot dry weight of Gly-R populations up to 70%. Consistent with poor control, cloransulam-methyl, tembotrione-, and topramezone-treated Gly-R plants produced greater shoot dry weight among all treatments (Table 5.8).

In summary, the PRE herbicide field trials suggest that acetochlor + atrazine, pyroxasulfone + atrazine, and S-metolachlor + atrazine + mesotrione would provide effective season-long kochia control in corn/grain sorghum. Sulfentrazone and metribuzin would provide effective residual control of kochia in soybean. Evident from the POST herbicide field trials, paraquat plus atrazine, paraquat plus linuron, and paraquat plus metribuzin would be effective burndown programs for kochia control in corn/grain sorghum, corn/fallow, and soybean, respectively. POST kochia control with glyphosate was 88% 5 WAT, and did not differ from bromoxynil + fluroxypyr (labeled in wheat/barley), tembotrione + atrazine (labeled in corn), and topramezone + atrazine (labeled in corn) programs.

Results from the greenhouse experiments suggest that atrazine + tembotrione or atrazine + topramezone would be an effective POST option for Gly-R kochia control in Gly-R corn. Lactofen or fomesafen could potentially provide effective control of Gly-R kochia in Gly-R soybean. Additionally, cloransulam-methyl would be an effective alternative product for Gly-R kochia control in Gly-R soybean, unless kochia plants are resistant to the ALS-inhibitor herbicide.

In conclusion, PRE soil-residual herbicide should serve as a foundation for kochia control programs. As kochia populations with resistance to group 9 (glyphosate), group 2 (sulfonylureas and imidazolinones), group 5 (atrazine), or group 4 herbicides (dicamba, fluroxypyr) continue to spread and impact larger acreages in the northwestern and north central US, incorporation of additional effective modes of action (investigated in this research) will be needed to control kochia. Kochia is a highly variable species, and the

results reported in this paper may not be consistent for all populations. Growers managing kochia in their fields should pay greater attention to the response of kochia to the herbicides they use, and use a diversity of herbicides and other weed management techniques to avoid selecting for additional herbicide resistance in kochia populations on their farms.

Acknowledgements

The authors would like to acknowledge Nicholas Reichard and Shane Leland for their technical assistance. Partial funding from Monsanto Company to conduct this research is gratefully acknowledged.

Table 5.1. Mean monthly air temperature and total precipitation at the MSU Southern Agricultural Research Center near Huntley, MT.

	Temperature			Total Precipitation		
Month	2011	2012	2013	2011	2012	2013
	C			mm		
March	3	8	3	20.1	45.0	5.8
April	6	10	6	68.6	22.6	29.7
May	11	13	14	237.5	82.3	154.2
June	17	20	19	39.1	9.9	35.8
July	23	25	23	67.3	12.2	16.5
August	23	22	23	16.0	9.1	36.6

Table 5.2. List of PRE herbicides treatments for kochia control in 2011, 2012, and 2013 at the MSU Southern Agricultural Research Center near Huntley, MT.

Herbicide(s)	Trade name	Application rate (kg ai or ae ha-1)	Manufacturer
Acetochlor + atrazine	HarnessXtra5.6L	2.604 + 2.100	Monsanto Company, 800 North Lindberg Ave., St. Louis, MO
Acetochlor + flumetsulam + clopyralid	TripleFlex	1.047 + 0.033 + 0.106	Monsanto Company
Dicamba	Rifle	0.280	Loveland Products Inc., Greeley, CO
Dicamba + 2,4-D amine	Rifle + Weedar 64	0.280 + 0.560	Loveland Products Inc. and Nufarm, Inc. Burr Ridge, IL
Dicamba + pendimethalin	Rifle + Prowl®H2O	0.280 + 0.798	Loveland Products Inc. and BASF Corporation, 26 Davis Drive, Research Triangle Park, NC 27709
Flumioxazin	Valor	0.070	Valent U.S.A. Corporation, Walnut Creek, CA
Flumioxazin + pyroxasulfone	Fierce	0.188	Valent U.S.A. Corporation
Isoxaflutole	Balance®flexx	0.062	Bayer Crop Science, Research Triangle Park, NC 27709
Metribuzin	Sencor® 75DF	0.425	Bayer Crop Science
Metribuzin + linuron	Sencor® 75DF + Linex® 4L	0.425 + 0.840	Bayer Crop Science and Tessenderlo Kerley Inc., Phoenix, AZ
Pyroxasulfone	Zidua	0.118	BASF Corporation
Pyroxasulfone + atrazine	Zidua + AAtrex® 4L	0.118 + 0.560	BASF Corporation and Syngenta Crop Protection, Inc. Greensboro, NC
Saflufenacil + 2,4-D	Sharpen + Weedar 64	0.025 + 0.560	BASF Corporation and Nufarm, Inc.
S-metolachlor + atrazine + mesotrione	Lumax	0.855 + 0.319 + 0.085	Syngenta Crop Protection
Sulfentrazone	Spartan	0.210	FMC Corporation, Philadelphia, PA

Table 5.3. Average kochia density and application dates of PRE and POST herbicides in 2011, 2012, and 2013 at the MSU Southern Agricultural Research Center near Huntley, MT.

	Kochia density		Herbicide application date	
Years	PRE[a]	POST[b]	PRE	POST
	Plants m^{-2}			
2011	54	79	April 26	June 12
2012	78	94	April 24	June10
2013	69	108	April 28	June 14

[a] In the PRE herbicide study, kochia densities were recorded 3 wk after emergence in the nontreated plots.

[b] In the POST herbicide study, kochia densities were recorded 1 d prior to the application of the POST herbicides.

Table 5.4. List of burndown and in-crop POST herbicide treatments for kochia control in 2011, 2012, and 2013 at the MSU Southern Agricultural Research Center near Huntley, MT.

Herbicide(s)	Trade name	Application rate (kg ai or ae ha^{-1})	Manufacturer
Bromoxynil + fluroxypyr	StaraneNXT	0.558 + 0.140	Dow AgroScience LLC, Indianapolis, IN
Bromoxynil + pyrasulfotole	Huskie	0.229 + 0.040	Bayer Crop Science
Bromoxynil + MCPA	Bronate Advanced	0.560 + 0.560	Bayer Crop Science
Carfentrazone-ethyl + 2,4-D amine	Aim + Weedar 64	0.034 + 0.560	FMC Corporation and Nufarm, Inc.
Cloransulam-methyl	FirstRate	0.017	Dow AgroScience LLC
Dicamba	Rifle	0.280	Loveland Products Inc.
Diflufenzopyr + dicamba + 2,4-D	Distinct + Weedar 64	(0.056 + 0.140) + 0.560	BASF Corporation and Nufarm, Inc.
Fomesafen	Flexstar	0.459	Syngenta Crop Protection
Glyphosate	Roundup WeatherMax	1.260	Monsanto Company
Glufosinate	Liberty	0.593	Bayer Crop Science
Lactofen	Cobra	0.218	Valent U.S.A. Corporation
Paraquat	Gramoxone Inteon	0.840	Syngenta Crop Protection
Paraquat + atrazine	Gramoxone Inteon + AAtrex	0.840 + 0.560	Syngenta Crop Protection
Paraquat + linuron	Gramoxone Inteon + linex	0.840 + 0.840	Syngenta Crop Protection
Paraquat + metribuzin	Gramoxone Inteon + Sencor75DF	0.840 + 0.425	Syngenta Crop Protection and Bayer Crop Science
Saflufenacil	Sharpen	0.025	BASF Corporation
Saflufenacil + 2,4-D	Sharpen + Weedar 64	0.025 + 0.560	BASF Corporation and Nufarm, Inc.
Saflufenacil + linuron	Sharpen + Linex	0.025 + 0.840	BASF Corporation and Nufarm, Inc.
S-metolachlor + mesotrione	Dual Magnum + Callisto	1.866 + 0.184	Syngenta Crop Protection
Tembotrione + atrazine	Laudis + AAtrex	0.089 + 0.560	Bayer Crop Science and Syngenta Crop Protection
Topramezone + atrazine	Impact + AAtrex	0.018 + 0.560	Amvac Chemical Corporation, Los Angeles, CA and Syngenta Crop Protection
Thifensulfuron-methyl + tribenuron-methyl + 2,4-D	HarmonyExtra SG + Weedar 64	0.017 + 0.008 + 0.560	DuPont, Wilmington, DE and Nufarm, Inc.

Table 5.5. List of alternative POST herbicides for glyphosate-resistant (Gly-R) kochia control.

Herbicide(s)[a,b]	Trade name	Application rate (kg ai or ae ha^{-1})	Manufacturer
Cloransulam-methyl[c]	FirstRate	0.017	Dow AgroScience LLC, Indianapolis, IN
Fomesafen[c]	Flexstar	0.459	Syngenta Crop Protection, Inc. Greensboro, NC
Lactofen[d]	Cobra	0.218	Valent U.S.A. Corporation, Walnut Creek, CA
S-metolachlor + mesotrione[c]	Dual Magnum + Callisto	1.866 + 0.184	Syngenta Crop Protection, Inc. Greensboro, NC
Tembotrione[d]	Laudis	0.089	Bayer CropScience, Research Triangle Park, NC
Tembotrione + atrazine[d]	Laudis + AAtrex	0.089 + 0.560	Bayer CropScience and Syngenta Crop Protection
Topramezone[d]	Impact	0.018	Amvac Chemical Corporation, Los Angeles, CA
Topramezone + atrazine[d]	Impact + AAtrex	0.018 + 0.560	Amvac Chemical Corporation and Syngenta Crop Protection

[a] Herbicides are labeled for corn or soybean
[b] All treatments were applied to 8- to 10-cm tall kochia plants.
[c] Nonionic surfactant (NIS) at 0.25% (v/v) was included.
[d] Methylated seed oil (MSO) at 1% (v/v) was included.

Table 5.6. Kochia control at 8, 10, and 12 wk after treatment (WAT) with various PRE herbicide treatments at the MSU Southern Agricultural Research Center near Huntley, MT[a,b].

Herbicide(s)	Rate(s)	8 WAT		10 WAT		12 WAT	
	(kg ai or ae ha^{-1})	%		%		%	
Acetochlor + atrazine	2.604 + 2.100	100	a	100	a	100	a
Acetochlor + flumetsulam + clopyralid	1.047 + 0.033 + 0.106	66	d	38	f	23	g
Dicamba	0.280	89	bc	60	e	40	f
Dicamba + 2,4-D	0.280 + 0.56	84	c	62	e	41	f
Dicamba + pendimethalin	0.280 + 0.798	94	ab	74	d	44	f
Flumioxazin	0.070	66	d	54	e	38	f
Flumioxazin + pyroxasulfone	0.070 + 0.118	90	bc	81	cd	70	e
Isoxaflutole	0.062	94	ab	76	d	71	de
Metribuzin	0.425	95	ab	89	bc	83	bcd
Metribuzin + linuron	0.425 + 0.840	96	ab	92	ab	80	cde
Pyroxasulfone	0.118	61	d	36	f	18	g
Pyroxasulfone + atrazine	0.118 + 0.560	93	ab	89	bc	83	bcd
Saflufenacil + 2,4-D	0.025 + 0.560	66	d	35	f	14	g
S-metolachlor + atrazine + mesotrione	0.855 + 0.319 + 0.085	99	a	98	a	93	ab
Sulfentrazone	0.210	97	ab	96	ab	91	abc

[a] PRE treatments were applied prior to kochia emergence in the field.

[b] Means within a column followed by similar letters are not significantly different based on Fisher's Protected LSD test at $P < 0.05$.

Table 5.7. Kochia control at 1, 3, and 5 wk after treatment (WAT) with various POST herbicide treatments at MSU Southern Agricultural Research Center near Huntley, MT.

Herbicide(s)[a]	Rate(s)	1 WAT[e]		3 WAT		5 WAT	
	(kg ai or ae ha^{-1})	%					
Bromoxynil + fluroxypyr[d]	0.558 + 0.140	95	bcd	92	cde	82	bcd
Bromoxynil + pyrasulfotole[c,d]	0.229 + 0.040	92	de	85	fg	77	de
Bromoxynil + MCPA	0.560 + 0.560	87	fg	77	jk	65	fg
Carfentrazone-ethyl + 2,4-D[d]	0.034 + 0.560	87	fg	82	ghi	60	gh
Cloransulam-methyl[d]	0.017	35	l	32	o	18	m
Dicamba	0.280	82	h	78	ij	77	de
Diflufenzopyr + dicamba + 2,4-D[d]	0.056 + 0.140 + 0.560	83	gh	82	ghi	78	cde
Fomesafen[d]	0.459	77	i	72	l	40	l
Glyphosate[c]	1.260	95	bcd	93	cd	88	b
Glufosinate[c]	0.593	87	fg	73	kl	52	ij
Lactofen[b]	0.218	88	ef	80	hij	57	hi
Paraquat[d]	0.840	99	ab	95	bc	85	bc
Paraquat + atrazine[d]	0.840 + 0.560	100	a	100	a	100	a
Paraquat + linuron[d]	0.840 + 0.840	99	ab	99	ab	98	a
Paraquat + metribuzin[d]	0.840 + 0.425	99	ab	98	ab	98	a
Saflufenacil[b,c]	0.025	90	ef	82	ghi	67	ef
Saflufenacil + 2,4-D[b,c]	0.025 + 0.560	97	abc	83	gh	72	ef
Saflufenacil + linuron[b,c]	0.025 + 0.840	95	bcd	90	de	78	cde
S-metolachlor + mesotrione[d]	1.866 + 0.184	62	j	57	m	48	jk
Tembotrione + atrazine[b]	0.089 + 0.560	93	cde	88	ef	85	bc
Topramezone + atrazine[b]	0.018 + 0.560	83	gh	83	gh	80	cd
Thifensulfuron + tribenuron + 2,4-D[d]	0.017 + 0.008 + 0.560	57	k	48	n	43	kl

[a]Herbicide treatments were applied to 8- to 10-cm-tall kochia plants in the field.[b] Methylated seed oil (MSO) at 1% v/v was included.[c] Ammonium sulfate (AMS) at 2 % w/v was included.[d] Nonionic surfactant (NIS) at 0.25% v/v was included.[e] Means within a column followed by similar letters are not significantly different based on Fisher's Protected LSD test at $P < 0.05$.

Table 5.8. Control and shoot dry weight (percentage of nontreated) of Gly-S and Gly-R kochia populations from Montana 3 wks after treatment with various POST herbicides labeled for corn or soybean [a, b].

Herbicide(s)	Rate(s)	Gly-S[e]		GIL01		CHES01		CHES02		JOP01		P value
	(kg ai or ae ha^{-1})	% Control										
Cloransulam-methyl[c]	0.017	93	a	37	c	29	e	12	E	12	d	0.0002
Fomesafen[c]	0.459	87	bc	88	a	84	b	71	B	73	ab	< 0.0001
Lactofen[d]	0.218	91	ab	89	a	89	ab	84	A	74	ab	0.0007
S-metolachlor + mesotrione[c]	1.866 + 0.184	78	d	64	b	77	c	64	C	71	b	0.0018
Tembotrione[d]	0.089	56	e	41	c	38	d	34	D	33	c	< 0.0001
Tembotrione + atrazine[d]	0.089 + 0.560	94	a	88	a	88	ab	76	B	78	a	< 0.0001
Topramezone[d]	0.018	11	f	11	d	28	e	33	D	16	d	0.0004
Topramezone + atrazine[d]	0.018 + 0.560	84	c	88	a	91	a	75	B	72	b	0.0001
		Dry weight (% of nontreated)										
Cloransulam-methyl[c]	0.017	8	f	66	b	77	a	86	A	76	a	< 0.0001
Fomesafen[c]	0.459	19	d	25	d	27	c	38	C	35	d	0.0057
Lactofen[d]	0.218	17	de	28	d	21	d	29	D	36	cd	0.0223
S-metolachlor + mesotrione[c]	1.866 + 0.184	34	c	44	c	30	c	42	C	43	c	0.0399
Tembotrione	0.089	60	b	60	b	58	b	74	B	54	b	0.0357
Tembotrione + atrazine[d]	0.089 + 0.560	11	ef	21	d	20	d	34	cd	36	d	< 0.0001
Topramezone	0.018	88	a	88	a	77	a	74	B	69	a	0.0143
Topramezone + atrazine[d]	0.018 + 0.560	17	de	23	d	18	d	36	cd	41	d	< 0.0001

[a] Abbreviations: Gly-S, glyphosate-susceptible population, Huntley, MT; Gly-R, glyphosate-resistant; GIL01, Gly-R population, Gildford, MT; JOP01, Gly-R population, Joplin, MT; CHES01, Gly-R population # 1, Chester, MT; CHES02, Gly-R population # 2, Chester, MT.

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

[c] Nonionic surfactant (NIS) at 0.25% (v/v) was included.

[d] Methylated seed oil (MSO) at 1% (v/v) was included.

[e] For percent control and shoot dry weight, means for a kochia population within a column followed by similar lowercase letters are not significantly different based on Fisher's Protected LSD test at $P < 0.05$.

Literature Cited

Baker DV, Withrow JR, Brown CS, Beck KG (2010) Tumbling: Use of diffuse knapweed (*Centaurea diffusa*) to examine an understudied dispersal mechanism. Invasive Plant Sci Manage 3:301-309

Beckie HJ (2006) Herbicide-resistant weeds: Management tactics and practices. Weed Technol 20:793-814

Beckie HJ, Blackshaw RE, Low R, Hall LM, Sauder CA, Martin S, Brandt EN, Shirriff SW (2013) Glyphosate- and acetolactate synthase inhibitor-resistant kochia (*Kochia scoparia*) in western Canada. Weed Sci 61:310-318

Christoffoleti PJ, Westra PB, Moore F (1997) Growth analysis of sulfonylurea-resistant and -susceptible kochia (*Kochia scoparia*). Weed Sci 45:691–695

Cranston HJ, Kern AJ, Hackett JL, Miller EK, Maxwell BD, Dyer WE (2001) Dicamba resistance in kochia. Weed Sci 49:164–170

Dahl GK, Dexter AG, Nalewaja JD (1982) Kochia competition and control in wheat. Proc North Cent Weed Control Conf 37:15-16

Dille JA, Stahlman PW, Geier PW, Riffel JD, Currie RS, Wilson RG, Sbatella GM, Westra P, Kniss AR, Moechnig MJ, Cole RM (2012) Kochia emergence profiles across the central Great Plains. Proc Weed Sci Soc Am 52:122

Durgan BR, Dexter AG, Miller SD (1990) Kochia (*Kochia scoparia*) interference in sunflower (*Helianthus annus*). Weed Technol 4:52–56.

Eberlein CV, Fore ZA (1984) Kochia biology. Weeds Today 15:5–6

Eberlein CV, Guttieri MJ, Fletcher FN (1993) Broadleaf weed control in potatoes (*Solanum tuberosum*) with postemergence directed herbicides. Weed Technol 7:298-303

Forcella F (1985) Spread of kochia in the northwestern United States. Weeds Today 16:4–6

Friesen LF, Beckie HJ, Warwick SI, Van Acker RC (2009) The biology of Canadian weeds. 138. *Kochia scoparia* (L.) Schrad. Can J Plant Sci 89:141–167

Heap I (2014) International Survey of Herbicide Resistant Weeds. http://www.weedscience.org Accessed: January 09, 2014

Hutchinson PJS, Hancock DM, Beutler BR (2005) Efficacy of reduced sulfentrazone rates applied preemergence with metribuzin in potato (*Solanum tuberosum*). Weed Technol 19:954-958

Jha P, Kumar V, Reichard N (2013) Preemergence residual herbicides: A valuable tool for weed control in glyphosate-resistant corn. Proc Weed Sci Soc Am 33

Knezevic SZ, Datta A, Scott J, Klein RN, Golus J (2009) Problem weed control in glyphosate-resistant soybean with glyphosate tank mixes and soil-applied herbicides. Weed Technol 23: 507-512

Kumar V, Jha P, Reichard N (2014) Occurrence and characterization of kochia (*Kochia scoparia*) populations with resistance to glyphosate in Montana. Weed Technol 28:122-130

Lloyd KL, Johnson JM, Gover AE, Sellmer JC (2011) Preemergence and postemergence suppression of kochia on rights-of-way. Weed Technol 25:292–297

Mengistu LW, Messersmith CG (2002) Genetic diversity of kochia. Weed Sci 50: 498-503

Nandula VK, Manthey FA (2002) Response of kochia (*Kochia scoparia*) inbreds to 2,4-D and dicamba. Weed Technol 16:50-54

Norsworthy, JK, Ward SM, Shaw DR, Llewellyn RS, Nichols RL, Webster TM, Bradley KW, Frisvold G, Powles SB, Burgos NR, Witt WW, Barrett M (2012) Reducing the risks of herbicide resistance: Best management practices and recommendations. Weed Sci 60:31-62

Preston C, Belles DS, Westra PH, Nissen SJ, Ward SM (2009) Inheritance of resistance to the auxinic herbicide dicamba in kochia (*Kochia scoparia*). Weed Sci 57:43-47

Primiani MM, Cotterman JC, Saari LL (1990) Resistance of *Kochia scoparia* to sulfonylurea and imidazolinone herbicides. Weed Technol 4:169-172

Reddy SS, Stahlman PW, Geier PW, Thompson CR (2012) Weed control and crop safety with premixed s-metolachlor and sulfentrazone in sunflower. Am J Plant Sci 3:1625-1631

Sbatella GM, Wilson RG (2010) Isoxaflutole shifts kochia (*Kochia scoparia*) populations in continuous corn. Weed Technol 24:392-396

Schwinghamer TD, Van Acker RC (2008) Emergence timing and persistence of kochia (*Kochia Scoparia*). Weed Sci 56:37-41

Stahlman PW, Olson BLS, Thompson, CR, Zollinger RK (2010) Pyroxasulfone (KIH-485) for weed control in sunflower. Pages 1-7 *in* Proc of the 1st Australian Summer Grains Conference. Gold Coast, Australia

Stallings GP, Thill DC, Mallory-Smith CA, Shafii B (1995) Pollen-mediated gene flow of sulfonylurea-resistant kochia (*Kochia scoparia*). Weed Sci 43:95–102

Stephenson DO IV, Bond JA, Landry RL, Edwards HM (2013) Effect of coapplied glyphosate, pyrithiobac, pendimethalin, or *S*-metolachlor on cotton injury, growth, and yield. Weed Technol 27:305-309

Tonks DJ, Westra P (1997) Control of sulfonylurea-resistant kochia (*Kochia scoparia*). Weed Technol 11:270-276

Waite J, Thompson CR, Peterson DE, Currie RS, Olson BL S, Stahlman PW, Khatib KA (2013) Differential kochia (*Kochia scoparia*) populations response to glyphosate. Weed Sci 61:193-200

Weatherspoon DM and Schweizer EE (1971) Competition between sugarbeets and five densities of kochia. Weed Sci 19:125-128.

Wicks GA, Martin AR, Mahnken GW (1993) Control of triazine-resistant kochia (*Kochia scoparia*) in conservation tillage corn (*Zea mays*). Weed Sci 41:225–231

Wicks GA, Martin AR, Haack AE, Mahnken GW (1994) Control of triazine-resistant kochia (*Kochia scoparia*) in sorghum (*Sorghum bicolor*). Weed Technol 8:748–753

Wolf R, Clay SA, Wrage LJ (2000) Herbicide strategies for managing kochia (*Kochia scoparia*) resistant to ALS-inhibiting herbicides in wheat (*Triticum aestivum*) and soybean (*Glycine max*). Weed Technol 14:268-273

CHAPTER 6

INFLUENCE OF HERBICIDES APPLIED POSTHARVEST IN WHEAT STUBBLE ON CONTROL, FECUNDITY, AND PROGENY FITNESS OF *KOCHIA SCOPARIA* IN THE US GREAT PLAINS

Contribution of Authors and Co-Authors

Manuscript in Chapter 6

Author: Vipan Kumar

Contributions: Conceived and implemented the study design. Conducted experiments, reviewed the literature, and wrote the manuscript.

Co-Author: Prashant Jha

Contributions: Advisement, provided field expertise and feedback on manuscript drafts

Manuscript Information Page

Vipan Kumar, Prashant Jha
Crop Protection Journal
Status of Manuscript:
___Prepared for submission to a peer-reviewed journal
___Officially submitted to a peer-review journal
___Accepted by a peer-reviewed journal
_×_Published in a peer-reviewed journal

Published by Elsevier Limited
Published in 2015, Volume 71 (doi:10.1016/j.cropro.2015.02.016)

Abstract

Field, greenhouse, and laboratory experiments were conducted at the Montana State University Southern Agricultural Research Center, Huntley, MT, USA, in 2012 and 2013, to evaluate the effectiveness of various postharvest-applied herbicides on late-season control, fecundity, seed viability, and progeny fitness of *Kochia scoparia* (kochia) in wheat stubble. Paraquat + atrazine, paraquat + linuron, and paraquat + metribuzin applied at the early bloom stage were the most effective postharvest treatments for late-season control (100%) at 28 d after treatment (DAT), biomass reduction (70 to 73%), and seed prevention of *K. scoparia*, and did not differ from glyphosate, glufosinate, saflufenacil + 2, 4-D, saflufenacil + atrazine, tembotrione + atrazine, or topramezone + atrazine treatments. Dicamba alone, dicamba + 2, 4-D, or diflufenzopyr + dicamba + 2, 4-D applied at the early bloom stage were ineffective, with < 70% late-season control, < 45% biomass reduction, and < 55% seed reduction of *K. scoparia*. In the absence of a postharvest herbicide, uncontrolled kochia plants at a density of 8 to 10 plants m^{-2} contributed > 100,000 seeds m^{-2}. Addition of atrazine to dicamba improved late-season control (80%) and seed reduction (78%) compared to dicamba alone, and reduced seed viability and 100-seed weight. There was no significant effect of any of the dicamba-containing herbicides applied at the early bloom stage on *K. scoparia* progeny fitness, including height, width, primary branches, and shoot dry weight of seedlings at 42 d after planting (DAP). The effective postharvest-applied herbicides investigated in this research should be utilized to prevent late-season *K. scoparia* seed bank replenishment in wheat, and as a component of herbicide resistance management program for the containment of

glyphosate- and/or acetolactate synthase (ALS)-inhibitor-resistant *K. scoparia* in wheat-based crop rotations in the U.S. Great Plains.

Key words: Late-season weed control; postharvest herbicide; herbicide-resistant; seed bank; fecundity; fitness.

1. Introduction

Kochia scoparia (L.) Schrad. (kochia) is one of the most problematic summer annual broadleaf weeds in irrigated and dryland cropping systems of the U. S. Great Plains (Eberlein and Fore, 1984; Forcella, 1985; Wicks et al., 1994). The invasiveness of *K. scoparia* is attributed to its early seedling emergence (Schwinghamer and Van Acker, 2008), rapid growth rate with C_4 photosynthetic pathway (Christoffoleti et al., 1997; Friesen et al., 2009), heat and salt tolerance (Friesen et al., 2009), high fecundity (>50,000 seeds plant^{-1}) (Stallings et al., 1995), and long-distance seed dispersal by wind-mediated tumble mechanism (Baker et al., 2010; Friesen et al., 2009). In late autumn, a fully-matured *K. scoparia* plant breaks off at the base of the stem (absission layer) near the soil surface and tumbles with the prevailing wind, dispersing seeds across the landscape (Baker et al., 2010). Furthermore, it exhibits wide genetic diversity due to its protogynous nature of flowering with a high degree of out-crossing and pollen-mediated gene flow (Mengistu and Messersmith, 2002; Stallings et al., 1995). *K. scoparia* has been reported to cause yield reductions as high as 95% in agronomic crops grown in the region, including wheat (Dahl et al., 1982; Durgan et al., 1990; Weatherspoon and Schweizer, 1971; Wicks et al., 1993, 1994).

K. scoparia has evolved resistance to several herbicide modes of action including photosystem II (PS II) inhibitors (atrazine), acetolactate synthase (*ALS*) inhibitors (sulfonylurea and imidazolinone herbicides), and synthetic auxins (dicamba and fluroxypyr) in the northern and central Great Plains of U. S. (Cranston et al., 2001; Heap, 2014; Preston et al., 2009; Primiani et al., 1990). In 2007, *K. scoparia* populations

resistant to glyphosate were first detected in Kansas, US (Heap, 2014; Waite et al., 2013). Since then, glyphosate-resistant *K. scoparia* has been reported in six other states in this region and in two provinces of Canada (Beckie et al., 2013; Heap, 2014; Kumar et al., 2014). In 2013, *K. scoparia* populations with 4.6- to 11-fold levels of resistance to glyphosate have been confirmed in chemical-fallow (chemical-fallow and wheat rotation) fields in Hill, Liberty, and Toole Counties of Montana (Kumar et al., 2014). The increased occurrence of glyphosate-resistant *K. scoparia* is a potential threat to glyphosate-resistant cropping systems, and to no-till chemical fallow-based crop rotations common in this region.

Major crop rotations in the semi-arid dryland region of the U.S. Great Plains include wheat-chemical fallow, wheat-corn/grain sorghum-chemical fallow, and/or wheat-summer crop-chemical fallow (Anderson, 1996), where *K. scoparia* poses a serious problem. *K. scoparia* has an extended period of emergence (Dille et al., 2012), with cohorts emerging as early as April through late June/early July in wheat or in chemical-fallow systems (Anderson, 1996). Cohorts that escape early-season POST herbicide application(s) or late-emerging cohorts in wheat often remain in vegetative stage at the time of wheat maturity, and hinder mechanical harvest. Moreover, these cohorts cause substantial late-season replenishment of the soil seed bank (Mickelson et al., 2004). It has been reported that *K. scoparia* plants after decapitation at wheat harvest can regrow, and produce up to 5,710 seeds plant^{-1} by late autumn (Mickelson et al., 2004). It is to be noted that late-season weed escapes are more problematic in wheat due to reliance on total POST programs with a lack of effective soil residual herbicides.

Late-season or postharvest herbicide treatments are often targeted at the early bloom stage or initiation of seed set to reduce/prevent late-season weed seed rain and seed bank increase (Bennet and Shaw, 2000; Jha and Norsworthy, 2012; Mickelson et al., 2004). Reported in other weed species such as *Amaranthus palmeri* L. Wats., glufosinate (0.820 kg ha^{-1}), 2, 4-D (1.06 kg ha^{-1}), or dicamba (0.280 kg ha^{-1}) applied at the inflorescence initiation stage reduced seed production of treated plants by 95%, and seed viability by 39 to 51% (Jha and Norsworthy, 2012). A single late-season application of glyphosate, 2, 4-D, dicamba, or glufosinate in *Chenopodium album* L., *Amaranthus retroflexus* L., *Abutilon theophrasti* Medik., and *Senna obtusifolia* L. reduced seed production up to 99% (Biniak and Aldrich, 1986; Fawcett and Slife, 1978; Taylor and Oliver, 1997).

Seed production from late-season weed escapes can favor the evolution of herbicide resistance due to the likelihood of resistant individuals being present in those weed escapes (Bagavathiannan and Norsworthy, 2012). Late-season herbicide applications in postharvest wheat stubble would serve as an additional tool for growers to manage glyphosate-resistant *K. scoparia* weed escapes and seed bank additions. Although a study on postharvest *K. scoparia* management has previously been conducted, only a few POST herbicides (glyphosate, 2, 4-D amine, and paraquat) were tested (Mickelson et al., 2004). Additionally, the information on the effect of postharvest-applied (late-season) herbicides on *K. scoparia* seed viability and progeny seedling fitness is lacking. The information generated from this research can be potentially utilized by growers to manage herbicide-resistant *K. scoparia* seed bank in wheat-chemical

fallow, wheat-corn/grain sorghum-chemical fallow, or wheat-soyabean-chemical fallow rotations in this region. The objectives of this research were (1) to evaluate effective herbicide programs applied postharvest in wheat stubble on *K. scoparia* control and biomass reduction, and (2) to determine their impact on *K. scoparia* seed production, 100-seed weight, seed viability, and progeny seedling fitness.

2. Materials and Methods

2.1. Field Experiments

Field experiments (45° 54' 50.11N, 108° 14' 53.99W) were conducted at the Montana State University (MSU) Southern Agricultural Research Center (SARC) near Huntley, MT, USA, in 2012 and 2013, to evaluate the effectiveness of herbicides (labeled in crops grown in rotation with wheat including corn, grain sorghum, soyabean, and/or chemical-fallow) applied postharvest in wheat stubble for *K. scoparia* control. The soil type at the study site was a Fort Collins clay loam (fine-loamy, mixed, superactive, mesic Aridic Haplustalfs) with organic matter content of 2.8% and pH of 7.8. The experimental site was under no-till dryland wheat-fallow rotation prior to the initiation of the study. During late autumn of 2012 and 2013, fully-matured *K. scoparia* seeds were collected from a naturally infested area at the MSU-SARC research farm. The weed population was known to be susceptible to all herbicides tested in this study. The seeds were broadcasted in early spring prior to the spring wheat planting to obtain uniform *K. scoparia* densities in the test plots. Spring wheat variety 'Vida' (70 kg ha^{-1}) was planted with a no-till drill at a row spacing of 15 to 18 cm on April 10, 2012 and April 6, 2013,

respectively. The test plots were established under dryland wheat production, with no supplemental irrigation, and fertilized with Nitrogen-Phosphorus-Potash as per the MSU recommendations for spring wheat production (Jacobson et al., 2005). Wheat was harvested with a plot-combine leaving approximately 30 to 35 cm tall wheat stubble on August 14, 2012 and August 12, 2013. Postharvest herbicide programs and their application rates are summarized in Table 6.1. A nontreated control was included for comparison. Herbicide treatments were applied using a handheld CO_2-pressurized backpack boom sprayer equipped with flat-fan nozzles (TeeJet 8001XR, Spraying Systems Co., P.O. Box 7900, Wheaton, IL), calibrated to deliver 94 L ha^{-1} of spray solution at 276 kPa. All treatments were applied 2 to 3 wk following harvest of the wheat crop, when decapitated *K. scoparia* plants had noticeable regrowth, and were at the early bloom stage (45 to 50 cm tall). Herbicide treatments were applied on September 3, 2012 and September 7, 2013.

The treatments were arranged in a randomized complete block design, with four replications. Each experimental plot was 5 m long by 2 m wide. *K. scoparia* control was visually assessed at 7, 14, and 28 d after treatment (DAT) on a scale of 0 to 100%, (where 0 equals no control and 100 equals complete control/plant death). Visual assessments for percent control were based on general chlorosis or necrosis of treated compared to nontreated plants. At 28 DAT, plants from a 1-m^2 quadrat at the center of each plot were harvested at the soil level. The inflorescence was removed from the plant by shaking with hand, and the remaining plant sample was oven-dried at 60 $^{\circ}$C for 3 d to determine plant dry weight, calculated as a percentage of the nontreated control. Seeds were manually

separated from the inflorescence; the coarse debris was removed using a 2-mm mesh size sieve, and the small debris was removed with an air-propelled column blower. Weight of a sample of 100 seeds, total seed weight m^{-2}, and number of seeds m^{-2} were determined. Seed production from the treated plots was calculated as a percentage of reduction from the nontreated control.

2.2. Seed Viability

A 100-seed subsample was randomly selected from each plot sample of *K. scoparia*. Seeds were placed on double layers of filter paper (Whatman®, Grade 2, Sigma-Aldrich Inc., St. Louis, MO 63178, USA) moistened with 10 ml deionized water in 10-cm-diam petri dishes (Sigma-Aldrich). Experiments were conducted in a completely randomized design with four replications (25 seeds per petri dish), and repeated. Petri dishes were placed in the dark in an incubator (VMR International, Sheldon Manufacturing Inc., Cornelius, OR 97113, USA) at a constant temperature of 24 °C, which is considered optimum for seed germination of *K. scoparia* (Al-Ahmadi and Kafi, 2006). Light is not required for *K. scoparia* seed germination (Everitt et al., 1983). Seeds were considered germinated when the radicals emerged and the tip of the radicle was uncoiled (Young et al., 1981). The number of germinated seeds was counted daily until 15 d. Non-germinated seeds were tested for viability using a 1% w/v tetrazolium chloride solution (Sbatella and Wilson, 2010). Seed viability was estimated as the percentage of total seeds that germinated plus those tested positive in tetrazolium chloride test.

2.3. Progeny Seedling Fitness

A 50-seed subsample of *K. scoparia* was randomly selected from each plot sample, and sown in germination trays (53 cm × 35 cm × 15 cm) filled with a commercial potting mix (VermiSoil™, Vermicrop Organics, 4265 Duluth Avenue, Rocklin, CA, USA) in the greenhouse at MSU-SARC. Emerged seedlings were thinned to obtain 10 uniform plants per tray. Treatments were arranged in a completely randomized design with four replications, and experiments were repeated. The greenhouse was maintained at 26/23 ± 3 ºC day/night temperature and 16/8 h photoperiod. Plants were watered daily to avoid moisture stress, and fertilized (Miracle-Gro water-soluble fertilizer [24-8-16], Scotts Miracle-Gro Products Inc., 14111 Scottslawn Road, Marysville, OH, USA) once weekly to maintain good growth. Growth parameters including seedling height, width, and number of primary branches per seedling were recorded 42 d after planting (DAP). The aboveground biomass from each tray was harvested at 42 DAP, dried at 60 °C for 3 d, and the average shoot dry weight per seedling was determined.

2.4. Statistical Analyses

All data were subjected to ANOVA using the MIXED procedure in SAS (Statistical Analysis Systems®, version 9.2, SAS Institute Inc., SAS Campus Drive, Cary, NC 27513, USA). Variances were divided into random effects (year or run, replication nested within a year or run, and interactions involving either of these two variables) and fixed effects (herbicide treatment). Data was transformed before analysis to improve the

normality of residuals and homogeneity of variance. Data on visually assessed percent control, dry weight (percentage of nontreated), percentage seed reduction, and percent seed viability were arcsine-transformed, and data on progeny seedling height, width, number of primary branches, shoot dry weight, and 100-seed weight were square-root-transformed. Non-transformed means are presented in tables based on the interpretation from the transformed data. Means were separated using Fisher's Protected LSD test at $P < 0.05$.

3. Results and Discussion

3.1. K. scoparia Control

K. scoparia densities ranged from 8 to 10 plants m^{-2} in the postharvest wheat stubble plots in in 2012 and 2013. Averaged over years, a single postharvest application of paraquat + atrazine, paraquat + linuron, or paraquat + metribuzin at the early bloom stage (45 to 50 cm tall plants) provided complete control of *K. scoparia* at 28 DAT, and did not differ from paraquat, glyphosate, dicamba + glyphosate, saflufenacil + atrazine, or saflufenacil + 2, 4-D treatments (Table 6.2). Glyphosate activity on *K. scoparia* was much slower compared to the contact activity of glufosinate, paraquat- or saflufenacil-based treatments, evident from the differences in control ratings at 7 vs. 14 DAT. Also in a greenhouse study, paraquat when tank-mixed with the PS II-inhibitors including atrazine (0.56 kg ha^{-1}), linuron (0.84 kg ha^{-1}), or metribuzin (0.42 kg ha^{-1}) provided complete control of 8- to 10-cm tall *K. scoparia* plants 7 DAT (Kumar et al., 2014). Control 28 DAT with glufosinate, tembotrione + atrazine, and + topramezone atrazine

was 91 to 95% (Table 6.2). Dicamba applied alone was the least effective postharvest herbicide treatment (47% control at 28 DAT); however, tank-mixing dicamba with atrazine improved *K. scoparia* control (80% at 28 DAT). Furthermore, the control was higher than dicamba + 2, 4-D (55% control at 28 DAT) or diflufenzopyr + dicamba + 2, 4-D (67% only at 28 DAT) treatment. Also reported in other broadleaf weed species such as *A. palmeri* and *S. obtusifolia.*, control from a late-season (early bloom stage of the weed) application of dicamba (0.28 kg ha^{-1}) or 2, 4-D (1.06 kg ha^{-1}) did not exceed 70% (Jha and Norsworthy, 2012; Taylor and Oliver, 1997).

3.2. Plant Dry Weight and Seed Production

All postharvest herbicide treatments were comparable for *K. scoparia* biomass reduction, which ranged from 68 to 73% of the nontreated, except dicamba, dicamba + 2, 4-D, dicamba + atrazine, and diflufenzopyr + dicamba + 2, 4-D treatments (Table 6.3). Among dicamba-containing treatments, dicamba + atrazine provided greater biomass reduction, although it was < 50%.

All herbicides tested prevented late-season *K. scoparia* seed production when applied at the early bloom stage in the postharvest wheat stubble, except dicamba, dicamba + 2, 4-D, dicamba + atrazine, and diflufenzopyr + dicamba + 2, 4-D (Table 6.3). Dicamba and dicamba + 2, 4-D reduced seed production by only 46 and 53% of the nontreated check (104,100 seeds m^{-2}) respectively, which were lower than the 78% seed reduction by dicamba + atrazine. Diflufenzopyr + dicamba + 2, 4-D was the least effective treatment in reducing late-season *K. scoparia* seed production. Consistent with

our results, Mickelson et al., (2004) reported that a late-season application of paraquat (0.701 kg ha^{-1}) and glyphosate (0.631 kg ha^{-1}) reduced *K. scoparia* seed production by 97 to 99%. Similarly, up to 99% reduction in seed production of *S. obtusifolia*, *Abutilon theophrasti* Medik., *Ipomoea lacunosa* L., and *Sida spinosa* L. has been reported with glyphosate, applied even at lower rates of 0.42 to 0.84 kg ha^{-1} at early flowering to early seed-set stage of the weeds (Biniak and Aldrich, 1986; Clay and Griffin, 2000; Thomas et al., 2005; Walker and Oliver, 2008). Although dicamba (0.280 kg ha^{-1}) and 2, 4-D (1.06 kg ha^{-1}) applied at the first visible sign of inflorescence reduced seed production of *A. palmeri* plants by 75 and 84%, respectively, (Jha and Norsworthy, 2012), the herbicides were not effective in reducing late-season *K. scoparia* seed bank additions in the postharvest wheat stubble.

3.3. Hundred Seed Weight and Seed Viability

The data shown in Table 6.4 represents only those treatments in which *K. scoparia* survivors did produce seed following a postharvest herbicide application in wheat stubble, and the nontreated check. Dicamba alone, dicamba + 2, 4-D, and diflufenzopyr + dicamba + 2, 4-D treatments applied at the early bloom stage of *K. scoparia* did not influence the 100-seed weight compared to the nontreated check (Table 6.4). Also reported in other weed species, such as *A. palmeri*, dicamba (0.28 kg ha^{-1}) applied at the early inflorescence stage had no effect on the 100-seed weight of the treated plants (Jha and Norsworthy, 2012). However, addition of atrazine to dicamba reduced the 100-seed weight of treated plants, which was 100 mg compared to 130 mg

for the nontreated check (Table 6.4). Seed weight is an indicator of seedling vigor (Steadman et al., 2006).

Furthermore, dicamba + atrazine reduced viability of *K. scoparia* seeds produced by the treated plants compared to the nontreated (Table 6.4). None of the other dicamba-containing treatments affected seed viability. The reduction in seed viability may be due to the abscission of floral structures or inhibition of seed development causing immature embryos or dead seeds (Isaacs et al., 1989; Steadman et al., 2006). Our results indicate a possible negative effect of atrazine on seed viability and seedling vigor of *K. scoparia* plants treated at the early bloom stage.

3.4. Progeny Seedling Fitness

The data shown in Table 6.4 represents only those treatments in which *K. scoparia* survivors did produce seed following a postharvest herbicide application in wheat stubble, and the nontreated check. Growth attributes including height, width, number of primary branches, and shoot dry weight of progeny seedlings of *K. scoparia* did not differ among the dicamba-containing postharvest-applied herbicides at 42 DAP (Table 6.4), and averaged 36.4 cm, 19.1 cm, 17, and 2.8 g plant^{-1}, respectively. The results confirm that a late-season (early bloom stage) application of dicamba or 2, 4-D in postharvest wheat stubble will have minimal influence on the competitive ability of *K. scoparia* seedlings (obtained from the treated plants) in the following season.

4. Conclusions

In summary, a single application of paraquat alone or tank-mixed with atrazine/ linuron/ metribuzin, saflufenacil + atrazine, saflufenacil + 2, 4-D, glyphosate alone or tank-mixed with dicamba, glufosinate, tembotrione + atrazine, or topramezone + atrazine at the early bloom stage of *K. scoparia* prevented late-season seed rain and seed bank additions in postharvest wheat stubble. The "zero seed tolerance" approach is one of the best management practices (BMPs) for the containment of herbicide resistance (Norsworthy et al., 2012). This strategy becomes more imperative for a species like *K. scoparia*, with prolific seed production (Stallings et al., 1995) and rapid seed bank turnover (due to low dormancy and high seedling recruitment) (Dille et al., 2012). A late-season application of dicamba alone or tank-mixed with 2, 4-D and/or diflufenzopyr was not effective in controlling *K. scoparia* in postharvest wheat stubble. Nevertheless, dicamba when tank-mixed with atrazine reduced seed production, seed weight and seed viability of treated plants, which would be an additional tool to manage *K. scoparia* infestation in the subsequent corn/grain sorghum crop.

Late-season weed escapes often have limited influence on crop yield (Zimdahl, 2004). Also, growers have to incur additional costs to use late-season (postharvest) herbicides. However, growers need to be educated that besides reducing *K. scoparia* infestation in the following season/crop, preventing late-season seed bank inputs is crucial for the containment of glyphosate and multiple herbicide (glyphosate and ALS-inhibitor)-resistant populations of the weed, which continue to spread and impact larger acreages in the wheat-based cropping systems of the US Great Plains. Growers should

proactively utilize the effective late-season (early bloom stage) herbicide programs evaluated in this research to prevent potentially high late-season seed bank increase of *K. scoparia* in wheat stubble (>100,000 seeds m^{-2} produced by nontreated plants in this study). Additionally, they should pay attention to the recommended usage of these tested postharvest herbicides in wheat stubble to avoid crop injury concerns in the rotational crop (corn/grain sorghum, soyabean, chemical-fallow), and integrate non-chemical weed control tactics to mitigate the occurrence of any new or multiple herbicide-resistant *K. scoparia* strain on their farm fields.

Acknowledgments

The authors thank Mr. Nicholas Reichard and Mr. Shane Leland for their technical assistance in this research. Funding obtained from Monsanto Company and Montana Wheat and Barley Committee to conduct this research is gratefully acknowledged.

Table 6.1. List of postharvest-applied herbicides for *Kochia scoparia* control in wheat stubble during 2012 and 2013 at the Montana State University, Southern Agricultural Research Center near Huntley, MT, USA.

Herbicide(s)	Application rate(s)	Trade name	Manufacturer
	kg ha^{-1}		
Dicamba	0.56 ae	Rifle	Loveland Products Inc.
Dicamba + 2,4-D	0.56 ae + 0.56 ae	Rifle + Weedar 64	Loveland Products Inc. and Nufarm, Inc.
Dicamba + atrazine	0.56 ae + 0.56 ai	Rifle + AAtrex	Loveland Products Inc. and Syngenta Crop Protection
Dicamba + glyphosate	0.56 ae + 1.26 ae	Rifle + Roundup WeatherMax	Loveland Products Inc. and Monsanto Company
Diflufenzopyr + dicamba + 2,4-D	0.06 ai + 0.15 ae + 0.56 ae	Rifle + Distinct + Weedar 64	BASF Corporation and Nufarm, Inc.
Glyphosate	1.26 ae	Roundup WeatherMax	Monsanto Company
Glufosinate	0.59 ai	Liberty	Bayer Crop Science
Paraquat	0.84 ai	Gramoxone Inteon	Syngenta Crop Protection
Paraquat + atrazine	0.84 ai + 0.56 ai	Gramoxone Inteon + AAtrex	Syngenta Crop Protection
Paraquat + linuron	0.84 ai+ 0.84 ai	Gramoxone Inteon + Linex	Syngenta Crop Protection
Paraquat + metribuzin	0.84 ai + 0.84 ai	Gramoxone Inteon + Sencor 75DF	Syngenta Crop Protection and Bayer Crop Science
Saflufenacil + atrazine	0.05 ai + 0.56 ai	Sharpen + AAtrex	BASF Corporation and Syngenta Crop Protection
Saflufenacil + 2,4-D	0.05 ai + 0.56 ai	Sharpen + Weedar 64	BASF Corporation and Nufarm, Inc.
Tembotrione + atrazine	0.09 ai + 0.56 ai	Laudis + AAtrex	Bayer Crop Science and Syngenta Crop Protection
Topramezone + atrazine	0.02 ai + 0.56 ai	Impact + AAtrex	Amvac Chemical Corporation and Syngenta Crop Protection

Table 6.2. Visual ratings of *Kochia scoparia* at 7, 14, and 28 d after treatment (DAT) of postharvest-applied herbicides in wheat stubble averaged over 2012 and 2013 at Montana State University, Southern Agricultural Research Center near Huntley, MT, USA.

Herbicide(s)[a]	Rate(s)	7 DAT	14 DAT	28 DAT
	kg ha^{-1}		%	
Dicamba[c,d]	0.56 ae	18	23	47
Dicamba + 2,4-D[c,d]	0.56 ae + 0.56 ae	21	36	55
Dicamba + atrazine[b]	0.56 ae + 0.56 ai	47	63	80
Dicamba + glyphosate[d]	0.56 ae + 1.26 ae	36	86	98
Diflufenzopyr + dicamba + 2,4-D	0.06 ai + 0.15 ae + 0.56 ae	41	54	67
Glyphosate[c]	1.26 ae	18	80	97
Glufosinate[c]	0.59 ai	67	87	95
Paraquat	0.84 ai	87	95	98
Paraquat + atrazine	0.84 ai + 0.56 ai	94	100	100
Paraquat + linuron[d]	0.84 ai + 0.84 ai	95	99	100
Paraquat + metribuzin	0.84 ai + 0.84 ai	97	100	100
Saflufenacil + atrazine[b,c]	0.05 ai + 0.56 ai	75	87	97
Saflufenacil + 2,4-D[b,c]	0.05 ai + 0.56 ai	76	87	96
Tembotrione + atrazine[b]	0.09 ai + 0.56 ai	37	79	91
Topramezone + atrazine[b]	0.02 ai + 0.56 ai	30	78	93
LSD (0.05)	——	5	4	4

[a] Herbicide treatments were applied 2 to 3 wk following the harvest of wheat, at the early bloom stage of 45- to 50-cm-tall *K. scoparia* plants, with an average density of 8 to 10 plants m^{-2} in wheat stubble.
[b] Methylated seed oil (MSO) at 1% v/v was included.
[c] Ammonium sulfate (AMS) at 2 % w/v was included.
[d] Nonionic surfactant (NIS) at 0.5% v/v was included.

Table 6.3. *Kochia scoparia* plant dry weight and seed reduction with the postharvest-applied herbicides in wheat stubble averaged over 2012 and 2013 at the Montana State University, Southern Agricultural Research Center near Huntley, MT, USA.

Herbicide (s)[a]	Rate (s)	Dry weight[e]	Seed reduction[e]
	kg ha^{-1}	—— % of nontreated ——	
Dicamba[c,d]	0.56 ae	64	46
Dicamba + 2,4-D[c,d]	0.56 ae + 0.56 ae	57	53
Dicamba + atrazine[b]	0.56 ae + 0.56 ai	51	78
Dicamba + glyphosate[d]	0.56 ae + 1.26 ae	30	100
Diflufenzopyr + dicamba + 2,4-D	0.06 ai + 0.15 ae + 0.56 ae	60	32
Glyphosate[c]	1.26 ae	31	100
Glufosinate[c]	0.59 ai	32	100
Paraquat	0.84 ai	31	100
Paraquat + atrazine	0.84 ai + 0.56 ai	29	100
Paraquat + linuron[d]	0.84 ai+ 0.84 ai	30	100
Paraquat + metribuzin	0.84 ai + 0.84 ai	27	100
Saflufenacil + atrazine[b,c]	0.05 ai + 0.56 ai	31	100
Saflufenacil + 2,4-D[b,c]	0.05 ai + 0.56 ai	28	100
Tembotrione + atrazine[b]	0.09 ai + 0.56 ai	32	100
Topramezone + atrazine[b]	0.02 ai + 0.56 ai	31	100
LSD (0.05)	——	5	2.4

[a] Herbicide treatments were applied 2 to 3 wk following the harvest of wheat, at the early bloom stage of 45- to 50-cm-tall *K. scoparia* plants in wheat stubble.
[b] Methylated seed oil (MSO) at 1% v/v was included.
[c] Ammonium sulfate (AMS) at 2 % w/v was included.
[d] Nonionic surfactant (NIS) at 0.5% v/v was included.
[e] *K. scoparia* plants were harvested from a 1-m^2 quadrat placed at the center of each plot. Plant dry weight and seed production averaged 429 g and 104,100 seeds m^{-2}, respectively, in nontreated plots.

Table 6.4. Influence of postharvest-applied herbicides on *Kochia scoparia* seed weight, seed viability, and progeny seedling fitness averaged over 2012 and 2013 at the Montana State University, Southern Agricultural Research Center near Huntley, MT, USA.

Herbicide(s)[a]	Rate(s)	100-seed weight	Seed viability[b]	Seedling height[c]	Seedling width[c]	Primary branches[c]	Shoot dry weight[c]
	kg ha^{-1}	Mg	%	(cm)	(cm)	no. seedling^{-1}	g seedling^{-1}
Dicamba	0.56 ae	130	92	34.7	17.5	16	2.6
Dicamba + 2,4-D	0.56 ae + 0.56 ae	116	91	38.1	21.5	17	3.2
Dicamba + atrazine	0.56 ae + 0.56 ai	100	84	37.5	19.8	17	2.9
Diflufenzopyr + dicamba + 2,4-D	0.06 ai + 0.15 ae + 0.56 ae	117	92	34.7	17.6	17	2.6
Nontreated	—	130	92	37.2	19.2	16	2.8
LSD (0.05)	—	21	6	NS	NS	NS	NS

[a] Herbicide treatments were applied 2 to 3 wk following the harvest of wheat, at the early bloom stage of 45- to 50-cm-tall *K. scoparia* plants in wheat stubble. Only those treatments in which *K. scoparia* survivors produced seed following a postharvest herbicide application in wheat stubble, and the nontreated check are included.

[b] Seed viability was determined as the percentage of total seeds that germinated plus those tested positive in tetrazolium chloride test.

[c] Progeny growth parameters were recorded at 42 d after planting in the greenhouse.

References

Al-Ahmadi, M.J., Kafi, M., 2007. Cardinal temperatures for germination of *Kochia scoparia* (L.). J. Arid Environ. 68, 308–314.

Anderson, R.L., Nielsen, D.C., 1996. Emergence pattern of five weeds in the central Great Plains. Weed Technol. 10, 744–749.

Bagavathiannan, M.V., Norsworthy, J.K., 2012. Late-season seed production in arable weed communities: management implications. Weed Sci. 60, 325–334.

Baker, D.V., Withrow, J.R., Brown, C.S., Beck, K.G., 2010. Tumbling: use of diffuse knapweed (*Centaurea diffusa*) to examine an understudied dispersal mechanism. Invasive Plant Sci. Manage. 3, 301–309.

Beckie, H.J., Blackshaw, R.E., Low, R., Hall, L.M., Sauder, C.A., Martin, S., Brandt, E.N., Shirriff, S.W., 2013. Glyphosate- and acetolactate synthase inhibitor-resistant kochia (*Kochia scoparia*) in western Canada. Weed Sci. 61, 310–318.

Bennett, A.C., Shaw, D.R., 2000. Effect of preharvest desiccants on weed seed production and viability. Weed Technol. 14, 530–538.

Biniak, B.M., Aldrich, R.J., 1986. Reducing velvetleaf (*Abutilon theophrasti*) and giant foxtail (*Setaria faberi*) seed production with stimulated-roller herbicide applications. Weed Sci. 34, 256–259.

Christoffoleti, P.J., Westra, P.B., Moore, F., 1997. Growth analysis of sulfonylurea-resistant and -susceptible kochia (*Kochia scoparia*). Weed Sci. 45, 691–695.

Clay, P.A., Griffin, J.L., 2000. Weed seed production and seedling emergence responses to late-season glyphosate applications. Weed Sci. 48, 481–486.

Cranston, H.J., Kern, A.J., Hackett, J.L., Miller, E.K., Maxwell, B.D., Dyer, W.E., 2001. Dicamba resistance in kochia. Weed Sci. 49, 164–170.

Dahl, G.K., Dexter, A.G., Nalewaja, J.D., 1982. Kochia competition and control in wheat. In: North Central Weed Control Conference. Indianapolis, USA.

Dille, J.A., Stahlman, P.W., Geier, P.W., Riffel, J.D., Currie, R.S., Wilson, R.G., Sbatella, G.M., Westra, P., Kniss, A.R., Moechnig, M.J., Cole, R.M., 2012. Kochia emergence profiles across the central Great Plains. In: Weed Science Society of America Conference. Hawaii, USA.

Durgan, B.R., Dexter, A.G., Miller, S.D., 1990. Kochia (*Kochia scoparia*) interference in sunflower (*Helianthus annus*). Weed Technol. 4, 52–56.

Eberlein, C.V., Fore, Z.A., 1984. Kochia biology. Weeds Today. 15, 5–6.

Everitt, J.H., Alaniz, M.A., Lee, J.B., 1983. Seed germination characteristics of *Kochia scoparia*. J. Range Manage. 36, 646–648.

Fawcett, R.S., Slife, F.W., 1978. Effects of 2, 4-D and dalapon on weed seed production and dormancy. Weed Sci. 26, 543–547.

Forcella, F., 1985. Spread of kochia in the northwestern United States. Weeds Today. 16, 4–6.

Friesen, L.F., Beckie, H.J., Warwick, S.I., Van Acker, R.C., 2009. The biology of Canadian weeds. 138. *Kochia scoparia* (L.) Schrad. Can. J. Plant Sci. 89, 141–167.

Heap, I.M., 2014. International Survey of Herbicide Resistant Weeds. www.weedscience.org (accessed October 2014).

Isaacs, M.A., Murdock, E.C., Toler, J.E., Wallace, S.U., 1989. Effects of late-season herbicide applications on sicklepod (*Cassia obtusifolia*) seed production and viability. Weed Sci. 37, 761–765.

Jacobson, J., Jackson, G., Jones, C., 2005. Fertilizer guidelines for Montana crops. Montana State University EB, 161.

Jha, P., Norsworthy, J.K., 2012. Influence of late-season herbicide applications on control, fecundity, and progeny fitness of glyphosate-resistant palmer amaranth (*Amaranthus palmeri*) biotypes from Arkansas. Weed Technol. 26, 807–812.

Kumar, V., Jha, P., Reichard, N., 2014. Occurrence and characterization of kochia (*Kochia scoparia*) populations with resistance to glyphosate in Montana. Weed Technol. 28, 122–130.

Mickelson, J.A., Bussan, A.J., Davis, E.S., Hulting, A.G., Dyer, W.E., 2004. Postharvest kochia (*Kochia scoparia*) management with herbicides in small grains. Weed Technol. 18, 426–431.

Mengistu, L.W., Messersmith, C.G., 2002. Genetic diversity of kochia. Weed Sci. 50, 498–503.

Norsworthy, J.K., Ward, S.M., Shaw, D.R., Llewellyn, R.S., Nichols, R.L., Webster, T.M., Bradley, K.W., Frisvold, G., Powles, S.B., Burgos, N.R., Witt, W.W., Barrett,

M., 2012. Reducing the risks of herbicide resistance: Best management practices and recommendations. Weed Sci. 60, 31–62.

Preston, C., Belles, D.S., Westra, P.H., Nissen, S.J., Ward, S.M., 2009. Inheritance of resistance to the auxinic herbicide dicamba in kochia (*Kochia scoparia*). Weed Sci. 57, 43–47.

Primiani, M.M., Cotterman, J.C., Saari, L.L., 1990. Resistance of *Kochia scoparia* to sulfonylurea and imidazolinone herbicides. Weed Technol. 4, 169–172.

Sbatella, G.M., Wilson, R.G., 2010. Isoxaflutole shifts kochia (*Kochia scoparia*) populations in continuous corn. Weed Technol. 24, 392–396.

Schwinghamer, T.D., Van Acker, R.C., 2008. Emergence timing and persistence of kochia (*Kochia Scoparia*). Weed Sci. 56, 37–41.

Stallings, G.P., Thill, D.C., Mallory-Smith, C.A., Shafii, B., 1995. Pollen-mediated gene flow of sulfonylurea-resistant kochia (*Kochia scoparia*). Weed Sci. 43, 95–102.

Steadman, K.J., Eaton, D.M., Plummer, J.A., Ferris, D.J., Powles, S.B., 2006. Late-season non-selective herbicide applications reduce *Lolium rigidum* seed numbers, seed viability, and seedling fitness. Aust. J. Agric. Res. 57, 133–141.

Taylor, S.E., Oliver, L.R., 1997. Sicklepod (*Senna obtusifolia*) seed production and viability as influenced by late-season postemergence herbicide applications. Weed Sci. 45, 497–501.

Thomas, W.E., Pline-Srnic, W.P., Viator, R.A., Wilcut, J.W., 2005. Effects of glyphosate application timing and rate on sicklepod (*Senna obtusifolia*) fecundity. Weed Technol. 19, 55–61.

Waite J., Thompson, C.R., Peterson, D.E., Currie, R.S., Olson, B.L.S., Stahlman, P.W., Khatib, K.A., 2013. Differential kochia (*Kochia scoparia*) populations response to glyphosate. Weed Sci. 61, 193–200.

Walker, E.R., Oliver, L.R., 2008. Weed seed production as influenced by glyphosate applications at flowering across a weed complex. Weed Technol. 22, 318–325.

Weatherspoon, D.M., Schweizer, E.E., 1971. Competition between sugarbeets and five densities of kochia. Weed Sci. 19, 125–128.

Wicks, G.A., Martin, A.R., Mahnken, G.W., 1993. Control of triazine-resistant kochia (*Kochia scoparia*) in conservation tillage corn (*Zea mays*). Weed Sci. 41, 225–231.

Wicks, G.A., Martin, A.R., Haack, A.E., Mahnken, G.W., 1994. Control of triazine-resistant kochia (*Kochia scoparia*) in sorghum (*Sorghum bicolor*). Weed Technol. 8, 748–753.

Young, J.A., Evans, R.A., Stevens, R., Everett, R.L., 1981. Germination of *Kochia prostrata* seed. Agron. J. 73, 957–961.

Zimdahl, R.L., 2004. Weed-Crop competition. A Review, second ed. Blackwell publishing Ltd., Oxford, UK, p. 220

CHAPTER 7

INFLUENCE OF GLYPHOSATE TIMING ON KOCHIA (*KOCHIA SCOPARIA*) DEMOGRAPHICS IN GLYPHOSATE-RESISTANT SUGAR BEET

Contribution of Authors and Co-Authors

Manuscript in Chapter 7

Author: Vipan Kumar

Contributions: Conceived and implemented the study design. Conducted experiments, reviewed the literature, and wrote the manuscript.

Co-Author: Prashant Jha

Contributions: Advisement, provided field expertise and feedback on manuscript drafts

Manuscript Information Page

Vipan Kumar, Prashant Jha
Weed Technology Journal
Status of Manuscript:
_×_Prepared for submission to a peer-reviewed journal
___Officially submitted to a peer-review journal
___Accepted by a peer-reviewed journal
___Published in a peer-reviewed journal

Published by Allen Press, Inc.
To be submitted in 2015

Abstract

An increased occurrence of glyphosate-resistant (GR) kochia is a major threat to the sustainability of GR cropping systems, including GR sugar beet in the northwestern United States. Lack of effective and economical, alternative herbicide programs for weed control in sugar beet further exacerbates the problem. To develop glyphosate resistance management strategies in GR sugar beet, it is critical to understand the population dynamics and demographics of weeds in response to glyphosate application timing, and their overall impact on soil seed bank replenishment. To fulfill this objective, field experiments were conducted at the Montana State University Southern Agricultural Research Center near Huntley, Montana, in 2012 and 2013. Treatments included glyphosate timing as a single application at the 2-, 6-, or 10-leaf (lf) stage of sugar beet or sequential applications at 2-lf followed by (fb) 6-lf, 6-lf fb 10-lf, 2-lf fb 6-lf fb 10-lf, or 6-lf fb 10-lf fb canopy closure (CC) stage of sugar beet. Kochia emergence was monitored in three cohorts. Results indicated that kochia plants in cohort 1 and 2 were more competitive (greater biomass and seed production) than cohort 3, at least in the absence of glyphosate. Compared with a single glyphosate timing, sequential applications of glyphosate at 6-lf fb 10-lf stage prevented kochia survival, biomass and seed production in cohort 1 and cohort 2. Kochia plants in cohort 3 that emerged after the glyphosate application at the 10-lf stage, produced up to 1123 seeds m^{-2}, and an additional application at the CC stage of sugar beet was needed to prevent biomass and seed production from cohort 3 survivors. Irrespective of timing, sequential applications of glyphosate provided the highest root and sucrose yields in GR sugar beet. In conclusion,

growers should utilize three applications of glyphosate applied sequentially at 6-lf fb 10-lf fb CC stage of sugar beet to prevent kochia seed production in GR sugar beet.

Nomenclature: Glyphosate; kochia, *Kochia scoparia* (L.) Schrad; sugar beet, *Beta vulgaris* L.

Key words: Weed cohort, demographics, fecundity, glyphosate resistance, population dynamics, and herbicide resistance management.

Introduction

Sugar beet is a commercial row crop grown in the northwestern United States, including Montana. In 2013, Montana ranked sixth among sugar beet growing states in the US (USDA-NASS 2014). Weed management is one of the major challenges in sugar beet production (Carlson et al. 2008). Sugar beet is a slow growing crop and a poor competitor of weeds, especially at the early growth stages (Dexter and Zollinger 2003; Scott and Wilcockson 1976). After the introduction of glyphosate-resistant (GR) sugar beet in 2007, it has been rapidly adopted by growers, and comprises more than 95% of the total U.S. sugar beet production (Kniss 2010). Weeds emerge in multiple flushes (cohorts) throughout the sugar beet growing season, and two to three applications of glyphosate are often needed for season-long weed control in GR sugar beet production (Kniss 2010; Wilson et al. 2002). Because of high efficacy, broad-spectrum weed control, and flexibility in application timing provided by glyphosate, and with a limited alternative, effective and economic herbicide options in sugar beet, growers often rely solely on glyphosate for weed control in their GR sugar beet fields (Kemp et al. 2009). Glyphosate applications are timed relative to sugar beet growth stages, and optimizing the application timing to target weed cohorts that emerge at different growth stages of sugar beet is critical to prevent weed escapes from glyphosate and maintain long-term sustainability of GR sugar beet.

Kochia (*Kochia scoparia* L.) is a monoecious, summer annual broadleaf weed in the cropland and non-cropland areas of the US Great Plains (Eberlein and Fore 1984; Forcella 1985; Wicks et al. 1994). It is considered one of the most problematic weeds in

agronomic crops of this region, including sugar beet, wheat (*Triticum aestivum* L.), corn (*Zea mays* L.), sorghum (*Sorghum bicolor* L.), sunflower (*Helianthus annus* L.), and soybean (*Glycine max* L. Merr) (Waite et al. 2013). Season-long interference of kochia reduced soybean, sorghum, wheat, corn, and sugar beet yields by 30, 38, 58, 40, and 95%, respectively (Dahl 1982; Durgan et al. 1990; Waite et al. 2013; Weatherspoon and Schweizer 1969; Wicks et al. 1993, 1994), a much higher yield loss in sugar beet compared to other crops. The success of kochia as a major weed is attributed to its early and extended period of emergence (Dille et al. 2012; Schwinghamer and Van Acker 2008), rapid growth rate with a C_4 photosynthetic pathway (Christoffoleti et al. 1997; Friesen et al. 2009), heat and salt tolerance (Friesen et al. 2009), prolific seed production (>50,000 seeds plant^{-1}) (Stallings et al. 1995), and long-distance seed dispersal by tumble mechanism (Baker et al. 2010; Friesen et al. 2009). Kochia biotypes with resistance to sulfonylurea (ALS inhibitors), triazine (PS II inhibitors), synthetic auxins, and glyphosate have been reported in this region (Heap 2015; Kumar et al. 2014).

Kochia has a competitive advantage over sugar beet due to its early germination, rapid growth, and an extended period of emergence in the growing season (Dotzenko and Arp 1971; Weatherspoon and Schweizer 1969). Studies in other crops such as soybean and corn demonstrate that weed cohorts emerging early in the season are more competitive, with greater vegetative biomass and seed production, and contribute to a greater reduction in crop yield compared with the late-emerging cohorts (Jha et al. 2008; Knezevic et al. 1994; Norsworthy et al. 2007). The reduction in competing ability of late-emerging weed cohorts in a crop is mainly attributed to the shading effect of crop canopy

on germination/emergence and survival of those cohorts (Jha et al. 2008; Norsworthy et al. 2007). Nevertheless, late-emerging weed cohorts can contribute significantly to the replenishment of the weed seed bank in the soil (Jha et al. 2008; Norsworthy and Olivier 2001; Norsworthy et al. 2007; Steckel and Sprague 2004) which further warrants the need of multiple POST applications of glyphosate, especially in GR sugar beet. Late-emerging kochia seedlings at a density of 8 to 10 plants m^{-2} even after decapitation at wheat harvest produced up to 100,104 seeds m^{-2} (Kumar and Jha 2015), indicating the high fecundity potential of this weed species.

Two to three in-crop applications of glyphosate per growing season over multiple years may potentially select kochia populations with evolved resistance to glyphosate in GR sugar beet fields, as reported in GR soybean and corn in the central and northern Great Plains (Heap 2015). The problem is further exacerbated because of a lack of herbicides (PRE or POST) for effective kochia control in sugar beet (Kniss 2010; Kemp et al. 2009). From a resistance management standpoint, it is imperative to understand the response of individual kochia cohorts to glyphosate applications and their overall impact on the weed seed bank in GR sugar beet production system.

We hypothesized that kochia management in GR sugar beet can be improved by understanding the population dynamics and demographics (seedling emergence, survival, biomass, seed production, and seed viability) of kochia cohorts that emerge at different growth stages of sugar beet and their response to glyphosate application timings. There appears to be no published research on the influence of glyphosate application timings on the kochia demographics in GR sugar beet. The objectives of this research were to

understand the effect of glyphosate application timings on survival, vegetative biomass, seed production, and seed viability of kochia, and their ultimate impact on root and sucrose yields in GR sugar beet.

Materials and Methods

Field Experiment. Field experiments were conducted at the Montana State University (MSU) Southern Agricultural Research Center (SARC) near Huntley, MT in 2012 and 2013. The soil type at the study site was a Fort Collins clay loam (Fine-loamy, mixed, superactive, mesic Aridic Haplustalfs) with 2.8% organic matter and a pH of 7.8. The experimental site was under sugar beet-barley rotation for the past five yr prior to the initiation of the study. Seedbed preparation at the study site included disking in the fall followed by field cultivation and leveling in the spring before sugar beet planting. Fully matured kochia seeds were collected from a naturally infested area at the MSU-SARC research farm. Seeds were broadcasted 2 to 3 d before sugar beet planting to obtain uniform kochia infestation. The selected kochia population was susceptible to glyphosate at the field-use rate of 870 g ae ha^{-1} (data not shown). Each yr, sugar beet plots were fertilized with Nitrogen-Phosphorus-Potash as recommended by the Montana State University sugar beet production guide (Jacobson et al. 2005). A burn-down application of glyphosate at 870 g ha^{-1} was made prior to sugar beet planting to kill existing weeds in the field. GR sugar beet variety "BTS 36RR50Pro200" (Genuity® Brand, Monsanto Company, 800 North Lindbergh Blvd., St. Louis, MO 63167) was planted at a rate of 119,500 seeds ha^{-1} in four rows spaced 61 cm apart on April 27, 2012, and April 24,

2013. GR sugar beet seedlings emerged approximately 7 to 10 d after planting. Immediately after planting, permanent 1-m^2 quadrats were established at the center of each sugar beet plot to monitor the emergence and survival of kochia cohorts throughout the growing season. Kochia cohorts that emerged at different dates relative to the sugar beet growth stages were separately marked with different colored plastic sticks within a quadrat in each plot. The first kochia cohort (cohort 1) comprised of plants that emerged from planting to the 2-leaf (lf) stage of sugar beet. The second kochia cohort (cohort 2) comprised of plants that emerged between the 2-lf and the 6-lf stage of sugar beet, and third cohort (cohort 3) comprised of kochia plants that emerged after the 10-lf stage and before the canopy closure (CC) stage of sugar beet.

Glyphosate (Roundup PowerMax®, Monsanto Company, 800 North Lindbergh Blvd., St. Louis, MO) treatments included single and sequential applications at different timings: 1) single application at the 2-lf stage, 2) single application at the 6-lf stage, 3) single application at the 10-lf stage , 4) sequential application at 2-lf followed by (fb) 6-lf stage, 5) sequential applications at 6-lf fb 10-lf stage, 6) sequential application at 2-lf fb 6-lf fb 10-lf stage of sugar beet, 7) sequential application at 6-lf fb 10-lf fb CC of sugar beet, and 8) a nontreated control for comparison. Glyphosate at 1260 g ha^{-1} was applied at 2-lf and 6-lf stages; whereas, 870 g ha^{-1} of glyphosate was applied at 10-lf and CC stages of sugar beet. Glyphosate application dates and corresponding sugar beet growth stages have been summarized in Table 7.1. The test site was kept free from other grassy and broadleaf weeds by hand-weeding as and when needed. Treatments were applied with a CO_2-pressurized backpack sprayer equipped with flat-fan spray nozzles (TeeJet

8001XR, Spraying Systems Co., P.O. Box 7900, Wheaton, IL), calibrated to deliver 94 L ha^{-1} of spray solution at 276 kPa. Ammonium sulfate (AMS) at 2% w/v was included with each glyphosate treatment. The study site was irrigated four times each yr as per the local standard, with an overhead linear sprinkler irrigation system.

The experiments were conducted as a randomized complete block design. Each glyphosate timing treatment was replicated in four blocks. Each experimental plot was 10 m long by 3 m wide. Each plot contained sugar beet plants along with kochia. The experimental design included repeated measures because survival of kochia cohorts within a glyphosate timing treatment was assessed at different wk after sugar beet emergence (WAE) during the growing season. Kochia emergence and survival was monitored beginning at 3 WAE and until sugar beet harvest. Biomass and seed production of kochia in each cohort were measured only at harvest. At maturity, kochia plants in each cohort were harvested at the soil level from the 1-m^2 quadrats using hand pruners. After harvesting, inflorescences were separated from the shoots by hand to determine the vegetative biomass and seed production of kochia plants in each cohort. The vegetative biomass was oven-dried at 65 C for 5 d and weighed. Kochia inflorescences were air-dried at room temperature for 72 h, threshed by hand, sieved through a 2-mm mesh size screen, and blown using an air-propelled column blower to separate the seeds from the remaining debris. The 1000-seed weight and total seed weight of each kochia cohort was determined to estimate seed production. Two central rows of sugar beet in each plot were harvested using a mechanical digger on September 21, 2012, and September 26, 2013. Sugar beet roots were counted and weighed to estimate root

yield in each plot. Sugar beet root samples were sent to the Western Sugar Cooperative, Billings, MT, for percent sucrose analysis, and sucrose yield for each plot was determined.

Laboratory Experiment. Experiments were conducted to test the viability of kochia seeds produced by glyphosate-treated plants (escapes) in comparison to those from the nontreated check in each cohort. A 75-seed subsample was randomly selected from a plot sample for each kochia cohort (only those where survivors produced seeds). The germination assays were conducted in a completely randomized design with three replications (25 seeds/petri dish), and repeated in time. Seeds were placed on two layers of filter paper (Whatman®, Grade 2, Sigma-Aldrich Inc., St. Louis, MO 63178, USA) moistened with 10 ml deionized water in 10-cm-diam petri dishes (Sigma-Aldrich). Petri dishes were wrapped with aluminum foil to avoid moisture loss. Light is not required for kochia seed germination (Everitt et al. 1983). Therefore, all petri dishes were placed in the dark in an incubator (VMR International, Sheldon Manufacturing Inc., Cornelius, OR 97113, USA) set to a constant temperature of 24 C, an optimum temperature for seed germination of kochia (Al-Ahmadi and Kafi, 2007). Seeds were considered germinated when the radicals emerged and the tip of the radicle was uncoiled (Young et al. 1981). Germinated seeds were counted daily and removed until 15 d after incubation. Non-germinated seeds were tested for viability using a 1% w/v tetrazolium chloride solution (Sbatella and Wilson, 2010). Seed viability was determined as the percentage of total seeds that germinated plus those tested positive in the tetrazolium chloride assay.

Statistical Analyses. All data from field and laboratory experiments were subjected to ANOVA using PROC MIXED in SAS (Statistical Analysis Systems®, version 9.3, SAS Institute Inc., SAS Campus Drive, Cary, NC). For percent survival of each kochia cohort, a repeated measure analysis was performed. A linear model was developed for the randomized complete block with repeated measures design. It included the fixed effects of glyphosate timing, kochia cohort (when applicable), WAE, and their interactions. Error terms were defined for the variables and the repeated measure (WAE). Random effects in the model included yr (field experiment) or run (laboratory experiment), replication nested within a yr or run, and interactions involving either of these two variables. Data on percent survival were arcsine-square-root transformed; data on vegetative biomass and seed production were log-transformed to improve the normality of residuals and homogeneity of variance. The assumption test calculations were performed using PROC UNIVARIATE and PROC GLM in SAS, and the transformed data met both the assumptions. Nontransformed means are presented in tables based on the interpretation from the transformed data. Means were separated using Fisher's protected LSD test at $P < 0.05$ using the PDMIX800 macro in SAS (Saxton 1998).

Results and Discussion

Kochia Cohorts. Kochia emergence in cohorts 1, 2, and 3 was similar in 2012 and 2013 sugar beet growing seasons. Emergence in cohort 1, 2, and 3 averaged 192, 58, and 18 plants m^{-2}, respectively (data not shown). In each yr, emergence of kochia in cohorts 1, 2, and 3 occurred between May 10 to May 20, June 1 to June 10, and July 1 to July 12,

respectively, in sugar beet. Mean daily minimum and maximum air temperatures during the emergence periods were 8 and 25 C, 7 and 24 C, 14 and 33 C, respectively (Figure 7.1). Kochia plants in cohort 1 were 2 to 8 cm in height when glyphosate was applied at the 2-lf stage of sugar beet. Plants from cohort 1 that were not treated at the 2-lf stage of sugar beet attained up to 25 cm height by the 6-lf stage of sugar beet. Plants in cohort 2 were 6 to 8 cm in height by the 6-lf stage of sugar beet, and plants in cohort 3 were 2 to 4 cm tall by the CC stage of sugar beet. Majority of the kochia seed bank (>90%) emerged in cohorts 1 and 2. No kochia emergence was observed after CC stage of sugar beet (occurred in the first fortnight of July) in any of the two yr.

Kochia Survival. Percent survival of kochia plants in cohort 1 was affected by the interaction of glyphosate timing and WAE over which survival was monitored during the growing season ($P < 0.001$). Kochia survival in cohort 1 was only 1 to 2% at 5 WAE following a single application of glyphosate at the 2-lf stage of sugar beet (Table 7.2). Survival of cohort 1 at 5 WAE in plots that received a single glyphosate application at 6-lf or 10-lf or sequential application at 6-lf fb 10-lf, 6-lf fb 10-lf fb CC stage of sugar beet did not differ from the nontreated plots because glyphosate was not applied in those plots by 5 WAE. When a single application of glyphosate was made at the 6-lf stage of sugar beet, survival of cohort 1 was 2% at 8 WAE, and did not differ from the single application at the 2-lf or sequential application at 2-lf fb 6-lf stage of sugar beet; however, the survival was less than the 10-lf glyphosate timing treatment. This was expected because those plots did not receive any glyphosate by the 8 WAE evaluation date (Table 7.2). When a single application of glyphosate was further delayed to the 10-lf

stage of sugar beet, survival of cohort 1 was 3% at 11 WAE, and did not differ from any single or sequential application treatments. There was a decline in kochia survival in the nontreated plots from 97% at 5 WAE to 80% at 15 WAE. In the nontreated plots, kochia plants from cohort 1 grew above the sugar beet canopy and the sugar beet was at a competitive disadvantage; therefore, the decline in percent survival was more likely attributed to the intraspecific competition or density-dependent mortality of kochia plants in those plots. Also, in soybean, the early emerging cohort of Palmer amaranth (emerged between planting to V3 stage of soybean) was more competitive than the crop (Jha et al. 2008). At the final assessment date (15 WAE), only 1 to 2% survival of cohort 1 was observed in plots treated with a single application of glyphosate, irrespective of application timing, and none of the plants survived the sequential glyphosate treatments.

Similar to cohort 1, percent survival of cohort 2 was influenced by the interaction of glyphosate application timing and WAE over which the survival was monitored ($P = 0.0023$). Cohort 2 emerged between 2-lf and 6-lf stage of sugar beet; thus, escaped the first glyphosate application made at the 2-lf timing (Table 7.2). Furthermore, cohort 2 did not receive a 10-lf glyphosate treatment until 8 WAE; therefore, percent survival of cohort 2 at 2-lf and 10-lf glyphosate timing did not differ the nontreated control, and averaged 93% at 8 WAE. A single glyphosate application at the 6-lf stage of sugar beet reduced cohort 2 survival to 3 to 6% compared with 91% survival in the nontreated plots at 8 WAE. At 11 WAE, survival of cohort 2 treated with a single delayed application at the 10-lf stage or sequential application of glyphosate at 6-lf fb 10-lf or 6-lf fb 10-lf fb CC stage of sugar beet was 2 to 8% compared with 82% survival in nontreated plots.

There was a decline in the percent survival of cohort 2 from 91% at 8 WAE to 64% at 15 WAE in the nontreated plots. Density-dependent mortality in nontreated plots has been previously reported in other weed species such as Palmer amaranth (*Amaranthus palmeri*), pusley (*Richardia spp.*), and common waterhemp (*Amaranthus rudis*) grown with soybean (Jha et al. 2008; Steckel and Sprague 2004). Sequential applications of glyphosate at 6-lf fb 10-lf or 6 lf- fb 10-lf fb CC timings prevented any kochia survival from cohort 2.

Similar to cohort 1 and 2, percent kochia survival in cohort 3 was influenced by the interaction of glyphosate timing and WAE (P = 0.0031). Kochia plants in cohort 3 emerged after the 10-lf application timing and before the CC stage of sugar beet in both years; thus, the late-emerging cohort escaped single or sequential applications of glyphosate made at 2-lf, 6-lf, or 10-lf stage of sugar beet. This data suggests that kochia has an extended period of emergence in sugar beet. Following an application of glyphosate at the CC stage of sugar beet, survival of cohort 3 at 11 WAE (7 d after the CC glyphosate timing) was reduced to 51% compared with 88 to 92% survival in nontreated plots (Table 7.2). A relatively less mortality of the cohort 3 plants 7 d after the CC glyphosate timing might be because of poor spray coverage on the 2 to 4 cm tall kochia plants beneath the sugar beet canopy at the time of herbicide application; however, none of the treated plants of cohort 3 survived by 15 WAE. In the nontreated plots, a significant decline in survival of cohort 3 from 11 to 15 WAE was most likely attributed to the shading effect of sugar beet canopy on the late-emerging kochia seedlings and to the intraspecific competition from survivors of cohort 1 and 2.

Kochia Biomass. There was a significant interaction of glyphosate timing by cohort on kochia biomass production (P = 0.0012). Averaged over yr, total biomass production by kochia survivors in cohort 1, 2, and 3 in nontreated control was 799 g m^{-2}. Cohort 1 contributed 453 g m^{-2} compared with 329 and 17 g m^{-2} by cohort 2 and 3, respectively, of the total kochia biomass in nontreated control. This indicates that early emerging kochia cohorts are more competitive to sugar beet compared with the latter ones. Similar results have been previously reported with common lambsquarters (*Chenopodium album*), sunflower (*Helianthus annuus*), velvetleaf (*Abutilon theophrasti*), and Powell amaranth (*Amaranthus powellii*) interference in sugar beet (Schweizer and Bridge 1982; Schweizer 1983; Schweizer and Lauridson 1985; Weatherspoon and Schweizer 1969). Also reported in those studies, sugar beet is a poor competitor of weeds at the early growth stages or before canopy closure, and majority of the broadleaf weeds emerging early in the season grow tall and shade the sugar beet plants.

Kochia survivors (1 to 2% at 15 WAE) in cohort 1 from a single application of glyphosate at the 2-lf, 6-lf, or 10-lf stage of sugar beet produced 39 to 50 g m^{-2} of vegetative biomass compared with no biomass production in plots treated with a sequential glyphosate application (Table 7.3). Kochia survivors in cohort 2 that escaped a single glyphosate application at the 2-lf timing produced biomass similar to the plants in the nontreated control, which averaged 325 g m^{-2} (Table 7.3). Although the differences were not significant, the average biomass produced by survivors following a single glyphosate application at the 6-lf or 10-lf stage of sugar beet was 18 g m^{-2} in cohort 2 compared with 47 g m^{-2} in cohort 1 (Table 7.3). This was possibly because kochia plants

in cohort 1 were larger in size when a single application of glyphosate was delayed to the 6-lf or 10-lf stage of sugar beet compared with the cohort 2 plants. The data indicates that timing of glyphosate application is crucial to reduce kochia competitiveness in GR sugar beet, especially from the early emerging cohort. Similar to cohort 1, a sequential application of glyphosate at 6-lf fb 10-lf or 6-lf fb 10-lf fb CC stage of sugar beet prevented any biomass production from kochia cohort 2 (Table 7.3). Kochia biomass production by the late-emerging cohort 3 plants was 12 to 20 g m^{-2} in the absence of glyphosate, and interestingly, did not differ from the biomass produced by glyphosate survivors from cohort 1 and 2. An additional application of glyphosate at the CC stage of sugar beet was needed to prevent vegetative biomass production from cohort 3 (Table 7.3).

Kochia Seed Production. Kochia seed production was also influenced by the interaction of glyphosate timing and cohort ($P < 0.001$). Kochia plants from cohort 1, 2, and 3 produced a total of 318,362 seeds m^{-2} in the nontreated plots in GR sugar beet (Table 7.3). Cohort 1, 2, and 3 contributed 86, 13.6, and 0.4%, respectively, of the total kochia seed production in the nontreated plots. These results are consistent with previously reported studies in GR corn and soybean, where early-emerging cohorts of Palmer amaranth, pusley, and sicklepod (*Senna obtusifolia*) produced more seeds than the late-emerging cohorts (Jha et al. 2008; Massinga et al. 2001; Norsworthy et al. 2007). Among all single glyphosate timing treatments, the total seed production was highest with the 2-lf timing (59,009 seed m^{-2}) and least with the 6-lf timing (18,461 seeds m^{-2}). The greater total seed production at the 2-lf glyphosate timing was attributed to the cohort 2 plants

that emerged after the 2-lf glyphosate timing and were not treated until the 6-lf stage of sugar beet (Table 7.3).

A small proportion of kochia plants (1%) from cohort 1 that survived the single application at the 2-lf stage of sugar beet produced significant amount of seeds, which averaged 12,166 seeds m^{-2}; however, it was 73% less than the seed production from cohort 2 (Table 7.3). There were no differences in seed production from cohort 1 when glyphosate was applied as a single application at 2-lf, 6-lf, or 10-lf timing, and ranged from 12,166 to 16, 943 seeds m^{-2}. The 2-lf fb 6-lf glyphosate timing prevented seed production from cohort 1, and there was no advantage of an additional application at the 10-lf or CC timing.

In cohort 2, delaying a single glyphosate application to the 10-lf stage of sugar beet (60 to 65 cm tall kochia plants) increased seed production (5,139 seeds m^{-2}) from the survivors compared with the 6-lf timing (2,375 and 2,948 seeds m^{-2}), which indicates that size of kochia plants at the time of glyphosate application is crucial to reduce seed production. Sequential glyphosate applications at 6-lf fb 10-lf stage prevented seed production from cohort 2, and did not differ from sequential glyphosate treatments at 2-lf fb 6-lf fb 10-lf or 6-lf fb 10-lf fb CC (Table 7.3).

Uncontrolled kochia plants in cohort 3 that emerged late after the glyphosate application at the 10-lf stage of sugar beet produced 536 to 1,123 seeds m^{-2} (Table 7.3), enough to cause significant replenishment of the soil seed bank in a season. Glyphosate applied at the CC stage of sugar beet was required to prevent seed production from cohort 3. The results suggest that three sequential applications of glyphosate at the 6-lf fb 10-lf

fb CC stage of sugar beet will be needed to prevent kochia seed bank additions to the soil and prevent infestation in the following year.

Seed Viability. There was no significant effect of glyphosate timing, cohort, or their interaction on the seed viability of kochia obtained from the survivors in treated or nontreated plots ($P = 0.634$). Averaged across glyphosate timing and cohort, kochia seed viability was 97 to 100% (data not shown). Kochia exhibits low seed dormancy (<10%) (Friesen et al. 2009), thus, the majority of the seeds from the glyphosate-treated or nontreated kochia plants senesced in the fall (after sugar beet harvest) and lying on the soil surface are expected to germinate in the following spring.

GR Sugar Beet Root and Sucrose Yields. Sugar beet root and sucrose yields were influenced by the main effect of glyphosate timing ($P < 0.001$). Averaged over yr, a single application of glyphosate at 2-lf, 6-lf, or 10-lf stage of sugar beet prevented root and sucrose yield reductions by 55 and 54%, respectively, compared with the nontreated control (Table 7.4). Although differences were not significant for root yields, delaying a single application of glyphosate to the 10-lf stage reduced sucrose yield by 16% compared with the glyphosate treatment at the 2-lf or 6-lf stage of sugar beet. Furthermore, average root and sucrose yields in plots treated with a sequential application of glyphosate were 49,385 and 6,746 kg ha^{-1}, respectively, which were higher than the yields of 44,948 and 6,152 kg ha^{-1}, respectively, obtained in plots with a single glyphosate application at 2-lf, 6-lf, or 10-lf stage of sugar beet.

In conclusion, kochia exhibits an extended period of emergence in sugar beet (late April through mid-July), with three major flushes (cohorts) during the growing season. Cohorts 1 and 2 that emerged early in the season from planting through the 6-lf stage of sugar beet were more competitive compared to the cohort 3 that emerged after the 10-lf stage of sugar beet. At least two sequential applications of glyphosate at 6-lf fb 10-lf stage of sugar beet were needed to prevent kochia survival, biomass and seed production from cohorts 1 and 2, and ultimately to prevent root and sucrose yield reductions in GR sugar beet. However, the late-emerging kochia cohort survived through sugar beet harvest and produced up to 1,123 seed m^{-2}. An additional application of glyphosate at the CC stage of sugar beet was needed to prevent any seed production from cohort 3, although it did not influence sugar beet yields. Overall, this research will aid in optimizing glyphosate applications in GR sugar beet to prevent kochia seed additions to the soil seed bank and to prevent GR sugar beet yield reductions.

Kochia exhibits high genetic diversity within and among populations (Mengistu and Messersmith 2002); an important factor to be considered in a kochia-control program, especially with the occurrence of herbicide-resistant individuals in a field population. The recurrent use of glyphosate in the absence of alternative, economical PRE or POST herbicide options will tend to enhance the selection pressure for glyphosate resistance development in kochia in GR sugar beet. This may necessitate utilization of a "zero tolerance" approach to kochia seed bank addition in crops grown in rotation with sugar beet, such as barley or corn, by utilizing alternative effective site-of-action herbicides. Integration of tillage would be an important component of glyphosate-

resistant kochia management program in GR sugar beet. Data obtained from this research can be utilized in developing simulation models to predict the possible development of GR kochia in sugar beet fields, and to develop proactive resistance management efforts.

Acknowledgements

The authors thank Nicholas Reichard, Shane Leland and Tom Fischer for their valuable technical assistance. Funding obtained from the Western Sugar Cooperative to conduct this research is appreciated.

Table 7.1. List of glyphosate application dates corresponding to sugar beet growth stages in 2012 and 2013 at the Montana State University Southern Agricultural Research Center near Huntley, MT. [a, b]

	Glyphosate application date	
Growth stages	2012	2013
2-lf	May 22	May 20
6-lf	June 13	June 13
10-lf	June 29	July 2
CC	——	July 15

[a] Abbreviations: lf, leaf; CC, canopy closure; WAE, wk after sugar beet emergence.
[b] Glyphosate at 1260 g ha^{-1} was applied at 2-lf (3 WAE) and 6-lf (6 WAE) stages of sugar beet. Glyphosate at 870 g ha^{-1} was applied at 10-lf (8 WAE) and CC (10 WAE) stages of sugar beet. All glyphosate treatments included ammonium sulfate (AMS) at 2% w/v.

Table 7.2. Effect of glyphosate application timing on percent survival of kochia cohorts in glyphosate-resistant sugar beet averaged over 2012 and 2013 at the Montana State University Southern Agricultural Research Center near Huntley, MT.

		Kochia survival [a, b]									
Timing [c]	Cohort [d]	5 WAE		8 WAE		11 WAE		13 WAE		15 WAE	
		%									
2-lf	1	2	bA	2	bA	2	bA	1	bA	1	bA
6-lf		99	aA	2	bB	1	bB	1	bB	1	bB
10-lf		98	aA	96	aA	3	bB	2	bB	2	bB
2-lf fb 6-lf		1	bA	0	bA	0	bA	0	bA	0	bA
6-lf fb 10-lf		100	aA	2	bB	0	bB	0	bB	0	bB
2-lf fb 6-lf fb 10-lf		2	bA	0	bA	0	bA	0	bA	0	bA
6-lf fb 10-lf fb CC		100	aA	3	bB	0	bB	0	bB	0	bB
Nontreated		97	aA	96	aA	91	aAB	87	aB	80	aC
2-lf	2			94	aA	85	aB	74	aC	69	aC
6-lf				6	bA	6	bA	4	bA	3	bA
10-lf				93	aA	8	bB	6	bB	4	bB
2-lf fb 6-lf				5	bA	4	bA	2	bA	2	bA
6-lf fb 10-lf				6	bA	2	bAB	1	bB	0	bB
2-lf fb 6-lf fb 10-lf				3	bA	2	bA	0	bA	0	bA
6-lf fb 10-lf fb CC				4	bA	4	bA	0	bA	0	bA
Nontreated				91	aA	82	aB	73	bC	64	aD
2-lf	3					91	aA	82	aB	71	aC
6-lf						88	aA	77	abB	70	aB
10-lf						92	aA	79	aB	67	aC
2-lf fb 6-lf						90	aA	80	aB	69	aC
6-lf fb 10-lf						88	aA	75	abB	65	aC
2-lf fb 6-lf fb 10-lf						91	aA	81	aB	68	aC
6-lf fb 10-lf fb CC						51	bA	27	cB	0	bC
Nontreated						89	aA	75	abB	66	aC

[a] Abbreviations: lf, leaf; fb, followed by; CC, canopy closure; WAE, wk after sugar beet emergence.
[b] For each kochia cohort, means within a column followed by the same lowercase letters and means within a row followed by the same uppercase letters are not significantly different based on Fisher's protected LSD test at $P < 0.05$.
[c] Glyphosate at 1260 g ha^{-1} was applied at 2-lf (3 WAE) and 6-lf (6 WAE) stages of sugar beet. Glyphosate at 870 g ha^{-1} was applied at 10-lf (8 WAE) and CC (10 WAE) stages of sugar beet. All glyphosate treatments included ammonium sulfate (AMS) at 2% w/v. No glyphosate was applied to the "nontreated" plots.
[d] Cohort 1 comprised of plants that emerged from planting to the 2-lf stage of sugar beet. Cohort 2 comprised of plants that emerged between the 2-lf and the 6-lf stage of sugar beet, and cohort 3 comprised of kochia plants that emerged after the 10-lf stage and before the CC stage of sugar beet. Survival of the three cohorts were analyzed separately.

Table 7.3. Effect of glyphosate application timing on biomass and seed production of kochia cohorts in glyphosate-resistant sugar beet averaged over 2012 and 2013 at the Montana State University Southern Agricultural Research Center near Huntley, MT[a].

	Kochia biomass[b, c]								Kochia seed production							
Timing[d]	Cohort 1		Cohort 2		Cohort 3		Total		Cohort 1		Cohort 2		Cohort 3		Total	
	g m^{-2}								No. m^{-2}							
2-lf	39	bB	321	aA	20	aC	380	b	12,166	bB	45,736	aA	1,107	aC	59,009	b
6-lf	44	bcA	14	bA	19	aA	77	c	14,963	bA	2,375	cB	1,123	aB	18,461	d
10-lf	50	bcA	22	bA	20	aA	92	c	16,943	bA	5,139	bB	1,076	aB	23,158	c
2-lf fb 6-lf	0	cA	18	bA	15	aA	33	cd	0	cA	2,948	cA	793	aA	3,741	e
6-lf fb 10-lf	0	cA	0	bA	15	aA	15	d	0	cA	0	dA	712	aA	712	f
2-lf fb 6-lf fb 10-lf	0	cA	0	bA	12	aA	12	d	0	cA	0	dA	536	abA	536	f
6-lf fb10-lf fb CC	0	cA	0	bA	0	bA	0	d	0	cA	0	dA	0	bA	0	f
Nontreated	453	aA	329	aB	17	aC	799	a	273,898	aA	43,345	aB	1,119	aC	318,362	a

[a] Abbreviations: lf, Leaf; fb, followed by; CC, canopy closure; WAE, wk after sugar beet emergence.

[b] For each kochia cohort, means within a column followed by the same lowercase letters and means within a row followed by the same uppercase letters are not significantly different based on Fisher's protected LSD test at $P < 0.05$.

[c] Cohort 1 comprised of plants that emerged from planting to the 2-lf stage of sugar beet. Cohort 2 comprised of plants that emerged between the 2-lf and the 6-lf stage of sugar beet, and cohort 3 comprised of kochia plants that emerged after the 10-lf stage and before the CC stage of sugar beet.

[d] Glyphosate at 1260 g ha^{-1} was applied at 2-lf (3 WAE) and 6-lf (6 WAE) stages of sugar beet. Glyphosate at 870 g ha^{-1} was applied at 10-lf (8 WAE) and CC (10 WAE) stages of sugar beet. All glyphosate treatments included ammonium sulfate (AMS) at 2% w/v. No glyphosate was applied to the "nontreated" plots.

Table 7.4. Effect of glyphosate application timing on glyphosate-resistant sugar beet root and sucrose yields averaged over 2012 and 2013 growing seasons at the Montana State University Southern Agricultural Research Center near Huntley, MT. [a–c]

Timing	Root yield		Sucrose yield	
	kg ha^{-1}			
2-lf	45,760	b	6,529	b
6-lf	45,193	b	6,469	b
10-lf	43,890	b	5,458	c
2-lf fb 6-lf	50,115	a	6,789	a
6-lf fb 10-lf	48,689	a	6,702	a
2-lf fb 6-lf fb 10-lf	50,607	a	6,801	a
6-lf fb 10-lf fb CC	48,131	a	6,693	a
Nontreated	20,163	c	2,837	d

[a] Abbreviations: lf, leaf; fb, followed by; CC, canopy closure; WAE, wk after sugar beet emergence.
[b] Glyphosate at 1260 g ha^{-1} was applied at 2-lf (3 WAE) and 6-lf (6 WAE) stages of sugar beet. Glyphosate at 870 g ha^{-1} was applied at 10-lf (8 WAE) and CC (10 WAE) stages of sugar beet. All glyphosate treatments included ammonium sulfate (AMS) at 2% w/v. No glyphosate was applied to the "nontreated" plots.
[c] Means within a column followed by the same lowercase letters are not significantly different based on Fisher's protected LSD test at $P < 0.05$.

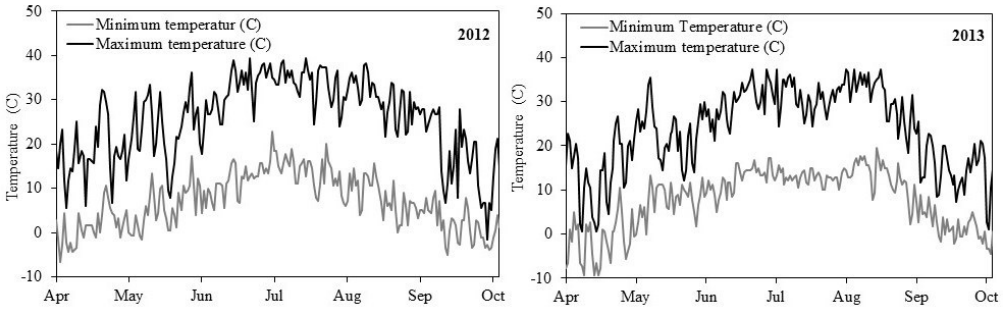

Figure 7.1. Daily maximum and minimum air temperature during the sugar beet growing seasons in 2012 and 2013 at the Montana State University Southern Agricultural Research Center near Huntley, MT.

Literature Cited

Al-Ahmadi MJ, Kafi M (2007) Cardinal temperatures for germination of *Kochia scoparia* (L.). J Arid Environ 68:308–314

Baker DV, Withrow JR, Brown CS, Beck KG (2010) Tumbling: Use of diffuse knapweed (*Centaurea diffusa*) to examine an understudied dispersal mechanism. Invasive Plant Sci Manage 3:301–309

Carlson AL, Luecke JL, Khan MFR, Dexter AG (2008) Survey of weed control and production practices on sugarbeet in eastern North Dakota and Minnesota in 2007. Sugarbeet Res Ext Rep 38:64–68

Christoffoleti PJ, Westra PB, Moore F (1997) Growth analysis of sulfonylurea-resistant and -susceptible kochia (*Kochia scoparia*). Weed Sci 45:691–695

Cranston HJ, Kern AJ, Hackett JL, Miller EK, Maxwell BD, Dyer WE (2001) Dicamba resistance in kochia. Weed Sci 49:164–170

Dahl GK, Dexter AG, Nalewaja JD (1982) Kochia competition and control in wheat. Proc North Cent Weed Control Conf 37:15–16

Dexter AG, Zollinger RK (2003) Weed control guide for sugarbeet. In 2002 Sugarbeet Research and Extension Reports. Fargo, ND: NDSU Extension Serv 33:3–34

Dille JA, Stahlman PW, Geier PW, Riffel JD, Currie RS, Wilson RG, Sbatella GM, Westra P, Kniss AR, Moechnig MJ, Cole RM (2012) Kochia emergence profiles across the central Great Plains. Proc Weed Sci Soc Am 52:122

Dotzenko AD, Arp AL (1971) Yield response of sugarbeets under various light intensities as influenced by kochia density. J Am Soc Sugarbeet Technol 16:479–481

Durgan BR, Dexter AG, Miller SD (1990) Kochia (*Kochia scoparia*) interference in sunflower (*Helianthus annus*). Weed Technol 4:52–56

Eberlein CV, Fore ZA (1984) Kochia biology. Weeds Today 15:5-6

Everitt JH, Alaniz MA, Lee JB (1983) Seed germination characteristics of kochia of *Kochia scoparia*. J Range Manag 36:646–648 \

Forcella F (1985) Spread of kochia in the northwestern United States. Weeds Today 16:4–6

Friesen LF, Beckie HJ, Warwick SI, Van Acker RC (2009) The biology of Canadian weeds. 138. *Kochia scoparia* (L.) Schrad. Can J Plant Sci 89:141–167

Heap I (2015) International Survey of Herbicide Resistant Weeds. http://www.weedscience.org Accessed: Mar 19, 2015

Jacobson J, Jackson G, Jones C (2005) Fertilizer Guidelines for Montana Crops. Bozeman, MT: Montana State University Extension Service Publication EB 161. 25 p

Jha P, Norsworthy JK, Bridges W Jr., Riley MB (2008) Influence of glyphosate timing and row width on Palmer amaranth (*Amaranthus palmeri*) and pusley (*Richardia* spp.) demographics in glyphosate-resistant soybean. Weed Sci 58:408–415

Kemp NJ, Taylor EC, Renner KA (2009) Weed management in glyphosate- and glufosinate-resistant sugar beet. Weed Technol 23:416–424

Knezevic SZ, Weise SF, Swanton CL (1994) Interference of redroot pigweed (*Amaranthus retroflexus*) in corn (*Zea mays*). Weed Sci 42:568–573

Kniss AR (2010) Comparison of conventional and glyphosate resistant sugar beet the year of commercial introduction in Wyoming. J Sugarbeet Res 47:127–134

Kumar V, Jha P, Reichard N (2014) Occurrence and characterization of kochia (*Kochia scoparia*) populations with resistance to glyphosate in Montana. Weed Technol 28:122-130

Kumar V, Jha P (2015) Influence of herbicides applied postharvest in wheat stubble on control, fecundity, and progeny fitness of *Kochia scoparia* in the US Great Plains. Crop Prot 71:144–149

Massinga RF, Currie RS, Horak MJ, Boyer J Jr. (2001) Interference of Palmer amaranth in corn. Weed Sci 49:202–208

Mengistu LW, Messersmith CG (2002) Genetic diversity of kochia. Weed Sci 50: 498-503

Norsworthy JK, Oliver LR (2001) Effect of seeding rate of drilled glyphosate-resistant soybean (*Glycine max*) on seed yield and gross profit margin. Weed Technol 15:284–292

Norsworthy JK, Jha P, Bridges W Jr. (2007) Sicklepod survival and fecundity in wide- and narrow-row glyphosate-resistant soybean (*Glycine max*). Weed Sci 55:252–259

Primiani MM, Cotterman JC, Saari LL (1990) Resistance of *Kochia scoparia* to sulfonylurea and imidazolinone herbicides. Weed Technol 4:169–172

Sbatella GM, Wilson RG (2010) Isoxaflutole shifts kochia (*Kochia scoparia*) populations in continuous corn. Weed Technol 24:392–396

Scott RK, Wilcockson SJ (1976) Weed biology and the growth of sugar beet. Ann Appl Bio 83:331–335

Schwinghamer TD, Van Acker RC (2008) Emergence timing and persistence of kochia (*Kochia Scoparia*). Weed Sci 56:37–41

Schweizer EE, Bridge LD (1982) Sunflower (*Helianthus annuus*) and velvetleaf (*Abutilon theophrasti*) interference in sugarbeets (*Beta vulgaris*). Weed Sci 30:514–519

Schweizer EE (1983) Common lambsquarters (*Chenopodium album*) interference in sugarbeets (*Beta vulgaris*). Weed Sci 31:5–8

Schweizer EE, Lauridson TC (1985) Powell amaranth (*Amaranthus powellii*) interference in sugarbeet (*Beta vulgaris*). Weed Sci 33:518–520

Stallings GP, Thill DC, Mallory-Smith CA, Shafii B (1995) Pollen-mediated gene flow of sulfonylurea-resistant kochia (*Kochia scoparia*). Weed Sci 43:95-102

Steckel LE, Sprague CL (2004) Late-season common waterhemp (*Amaranthus rudis*) interference in narrow- and wide-row soybean. Weed Technol 18:947–952

[USDA-NASS] United States Department of Agriculture– National Agricultural Statistics Service (2014) Montana's Rank in the Nation's Agriculture. http://www.nass.usda.gov/ Statistics_by_State/Montana/Publications/Annual_ Statistical_Bulletin/2014/23%20RankMTRank.pdf. Accessed: Mar 29, 2015

Waite J, Thompson CR, Peterson DE, Currie RS, Olson BLS, Stahlman PW, Khatib KA (2013) Differential kochia (*Kochia scoparia*) populations response to glyphosate. Weed Sci 61:193–200

Weatherspoon DM, Schweizer EE (1969) Competition between kochia and sugarbeets. Weed Sci 17:464–467

Wicks GA, Martin AR, Mahnken GW (1993) Control of triazine-resistant kochia (*Kochia scoparia*) in conservation tillage corn (*Zea mays*). Weed Sci 41:225–231

Wicks GA, Martin AR, Haack AE, Mahnken GW (1994) Control of triazine-resistant kochia (*Kochia scoparia*) in sorghum (*Sorghum bicolor*). Weed Technol 8:748–753

Wilson RG, Yonts CD, Smith JA (2002) Influence of glyphosate and glufosinate on weed control and sugarbeet (*Beta vulgaris*) yield in herbicide-tolerant sugarbeet. Weed Technol 16:66–73

Young JA, Evans RA, Stevens R, Everett RL (1981) Germination of *Kochia prostrata* seed. Agron J 73:957–961

CHAPTER 8

CONTROL OF VOLUNTEER GLYPHOSATE-RESISTANT CANOLA IN GLYPHOSATE-RESISTANT SUGAR BEET

Contribution of Authors and Co-Authors

Manuscript in Chapter 8

Author: Vipan Kumar

Contributions: Conceived and implemented the study design. Conducted experiments, reviewed the literature, and wrote the manuscript.

Co-Author: Prashant Jha

Contributions: Advisement, provided field expertise and feedback on manuscript drafts

Manuscript Information Page

Vipan Kumar, Prashant Jha
Weed Technology Journal
Status of Manuscript:
___Prepared for submission to a peer-reviewed journal
___Officially submitted to a peer-review journal
___Accepted by a peer-reviewed journal
_x_Published in a peer-reviewed journal

Published by Allen Press, Inc.
Published in 2015, Volume 29, Issue 1

Abstract

Occurrence of glyphosate-resistant (GR) canola volunteers in GR sugar beet is a management concern for growers in the Northern Great Plains. Field experiments were conducted at the Southern Agricultural Research Center near Huntley, MT, in 2011 and 2012 to evaluate effective herbicide programs to control volunteer GR canola in GR sugar beet. Single POST application of triflusulfuron methyl alone at the two-leaf stage of sugar beet was more effective at 35 compared with 17.5 g ai ha^{-1}. However, rate differences were not evident when triflusulfuron methyl was applied as a sequential POST (two-leaf followed by [fb] six-leaf stage of sugar beet) program (17.5 fb 17.5 or 35 fb 35 g ha^{-1}). Volunteer GR canola plants in the sequential POST triflusulfuron methyl–containing treatments produced little biomass (11 to 15% of nontreated plots) but a significant amount of seeds (160 to 661 seeds m^{-2}). Ethofumesate (4,200 g ai ha^{-1}) PRE followed by sequential POST triflusulfuron methyl (17.5 or 35 g ha^{-1}) provided effective control (94 to 98% at 30 d after treatment [DAT]), biomass reduction (97%), and seed prevention of volunteer GR canola. There was no additional advantage of adding either desmedipham + phenmedipham + ethofumesate premix (44.7 g ha^{-1}) or ethofumesate (140 g ha^{-1}) to the sequential POST triflusulfuron methyl–only treatments. The sequential POST ethofumesate-only (140 fb 140 g ha^{-1}) treatment provided poor volunteer GR canola control at 30 DAT, and the noncontrolled plants produced 6,361 seeds m^{-2}, which was comparable to the nontreated control (7,593 seeds m^{-2}). Sequential POST triflusulfuron methyl–containing treatments reduced GR sugar beet root and sucrose yields to 18 and 20%, respectively. Consistent with GR canola control, sugar beet root

and sucrose yields were highest (95 and 91% of hand-weeded plots, respectively) when the sequential POST triflusulfuron methyl–containing treatments were preceded by ethofumesate (4,200 g ha^{-1}) PRE. Growers should utilize these effective herbicide programs to control volunteer GR canola in GR sugar beet. Because of high canola seed production potential, as evident from this research, control efforts should be aimed at preventing seed bank replenishment of the GR canola volunteers.

Nomenclature: Desmedipham; ethofumesate; glyphosate; phenmedipham; triflusulfuron methyl; canola, Brassica napus L.; sugar beet, *Beta vulgaris* L.

Key words: Crop volunteer; glyphosate-resistant canola; glyphosate-resistant sugar beet; herbicide efficacy.

Introduction

Sugar beet is an important commercial crop grown in the Northern Great Plains. North Dakota and Minnesota are the leading sugar beet–producing states in this region (Ali 2004). Montana is ranked sixth, with 3.7% of the total U.S. sugar beet production in 2012 (USDA-NASS 2013). Major sugar beet–growing areas in Montana are located east of the continental divide along the Yellowstone River and areas surrounding its tributaries (Afanasiev 1964). Since its introduction in 2007, glyphosate-resistant (GR) sugar beet represents > 95% of total U.S. sugar beet production (Kniss 2010). The rapid adoption of GR sugar beet is due to effective broad-spectrum weed control efficacy, crop safety, and flexibility of glyphosate compared with conventional sugar beet herbicides (Kemp et al. 2009; Kniss et al. 2012; Wilson et al. 2002). Kniss (2010) estimated that less tillage and lower herbicide costs and greater sucrose yields (over 1.4 t ha^{-1}) with high-yielding GR cultivars could increase net economic returns by \$576 ha^{-1} in GR compared with conventional sugar beet.

Canola is another GR crop often grown in rotation with sugar beet, cereals, or soybean [*Glycine max* (L.) Merr.] in this region (North Dakota, Minnesota, Montana, Colorado, and Idaho) (USDA-ERS 2012). Canola seed loss through pod drop and pod shattering at harvest primarily contributes to volunteer problems in succeeding crop rotations (Cavalieri et al. 2014; Gulden et al. 2003). Seed losses at canola harvest could potentially add 3,000 seeds m^{-2} (equivalent to 5.9% of the canola crop yield) to the soil seed bank in a growing season (Gulden et al. 2003), which could be enough to cause high levels of infestation in the subsequent crop. Furthermore, viable seeds can persist in the

soil for 4 to 5 yr after the canola crop (Simard et al. 2002), although the majority of seeds germinate in the first year after harvest (Gulden et al. 2004; Harker et al. 2006). In a study conducted in western Canada, volunteer canola seedling recruitment was observed 1 to 3 yr after canola production (Gulden et al. 2004), which might be a concern for growers. Seed burial through tillage and secondary seed dormancy further contributes to the persistence of volunteer canola (Gulden et al. 2004). Glyphosate, glufosinate, and imidazolinone genetically engineered herbicide-resistant (HR) canola cultivars are commercially available. Nevertheless, canola is an allotetraploid with an average out-crossing rate of 21.8%, suggesting a potential for pollen-mediated dispersal of single or multiple HR traits to weedy relatives and conventional canola cultivars (Hall et al. 2000; Rakow and Woods 1987).

Crop volunteers that emerge in subsequent crops are weeds. These volunteers reduce yield of the planted crop by competing for nutrients, moisture, and light; serve as alternative hosts for diseases and insect pests; and interfere with harvest operations (O'Donovan et al. 2008; Ogg and Parker 2000; Williams and Boydston 2006). In western Canada, volunteer canola is rated as one of the most troublesome weeds in agronomic crops (Leeson et al. 2005). GR volunteers in GR crops further complicate the problem because of a limited choice of effective herbicides (Beckie and Owen 2007; Raybould and Gray 1994; York et al. 2005). Interference and yield losses caused by GR volunteers in GR crops such as corn (*Zea mays* L.), soybean, cotton (*Gossypium hirsutum* L.), or sugar beet in rotation have been previously reported (Deen et al. 2006; Kniss et al. 2012; Marquardt and Johnson 2013; York et al. 2005). For instance, early-season competition

from volunteer GR corn reduced GR soybean yields by 55 to 68% (Deen et al. 2006). Similarly, Kniss et al. (2012) reported that early-season interference of volunteer GR corn at a density of 1 plant m^{-2} can reduce sucrose yields by 19 to 45% in GR sugar beet.

Volunteer canola (non-HR) interference in rotational crops such as corn, soybean, wheat (*Triticum aestivum* L.), dry pea (*Pisum sativum* L.), flax (*Linum usitatissimum* L.), and sunflower (*Helianthus annuus* L.) has been documented (Jenks et al. 2006; Rainbolt et al. 2004). O'Donovan et al. (2008) reported that volunteer canola at densities of 50 plants m^{-2} reduced wheat yield by 13 to 49%, depending on the time of emergence of volunteers in the wheat crop. With recent increase in GR canola production in this region (Devine and Buth 2001), there is an increased concern for the occurrence of GR canola volunteers in GR crops grown in rotation, including GR sugar beet.

Herbicides are the most common and effective tools to control canola volunteers in agronomic crops (Beckie et al. 2006; Légère et al. 2006; Rainbolt et al. 2004). Alternative herbicides such as 2,4-D or MCPA (synthetic auxins, Group 4) in wheat, metribuzin (photosystem II inhibitor, Group 5) in soybean, and metsulfuron (ALS inhibitor, Group 2) have been found effective in controlling single-HR, multiple-HR, or non-HR canola volunteers (Beckie et al. 2004, 2006; Légère et al. 2006; Rainbolt et al. 2004). There appears to be no published information on herbicide options to control volunteer GR canola in GR sugar beet. Objectives of this research were to determine effective PRE and POST herbicide programs to control volunteer GR canola in GR sugar beet and to determine their effect on both root and sucrose yield.

Materials and Methods

Field experiments were conducted at the Montana State University Southern Agricultural Research Center near Huntley, MT, in 2011, and repeated in 2012. The soil type was Fort Collins clay loam (fine-loamy, mixed, superactive, mesic Aridic Haplustalfs) with 2.8% organic matter and pH 7.8. The test site was under a sugar beet–barley rotation for the past 5 yr before initiation of the study; glyphosate (840 g ae ha^{-1}) was used for weed control in GR sugar beet, and bromoxynil plus MCPA (1.12 kg ai ha^{-1}) and penoxaden (60 g ai ha^{-1}) was used for weed control in the barley crop. Seedbed preparation included disking in the fall followed by field cultivation and leveling in the spring before sugar beet planting. Test plots were fertilized with nitrogen–phosphorus–potash as per Montana State University recommendations for sugar beet production (Jacobson et al. 2005). A preseeding application of glyphosate at 840 g ae ha^{-1} was made to control existing weeds in the field. Selected field sites had no history of canola production; therefore, seeds of GR canola hybrid 'DKL 30-42' (Dekalb® Brand, Monsanto Company, 800 North Lindbergh Boulevard, St. Louis, MO 63167) were uniformly broadcast in each plot using a hand spreader at the rate of 1.5 kg ha^{-1} to simulate an infestation of volunteer GR canola. Canola seeds were incorporated into the soil 1.5 cm deep with a light harrow immediately before sugar beet planting. GR sugar beet variety 'BTS 39RR8N RP' (Genuity® Brand, Monsanto) was planted at a rate of 119,500 seeds ha^{-1} in six 61-cm rows on April 20, 2011, and April 26, 2012. GR canola seedlings emerged 3 to 5 d after planting, and GR sugar beet seedlings emerged approximately 7 to 10 d after planting. Sugar beet was irrigated using a overhead linear

sprinkler system as per the local standard. All herbicides were applied at their labeled field use rates as either single POST at the two-leaf stage of sugar beet or sequential POST at the two-leaf followed by (fb) six-leaf stage (15 d after the single POST) in the presence or absence of a PRE treatment. All treatments consisted of various rates and application timings of the following three herbicides: triflusulfuron methyl (Upbeet®, Dupont, Wilmington, DE), a prepackaged mixture of desmedipham + phenmedipham+ ethofumesate (Progress®, Bayer CropScience, Research Triangle Park, NC), and ethofumesate (Norton®, Bayer CropScience). Individual treatments are described in Tables 8.1–8.3. A nontreated control and a hand-weeded check were included for comparison. Plots were kept free from all other weeds by spraying glyphosate (840 g ae ha^{-1}) three times during the growing season. All herbicide treatments were applied with a CO_2-pressurized backpack sprayer equipped with flat-fan spray nozzles (TeeJet 8001XR, Spraying Systems Co., P.O. Box 7900, Wheaton, IL), calibrated to deliver 94 L ha^{-1} of spray solution at 276 kPa.

A randomized complete block design with four replications was used. Individual plots were 3 m wide by 10 m long. Canola densities were recorded within two 1-m^2 quadrats placed at the center of each plot. Percent control of volunteer GR canola was visually assessed at 7, 15, and 30 d after treatment (DAT) using a scale of 0% (no control) to 100% (complete control). At physiological maturity, aboveground canola biomass was hand-harvested from two 1-m^2 quadrats placed at the center of each plot, and the dry weight was determined after oven-drying at 60 C for 3 d. Canola seeds were separated from the pods, cleaned, and manually counted. Two center rows of sugar beet

were harvested in each plot using a mechanical digger on September 18, 2011, and September 21, 2012. Sugar beet root yield was recorded at this harvest. Root samples were sent to the Western Sugar Cooperative (Billings, MT) for percent sucrose analysis, and sucrose yield for each plot was calculated.

Statistical Analyses. All data were subjected to ANOVA using the MIXED procedure in SAS (version 9.2, SAS Institute Inc., SAS Campus Drive, Cary, NC 27513). Variances were divided into random effects (year, replication [year], and interactions involving either of these two variables) and fixed effects (herbicide treatments). Data on visual estimates of percent control and seed production (no. m^{-2}) were arcsine square root–transformed before analysis to improve the normality of residuals and homogeneity of variance. Canola biomass (% of nontreated) data was log transformed. Nontransformed means are presented in tables based on the analysis from the transformed data. Data from the nontreated plots were not included in the canola percent control analysis. Sugar beet root and sucrose yields were expressed as percentage of hand-weeded treatment. Means were separated using Fisher's protected LSD test at $P < 0.05$.

Results and Discussion

Volunteer GR Canola Control. Volunteer GR canola densities in the GR sugar beet plots averaged 28 to 34 plants m^{-2}. The ratings at 15 DAT represent visual estimates of percent control 15 d after the single POST (twp-leaf stage of sugar beet) or after the first application in the sequential POST (two-leaf followed by six-leaf stage of sugar beet) program; the second application in the sequential POST program was not made until 15 d

after the first treatment. Visual symptoms in canola from the PRE application of ethofumesate (4,200 g ha^{-1}) included delayed seedling emergence, leaf cupping or puckering, and stunted growth. Visual symptoms in canola from the POST triflusulfuron-containing programs included general chlorosis, stunting, and necrosis. Volunteer GR canola control 15 DAT with triflusulfuron methyl alone at the two-leaf stage of sugar beet (single or sequential POST program) was higher at the 35 g ha^{-1} (66% control) compared with 17 g ha^{-1} rate (56% control) (Table 8.2). Among all treatments, ethofumesate (4,200 g ha^{-1}) PRE followed by POST triflusulfuron methyl (17.5 or 35 g ha^{-1}) alone or in combination with ethofumesate (140 g ha^{-1}) at the two-leaf timing was the most effective, with control averaging 90 to 95%. Control 15 DAT was least (18%) with ethofumesate (140 g ha^{-1}) alone applied POST.

The 30 DAT ratings represent visual estimates of percent control 30 d after the single POST (two-leaf stage of sugar beet) or 15 d after the second application in the sequential POST (two-leaf followed by six-leaf stage of sugar beet) program. Ethofumesate PRE followed by sequential POST triflusulfuron methyl applied at 17.5 fb 17.5 g ha^{-1} or 35 fb 35 g ha^{-1} controlled volunteer GR canola by 94 to 98% in GR sugar beet 30 DAT, irrespective of adding ethofumesate (140 g ha^{-1}) POST (Table 8.2). Control 30 DAT with single POST triflusulfuron methyl was 71% at the 35 g ha^{-1} rate compared with 57% at the 17.5 g ha^{-1} rate. Furthermore, control with the single POST triflusulfuron methyl at the 35 g ha^{-1} rate did not differ from sequential POST triflusulfuron methyl treatments (17.5 or 35 g ha^{-1} fb 17.5 or 35 g ha^{-1}). Addition of desmedipham + phenmedipham + ethofumesate premix (44.7 g ha^{-1}) or ethofumesate

(140 g ha^{-1}) did not further improve control compared with the sequential POST triflusulfuron methyl–only treatments. Consistent with the 15 DAT rating, ethofumesate only applied sequential POST (140 fb 140 g ha^{-1}) was the least effective treatment.

Volunteer GR Canola Biomass. Consistent with the control ratings, ethofumesate (4,200 g ha^{-1}) PRE followed by sequential POST triflusulfuron methyl alone (either 17.5 fb 17.5 g ha^{-1} or 35 fb 35 g ha^{-1}) treatment reduced (97% average of nontreated) volunteer GR canola biomass the most; no additional benefit was observed from tank-mixing ethofumesate (140 g ha^{-1}) POST (Table 8.3). Single POST triflusulfuron methyl applied at 35 g ha^{-1} reduced GR canola biomass by 84% compared with 42% of the nontreated control when applied at 17.5 g ha^{-1}. Furthermore, canola biomass reduction with single POST triflusulfuron methyl at the 35 g ha^{-1} rate did not differ from any of the sequential POST triflusulfuron methyl–containing treatments and averaged 87% of the nontreated control (Table 8.3). Similar to the control ratings, no additional advantage was observed on GR canola biomass reduction by tank mixing either desmedipham + phenmedipham + ethofumesate (44.7 kg ha^{-1}) premix or ethofumesate (140 g ha^{-1}) with the sequential POST triflusulfuron methyl–only treatment. Noncontrolled volunteer GR canola in the sequential POST ethofumesate-only treatment produced a significant amount of biomass (i.e., 74% of the nontreated).

Volunteer GR Canola Seed Production. Consistent with effective control and biomass reduction, ethofumesate PRE (4,200 g ha^{-1}) followed by POST triflusulfuron methyl alone applied sequentially at either 17.5 fb 17.5 g ha^{-1} or 35 fb 35 g ha^{-1} prevented any

volunteer GR canola seed production (Table 8.3). There was no additional benefit of adding either ethofumesate or desmedipham + phenmedipham + ethofumesate premix to the sequential POST triflusulfuron methyl–only treatments. Seed production with a single POST triflusulfuron methyl was significantly less at the 35 g ha^{-1} (626 seeds m^{-2}) rate compared with the 17.5 g ha^{-1} (1,913 seeds m^{-2}) rate. Also, seed production with a single POST triflusulfuron methyl at the 35 g ha^{-1} rate did not differ from any of the sequential POST programs (in the absence of ethofumesate PRE), except for the sequential POST ethofumesate-only (140 fb 140 g ha^{-1}) treatment. Volunteer GR canola plants in the sequential POST ethofumesate-only treatment produced 6,361 seeds m^{-2}, which was no different from the nontreated control (7,593 seeds m^{-2}).

GR Sugar Beet Root and Sucrose Yields. Season-long interference of volunteer GR canola in nontreated control reduced GR sugar beet root and sucrose yields by 50 and 53% of the hand-weeded control, respectively (Table 8.4). Consistently, the ethofumesate (4,200 g ha^{-1}) PRE followed by sequential POST triflusulfuron methyl (17.5 or 35 g ha^{-1}) treatments had the highest root and sucrose yields, which averaged 95 and 92% of the hand-weeded control, respectively. After ethofumesate PRE, there was no benefit of adding ethofumesate (140 g ha^{-1}) to the POST sequential triflusulfuron methyl–only treatments for sugar beet yields. Root and sucrose yields (80 and 78% of hand-weeded, respectively) were significantly higher with the single POST triflusulfuron methyl applied at 35 compared with 17.5 g ha^{-1}. Furthermore, sugar beet yields with the single POST triflusulfuron methyl at 35 g ha^{-1} did not differ from any of the sequential POST triflusulfuron-containing programs in the absence of ethofumesate PRE. Among all

herbicide treatments, the lowest root (55% of hand-weeded) and sucrose yields (50% of hand-weeded) were obtained with the sequential POST ethofumesate-only treatment.

In conclusion, volunteer GR canola at densities of approximately 30 plants m^{-2} caused severe interference in GR sugar beet and reduced sucrose yields by 53%. If not managed, uncontrolled canola plants could add a significant number of seeds (7,593 seeds m^{-2}) to the soil seed bank for presence of GR volunteers in subsequent crops. Furthermore, the potential risk of pollen-mediated gene flow of the transgenic HR trait from the GR canola volunteers to weedy relatives and non-HR canola (Beckie et al. 2004; Hall et al. 2000) warrants attention for effective control of the volunteers. Based on this research, ethofumesate (4,200 g ha^{-1}) PRE followed by sequential POST triflusulfuron methyl (17.5 g ha^{-1}) would be an effective herbicide program to prevent seed bank replenishment of volunteer GR canola and prevent yield reductions in GR sugar beet. Canola can emerge earlier than sugar beet; nevertheless, plants exhibit winter hardiness, resulting in lower efficacy of POST herbicides applied in the spring (Légère et al. 2006). Therefore, targeting the seed bank with PRE herbicides early in the spring (e.g., ethofumesate PRE at the full, recommended rate in sugar beet) will aid in reducing volunteer canola interference in the crop. This field-based research is one of the first attempts to address this issue and will add to the knowledge on available herbicide tools to manage volunteer HR canola.

Acknowledgments

The authors thank Ms. Josefina O. Garcia, Mr. Nicholas Reichard, and Mr. Tom Fischer for their technical assistance in conducting the field experiments.

Table 8.1. Herbicide programs for volunteer GR canola control in GR sugar beet at the experimental site near Huntley, MT, in 2011 and 2012[a-d].

Herbicide(s)	Rate (s)	Timing (s)	Trade Names & Manufacturers
	(g ai or ae ha^{-1})		
Tri	17.5	2-lf	UpBeet®, DuPont, Wilmington, DE
Tri	35	2-lf	UpBeet®, DuPont, Wilmington, DE
Tri fb tri	17.5 fb 17.5	2-lf fb 6-lf	UpBeet®, DuPont, Wilmington, DE
Tri fb tri	35 fb 35	2-lf fb 6-lf	UpBeet®, DuPont, Wilmington, DE
Tri fb tri	17.5 fb 35	2-lf fb 6-lf	UpBeet®, DuPont, Wilmington, DE
Tri fb tri	35 fb 17.5	2-lf fb 6-lf	UpBeet®, DuPont, Wilmington, DE
(Tri + DPE) fb (tri + DPE)	(17.5 + 44.7) fb (17.5 + 44.7)	2-lf fb 6-lf	UpBeet®, DuPont, Wilmington, DE; Progress®, Bayer CropScience, Research Triangle Park, NC
(Tri + DPE) fb (tri + DPE)	(35 + 44.7) fb (35 + 44.7)	2-lf fb 6-lf	UpBeet®, DuPont, Wilmington, DE; Progress®, Bayer CropScience, Research Triangle Park, NC
(Etho + tri) fb (etho + tri)	(140 + 17.5) fb (140 + 17.5)	2-lf fb 6-lf	Nortron® SC, Bayer CropScience, Research Triangle Park, NC; UpBeet®, DuPont, Wilmington, DE
(Etho + tri) fb (etho + tri)	(140 + 35) fb (140 + 35)	2-lf fb 6-lf	Nortron® SC, Bayer CropScience, Research Triangle Park, NC; UpBeet®, DuPont, Wilmington, DE
Etho fb etho	140 fb 140	2-lf fb 6-lf	Nortron® SC, Bayer CropScience, Research Triangle Park, NC
Etho fb (etho + tri) fb tri	4200 fb (140 + 17.5) fb 17.5	PRE fb 2-lf fb 6-lf	Nortron® SC, Bayer CropScience, Research Triangle Park, NC; UpBeet®, DuPont, Wilmington, DE
Etho fb (etho + tri) fb tri	4200 fb (140 + 35) fb 35	PRE fb 2-lf fb 6-lf	Nortron® SC, Bayer CropScience, Research Triangle Park, NC; UpBeet®, DuPont, Wilmington, DE
Etho fb tri fb tri	4200 fb 17.5 fb 17.5	PRE fb 2-lf fb 6-lf	Nortron® SC, Bayer CropScience, Research Triangle Park, NC; UpBeet®, DuPont, Wilmington, DE
Etho fb tri fb tri	4200 fb 35 fb 35	PRE fb 2-lf fb 6-lf	Nortron® SC, Bayer CropScience, Research Triangle Park, NC; UpBeet®, DuPont, Wilmington, DE

[a] Abbreviations: GR, glyphosate-resistant; Tri, triflusulfuron methyl; DPE, desmedipham + phenmedipham + ethofumesate premix; etho, ethofumesate; fb, followed by; lf, true leaf.
[b] Application timing corresponded to sugar beet growth stages.
[c] All POST treatments were tank-mixed with glyphosate at 840 g ha^{-1}.
[d] All POST treatments included methylated seed oil (MSO) at 1.5 % (v/v) and ammonium sulfate (AMS) at 2% w/v, except POST ethofumesate alone treatment.

Table 8.2. Volunteer GR canola control in GR sugar beet with various herbicide programs at 15 and 30 d after treatment (DAT) at the experimental site near Huntley, MT, averaged over 2011 and 2012 [a-e].

			Canola control			
Herbicide(s)	Rate(s)	Timing (s)	15 DAT		30 DAT	
	(g ai or ae ha^{-1})		%			
Tri	17.5	2-lf	55	f	57	e
Tri	35	2-lf	65	de	71	d
Tri fb tri	17.5 fb 17.5	2-lf fb 6-lf	57	f	72	cd
Tri fb tri	35 fb 35	2-lf fb 6-lf	66	de	74	cd
Tri fb tri	17.5 fb 35	2-lf fb 6-lf	57	f	73	cd
Tri fb tri	35 fb 17.5	2-lf fb 6-lf	66	de	74	cd
(Tri + DPE) fb (tri + DPE)	(17.5 + 44.7) fb (17.5 + 44.7)	2-lf fb 6-lf	59	ef	74	cd
(Tri + DPE) fb (tri + DPE)	(35 + 44.7) fb (35 + 44.7)	2-lf fb 6-lf	68	cd	76	bc
(Etho + tri) fb (etho + tri)	(140 + 17.5) fb (140 + 17.5)	2-lf fb 6-lf	60	ef	73	cd
(Etho + tri) fb (etho + tri)	(140 + 35) fb (140 + 35)	2-lf fb 6-lf	69	cd	76	bc
Etho fb etho	140 fb 140	2-lf fb 6-lf	18	h	32	f
Etho fb (etho + tri) fb tri	4200 fb (140 + 17.5) fb 17.5	PRE fb 2-lf fb 6-lf	90	ab	95	a
Etho fb (etho + tri) fb tri	4200 fb (140 + 35) fb 35	PRE fb 2-lf fb 6-lf	95	a	98	a
Etho fb tri fb tri	4200 fb 17.5 fb 17.5	PRE fb 2-lf fb 6-lf	90	ab	94	a
Etho fb tri fb tri	4200 fb 35 fb 35	PRE fb 2-lf fb 6-lf	93	a	95	a

[a] Abbreviations: GR, glyphosate-resistant; Tri, triflusulfuron methyl; DPE, desmedipham + phenmedipham + ethofumesate; Etho, ethofumesate; lf, true-leaf; fb, followed by.

[b] Application timing corresponded to GR sugar beet growth stages.[c] All POST treatments were tank-mixed with glyphosate at 840 g ha^{-1}.

[d] All POST treatments included methylated seed oil (MSO) at 1.5 % (v/v) and ammonium sulfate (AMS) at 2% w/v, except POST ethofumesate alone treatment.

[e] Means within a column followed by similar letters are not significantly different based on Fisher's Protected LSD test at $P < 0.05$.

Table 8.3. Biomass and seed production of volunteer GR canola in GR sugar beet with various herbicide treatments at the experimental site near Huntley, MT, averaged over 2011 and 2012 [a-e].

Herbicide(s)	Rate(s)	Timing(s)	Biomass		Seed production	
	(g ai or ae ha^{-1})		% of nontreated		no. m^{-2}	
Tri	17.5	2-lf	58	b	1913	b
Tri	35	2-lf	16	cd	626	cde
Tri fb tri	17.5 fb 17.5	2-lf fb 6-lf	15	cd	661	cde
Tri fb tri	35 fb 35	2-lf fb 6-lf	14	d	400	de
Tri fb tri	17.5 fb 35	2-lf fb 6-lf	15	cd	609	cde
Tri fb tri	35 fb 17.5	2-lf fb 6-lf	11	de	567	cde
(Tri + DPE) fb (tri + DPE)	(17.5 + 44.7) fb (17.5 + 44.7)	2-lf fb 6-lf	12	de	250	e
(Tri + DPE) fb (tri + DPE)	(35 + 44.7) fb (35 + 44.7)	2-lf fb 6-lf	13	de	160	e
(Etho + tri) fb (etho + tri)	(140 + 17.5) fb (140 + 17.5)	2-lf fb 6-lf	12	de	165	e
(Etho + tri) fb (etho + tri)	(140 + 35) fb (140 + 35)	2-lf fb 6-lf	11	de	177	e
Etho fb etho	140 fb 140	2-lf fb 6-lf	74	a	6361	a
Etho fb (etho + tri) fb tri	4200 fb (140 + 17.5) fb 17.5	PRE fb 2-lf fb 6-lf	3	f	0	e
Etho fb (etho + tri) fb tri	4200 fb (140 + 35) fb 35	PRE fb 2-lf fb 6-lf	4	f	0	e
Etho fb tri fb tri	4200 fb 17.5 fb 17.5	PRE fb 2-lf fb 6-lf	3	f	0	e
Etho fb tri fb tri	4200 fb 35 fb 35	PRE fb 2-lf fb 6-lf	3	f	0	e
Nontreated	———	———	——		7593	a

[a] Abbreviations: GR, glyphosate-resistant; Tri, triflusulfuron methyl; DPE, desmedipham + phenmedipham + ethofumesate premix; Etho, ethofumesate; lf, true-leaf; fb, followed by.

[b] All POST treatments were tank-mixed with glyphosate at 840 g ha^{-1}.

[c] Application timing corresponded to sugar beet growth stages.

[d] All POST herbicide treatments included methylated seed oil (MSO) at 1.5 % (v/v) and ammonium sulfate (AMS) at 2% w/v, except POST ethofumesate alone treatment.

[e] Means within a column followed by similar letters are not significantly different based on Fisher's Protected LSD test at $P < 0.05$.

Table 8.4. Effect of herbicide treatments on root and sucrose yields of GR sugar beet at the experimental site near Huntley, MT, averaged over 2011 and 2012 [a-d].

Herbicide(s)	Rate(s)	Timing(s)	Root yield		Sucrose yield	
	(g ai or ae ha^{-1})		% of hand-weeded			
Tri	17.5	2-lf	67	f	67	g
Tri	35	2-lf	80	e	78	def
Tri fb tri	17.5 fb 17.5	2-lf fb 6-lf	79	e	79	def
Tri fb tri	35 fb 35	2-lf fb 6-lf	80	e	79	def
Tri fb tri	17.5 fb 35	2-lf fb 6-lf	81	de	80	def
Tri fb tri	35 fb 17.5	2-lf fb 6-lf	82	de	80	def
(Tri + DPE) fb (tri + DPE)	(17.5 + 44.7) fb (17.5 + 44.7)	2-lf fb 6-lf	79	e	77	ef
(Tri + DPE) fb (tri + DPE)	(35 + 44.7) fb (35 + 44.7)	2-lf fb 6-lf	85	cde	82	cde
(Etho + tri) fb (etho + tri)	(140 + 17.5) fb (140 + 17.5)	2-lf fb 6-lf	83	cde	80	def
(Etho + tri) fb (etho + tri)	(140 + 35) fb (140 + 35)	2-lf fb 6-lf	84	cde	82	cde
Etho fb etho	140 fb 140	2-lf fb 6-lf	55	g	50	h
Etho fb (etho + tri) fb tri	4200 fb (140 + 17.5) fb 17.5	PRE fb 2-lf fb 6-lf	92	ab	88	abc
Etho fb (etho + tri) fb tri	4200 fb (140 + 35) fb 35	PRE fb 2-lf fb 6-lf	96	a	92	ab
Etho fb tri fb tri	4200 fb 17.5 fb 17.5	PRE fb 2-lf fb 6-lf	95	a	92	ab
Etho fb tri fb tri	4200 fb 35 fb 35	PRE fb 2-lf fb 6-lf	96	a	94	a
Nontreated	———	———	50	g	47	h

[a] Abbreviations: GR, glyphosate-resistant; Tri, triflusulfuron methyl, DPE, desmedipham + phenmedipham + ethofumesate premix; Etho, ethofumesate; lf, true-leaf; fb, followed by.

[b] All POST treatments were tank mixed with glyphosate at 840 g ha^{-1}.

[c] Application timing corresponded to sugar beet growth stages.

[c] All POST treatments included methylated seed oil (MSO) at 1.5 % (v/v) and ammonium sulfate (AMS) at 2% w/v, except POST ethofumesate alone treatment.

[d] Means within a column followed by similar letters are not significantly different based on Fisher's Protected LSD test at $P < 0.05$.

Literature Cited

Afanasiev MM (1964) The effect of simulated hail injuries on yield and sugar content of beets. J Am Soc Sugar Beet Technol 13:225–237

Ali MB (2004) Characteristics and Production Costs of U.S. Sugarbeet Farms. Washington, DC: United States Department of Agriculture Economics Research Service Statistical Bulletin 974-8. http://www.ers.usda.gov/media/943070/sb974-8.pdf. Accessed June 7, 2014

Beckie HJ, Harker KN, Hall LM (2006) A decade of herbicide-resistant crops in Canada. Can J Plant Sci 86:1243–1264

Beckie HJ, Owen MDK (2007) Herbicide-resistant crops as weeds in North America. CAB Rev: Perspect Agric Vet Sci Nutr Nat Resour 2 (44):1–22

Beckie, HJ, Swartz GS, Nair HK, Warwick SI, Johnson E (2004) Multiple herbicide–resistant canola can be controlled by alternative herbicides. Weed Sci 52:152–157

Cavalieri A, Lewis DW, Gulden RH (2014) Pod drop and pod shatter are not closely related in canola. Crop Sci 54:1184–1188

Deen W, Hamill A, Shropshire C, Soltani N, Sikemma PH (2006) Control of volunteer glyphosate-resistant corn (*Zea mays*) in glyphosate-resistant soybean (*Glycine max*). Weed Technol 20:261–266

Devine MD, Buth JL (2001) Advantages of genetically modified canola. A Canadian perspective. Br Crop Prot Conf Weeds 1,2:367–372

Gulden RH, Shirtliffe SJ, Thomas AG (2003) Harvest losses of canola (*Brassica napus*) cause large seedbank inputs. Weed Sci 51:83–86

Gulden RH, Thomas AG, Shirtliffe SJ (2004) Secondary dormancy, temperature, and seed burial depth regulate seed bank dynamics in *B. napus*. Weed Sci 52:382–388

Hall LM, Topinka K, Huffman J, Davis L, Good A (2000) Pollen flow between herbicide-resistant *Brassica napus* is the cause of multiple-resistant *B. napus* volunteer. Weed Sci 48:688–694

Harker KN, Clayton GW, Blackshaw RE, O'Donovan JT, Johnson EN, Gan Y, Holm FA, Sapsford KL, Irvine RB, Van Acker RC (2006) Persistence of glyphosate-resistant canola in western Canadian cropping systems. Agron J 98:107–11

Jacobson J, Jackson G, Jones C (2005) Fertilizer Guidelines for Montana Crops. Bozeman, MT: Montana State University Extension Service Publication EB 161. 25 p

Jenks BM, Willoughby GP, Markle DM (2006) Volunteer canola control in corn, soybean, sunflower, dry pea, and flax. Abstract 24-4 *in* Proceedings of the ASA-CSSA-SSSA International Annual Meetings. US Canola Association. Indianapolis, IN: American Society of Agronomy/Crop Science Society of Agronomy/Soil Science Society of Agronomy

Kemp NJ, Taylor EC, Renner KA (2009) Weed management in glyphosate- and glufosinate-resistant sugar beet. Weed Technol 23:416–424

Kniss AG, Sbatella GM, Wilson RG (2012) Volunteer glyphosate resistant corn interference and control in glyphosate resistant sugar beet. Weed Technol 26:348–355

Kniss AR (2010) Comparison of conventional and glyphosate-resistant sugar beet the year of commercial introduction in Wyoming. J Sugarbeet Res 47:127–134

Leeson JY, Thomas AG, Hall LM, Brenzil C, Andrews T, Brown KR, Van Acker RC (2005) Prairie Weed Surveys of Cereal, Oilseed and Pulse Crops from the 1970s to the 2000s. Saskatoon, SK, Canada: Agriculture and Agri-Food Canada Weed Survey Series Publ. 05-1. 395 p

Légère A, Simard MJ, Johnson E, Stevenson EC, Beckie H, Blackshaw RE (2006) Control of volunteer canola with herbicides; effects of plant growth stage and cold acclimation. Weed Technol 20:485–493

Marquardt PT, Johnson WG (2013) Influence of clethodim application timing on control of volunteer corn in soybean. Weed Technol 27:645–648

O'Donovan JT, Harker KN, Dew DA (2008) Effect of density and time of removal of volunteer canola (*Brassica rapa* L.) on yield loss of wheat (*Triticum aestivum* L.). Can J Plant Sci 88:839–842

Ogg AG Jr, Parker R (2000) Control of Volunteer Crop Plants. Pullman, WA: Washington State University Extension Bull. EB1523:1–4

Rainbolt CR, Thill DC, Young FL (2004) Control of volunteer herbicide-resistant wheat and canola. Weed Technol 18:711–718

Rakow G, Woods D (1987) Out-crossing in rape and mustard under Saskatchewan prairie conditions. Can J Plant Sci 67:147–151

Raybould AF, Gray A (1994) Will hybrids of genetically modified crops invade natural communities? Trends Ecol Evol 9:85–89

Simard MJ, Légère A, Pageau D, Lajeunesse J, Warwick S (2002) The frequency and persistence of volunteer canola (*Brassica napus*) in Québec cropping systems. Weed Technol 16:433–439

[USDA-ERS] United States Department of Agriculture–Economic Research Service (2012) Canola Production. http://www.ers.usda.gov/topics/crops/soybeans-oil-crops/canola.aspx#canolaprod. Accessed June 7, 2014

[USDA-NASS] United States Department of Agriculture–National Agricultural Statistics Service (2013) Montana's Rank in the Nation's Agriculture. http://www.nass.usda.gov/Statistics_by_State/Montana/Publications/Annual_Statistical_Bulletin/2013/RankMTRank.pdf. Accessed May 6, 2014

Williams, MM, Boydston RA (2006) Volunteer potato interference in carrot. Weed Sci 54:94–99

Wilson RG, Yonts CD, Smith JA (2002) Influence of glyphosate and glufosinate on weed control and sugar beet (*Beta vulgaris*) yield in herbicide tolerant sugar beet. Weed Technol 16:66–73

York AC, Beam JB, Culpepper AS (2005) Control of volunteer glyphosate-resistant soybean in cotton. J Cotton Sci 9:102–10

CHAPTER 9

CONCLUSIONS

Occurrence of herbicide-resistant (HR) *K. scoparia* is an increasing challenge for growers in the northwestern United States, including Montana. This research work highlights the first report of glyphosate-resistant (GR) *K. scoparia* in Montana, and provides insights on the molecular/genetic mechanism of glyphosate resistance, growth and reproductive fitness, and alternative strategies for GR *K. scoparia* management in MT cropping systems. Glyphosate resistance in *K. scoparia* evolved under wheat-fallow rotation in the northern Montana, mainly due to repeated use of glyphosate (3 to 4 applications per season) in chemical fallow prior to winter wheat planting. In response to *K. scoparia* control failures with the field-use rate of glyphosate during summer of 2012, seeds of putative GR *K. scoparia* accessions were collected from Hill and Liberty Counties in northern Montana. Glyphosate-susceptible *K. scoparia* accessions were also collected from nearby fields under organic wheat production in Liberty (designated as SUS) and Yellowstone Counties (designated as GS). Based on whole-plant dose-response experiments, four putative GR *K. scoparia* accessions (GIL01, JOP01, CHES01, and CHES02) had 4.6 to 11-fold levels of glyphosate resistance relative to the GS *K. scoparia* accession. Furthermore, GR *K. scoparia* accessions (GIL01, JOP01, and CHES01) also exhibited 9.3- to 30-fold more resistance to premixed thifensulfuron methyl + tribenuron methyl + metsulfuron methyl (ALS-inhibitor herbicides) than the GS *K. scoparia* accession i.e. multiple herbicide-resistant (MHR) *K. scoparia*.

Laboratory experiments using PCR and qPCR techniques revealed that three GR *K. scoparia* (GIL01, JOP01, and CHES01) accessions had ~ 4 to 10 amplified copies of the *EPSPS* gene. The *ESPSP* gene amplification mechanism of glyphosate resistance was further confirmed with immunoblotting; all GR *K. scoparia* accessions accumulated higher EPSPS protein compared to the GS accession. Resistance to ALS-inhibitors in those GR *K. scoparia* accessions was conferred by $Pro_{197}Gln$ amino acid substitution (single point mutation).

To elaborate the effects of *EPSPS* gene amplification on fitness of GR *K. scoparia*, greenhouse experiments on inbred lines of GR (JOP01 and CHES01) and SUS *K. scoparia* accessions were conducted under different levels of intraspecific plant competition. Results revealed that the 2- to 14-fold amplification of *EPSPS* gene did not confer any vegetative growth- or fecundity-related fitness advantage or fitness penalty in GR *K. scoparia*. In another experiment, the *EPSPS* gene amplification had an additive effect on glyphosate resistance level. These results further suggest that GR *K. scoparia* with high *EPSPS* gene copy number will most likely persist in field populations, irrespective of glyphosate selection pressure. An integrated weed management approach will be required to manage GR *K. scoparia*.

To develop management strategies, several PRE and POST herbicides were tested for effectiveness on *K. scoparia* control under field and greenhouse conditions. Among PRE herbicide programs tested, acetochlor + atrazine, pyroxasulfone + atrazine, and S-metolachlor + atrazine + mesotrione provided effective season-long residual control of kochia. Sulfentrazone and metribuzin also provided effective *K. scoparia* control under

field conditions. Among POST herbicide programs tested, paraquat plus atrazine, paraquat plus linuron, and paraquat plus metribuzin were effective for *K. scoparia* control, and can be utilized in corn/grain sorghum, corn/fallow, and soybean, respectively. POST *K. scoparia* control with bromoxynil + fluroxypyr (labeled in wheat/barley), tembotrione + atrazine (labeled in corn), and topramezone + atrazine (labeled in corn) was not different from the glyphosate treatment. Growers should adopt these alternative and effective PRE and POST herbicide programs for managing GR *K. scoparia* in their farm fields.

In addition, late-season application of paraquat alone or tank-mixed with atrazine/ linuron/ metribuzin, saflufenacil + atrazine, saflufenacil + 2, 4-D, glyphosate alone or tank-mixed with dicamba, glufosinate, tembotrione + atrazine, or topramezone + atrazine prevented *K. scoparia* seed production in postharvest wheat stubble. Dicamba applied alone or tank-mixed with 2, 4-D and/or diflufenzopyr was not effective for *K. scoparia* control in postharvest wheat stubble; however, dicamba tank-mixed with atrazine reduced seed production, seed weight and seed viability of treated *K. scoparia* plants. These effective late-season herbicides evaluated in postharvest wheat stubble would serve as an additional tool to manage GR *K. scoparia* populations in the wheat-based semiarid cropping systems of the US Great Plains.

Demographic studies in GR sugar beet indicated that *K. scoparia* has an extended period of emergence (late April to mid-July) in sugar beet. The early emerging *K. scoparia* cohorts (cohort 1 and 2) were more aggressive and competitive than the late-emerging cohort (cohort 3). Results suggested that at least two sequential applications of

glyphosate at 6-lf fb 10-lf stage of sugar beet were required to prevent kochia survival, biomass and seed production from cohort 1 and 2, and to prevent sugar beet root and sucrose yield reductions. However, an additional application of glyphosate at the canopy closure stage of sugar beet will be needed to prevent seed production by the late-emerging cohort of *K. scoparia*.

In managing volunteer GR canola in GR sugar beet, results from a two-year field research indicated that volunteer GR canola at densities of approximately 30 plants m^{-2} reduced sucrose yields by 53%, and contributed significant amount of seeds (7,593 seeds m^{-2}) to the soil seed bank. Among herbicide programs tested, ethofumesate (4,200 g ha^{-1}) PRE followed by sequential POST triflusulfuron methyl (17.5 g ha^{-1}) would be an effective herbicide program to prevent seed bank replenishment of volunteer GR canola and prevent yield reductions in GR sugar beet.

Future research is needed to determine the frequency and geographical distribution of *K. scoparia* populations with evolved resistance to glyphosate, ALS-inhibitors, and/or auxinic herbicides (predominantly dicamba) in Montana and other states in the US Great Plains. Research efforts are required to understand the growth and reproductive fitness of GR *K. scoparia* in the presence of crop competition and glyphosate selection pressure under diverse environmental conditions. In addition, research on understanding the biology and ecology of *K. scoparia* should be emphasized to develop best management practices to prevent development of multiple herbicide-resistant *K. scoparia* populations in this region.

REFERENCES CITED

Afanasiev MM (1964) The effect of simulated hail injuries on yield and sugar content of beets. J Am Soc Sugar Beet Technol 13:225–237

Al-Ahmadi MJ, Kafi M (2007) Cardinal temperatures for germination of *Kochia scoparia* (L.). J Arid Environ 68: 308–314

Alcocer-Ruthling M, Thill DC, Shafii B (1992) Differential competitiveness of sulfonylurea resistant and susceptible prickly lettice (*Lactuca serriola*). Weed Technol 6:303–309

Alcorta M, Fidelibus MW, Steenwerth KL, Shrestha A (2011) Competitive effects of glyphosate-resistant and glyphosate-susceptible horseweed (*Conyza canadensis*) on young grapevines (*Vitis vinifera*). Weed Sci. 59:489–494

Ali MB (2004) Characteristics and Production Costs of U.S. Sugarbeet Farms. Washington, DC: United States Department of Agriculture Economics Research Service Statistical Bulletin 974-8. http://www.ers.usda.gov/media/943070/sb974-8.pdf. Accessed June 7, 2014

Anderson KS, Sammons RD, Leo GC, Sikorski JA, Benesi AJ, Johnson KA (1990) Observation by carbon-13 NMR of the EPSP synthase tetrahedral intermediate bound to the enzyme active site. Biochem. 29:1460–1465

Anderson RL, Nielsen DC (1996) Emergence pattern of five weeds in the central Great Plains. Weed Technol 10:744–749

Bagavathiannan MV, Norsworthy JK (2012) Late-season seed production in arable weed communities: management implications. Weed Sci 60:325–334

Baker DV, Withrow JR, Brown CS, Beck KG (2010) Tumbling: Use of diffuse knapweed (*Centaurea diffusa*) to examine an understudied dispersal mechanism. Invasive Plant Sci Manage 3:301-309

Baucom RS, Mauricio R (2004) Fitness costs and benefits of novel herbicide tolerance in a noxious weed. Proc Natl Acad Sci USA. 101:13386–13390

Beckie, HJ, Swartz GS, Nair HK, Warwick SI, Johnson E (2004) Multiple herbicide–resistant canola can be controlled by alternative herbicides. Weed Sci 52:152–157

Beckie HJ (2006) Herbicide-resistant weeds: Management tactics and practices. Weed Technol 20:793-814

Beckie HJ, Harker KN, Hall LM (2006) A decade of herbicide-resistant crops in Canada. Can J Plant Sci 86:1243–1264

Beckie HJ, Owen MDK (2007) Herbicide-resistant crops as weeds in North America. CAB Rev: Perspect Agric Vet Sci Nutr Nat Resour 2 (44):1–22

Beckie HJ, Blackshaw RE, Low R, Hall LM, Sauder CA, Martin S, Brandt EN, Shirriff SW (2013) Glyphosate- and acetolactate synthase inhibitor-resistant kochia (*Kochia scoparia*) in western Canada. Weed Sci 61:310-318

Bennett AC, Shaw DR (2000) Effect of preharvest desiccants on weed seed production and viability. Weed Technol 14: 530–538

Biniak BM, Aldrich RJ (1986) Reducing velvetleaf (*Abutilon theophrasti*) and giant foxtail (*Setaria faberi*) seed production with stimulated-roller herbicide applications. Weed Sci 34: 256–259

Bolt MW, Mahoney PA (1997) High-efficiency blotting of proteins of diverse sizes following sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Anal Biochem 247:185–192

Carlson AL, Luecke JL, Khan MFR, Dexter AG (2008) Survey of weed control and production practices on sugarbeet in eastern North Dakota and Minnesota in 2007. Sugarbeet Res Ext Rep 38:64–68

Cavalieri A, Lewis DW, Gulden RH (2014) Pod drop and pod shatter are not closely related in canola. Crop Sci 54:1184–1188

Chandi A, Milla-Lewis SR, Giacomini D, Westra P, Preston C, Jordan DL, York AC, Burton JD, Whitaker JR (2012) Inheritance of evolved glyphosate resistance in a North Carolina Palmer amaranth (*Amaranthus palmeri*) biotype. Int J Agron doi:10.1155/2012/176108

Christoffoleti PJ, Westra PB, Moore F (1997) Growth analysis of sulfonylurea-resistant and -susceptible kochia (*Kochia scoparia*). Weed Sci 45:691–695

Clay PA, Griffin JL (2000) Weed seed production and seedling emergence responses to late-season glyphosate applications. Weed Sci 48: 481–486

Cranston HJ, Kern AJ, Hackett JL, Miller EK, Maxwell BD, Dyer WE (2001) Dicamba resistance in kochia. Weed Sci 49:164–170

Dahl GK, Dexter AG, Nalewaja JD (1982) Kochia competition and control in wheat. Proc North Cent Weed Control Conf 37:15-16

Davis VM, Gibson KD, Bauman TT, Weller SC, Johnson WG (2009) Influence of weed management practices and crop rotation on glyphosate-resistant horseweed (*Conyza canadensis*) population dynamics and crop yield—years III and IV. Weed Sci 57:417–426

Délye C, Menchari Y, Michel S, Cadet É, Corre VL (2013) A new insight into arable weed adaptive evolution: mutations endowing herbicide resistance also affect germination dynamics and seedling emergence. Ann of Bot 111: 481–691

Deen W, Hamill A, Shropshire C, Soltani N, Sikemma PH (2006) Control of volunteer glyphosate-resistant corn (*Zea mays*) in glyphosate-resistant soybean (*Glycine max*). Weed Technol 20:261–266

Devine MD, Buth JL (2001) Advantages of genetically modified canola. A Canadian perspective. Br Crop Prot Conf Weeds 1, 2: 367–372

Dexter AG, Zollinger RK (2003) Weed control guide for sugarbeet. In 2002 Sugarbeet Research and Extension Reports. Fargo, ND: NDSU Extension Serv. 33:3–34

Dille JA, Stahlman PW, Geier PW, Riffel JD, Currie RS, Wilson RG, Sbatella GM, Westra P, Kniss AR, Moechnig MJ, Cole RM (2012) Kochia emergence profiles across the central Great Plains. Proc Weed Sci Soc Am 52:122

Donald WW, Prato T (1991) Profitable, effective herbicides for planting-time weed control in no-till spring wheat (*Triticum aestivum*). Weed Sci 39:83–90

Duke SO, Powles SB (2008) Glyphosate: a once-in-a-century herbicide. Pest Manag Sci 64:319–325

Durgan BR, Dexter AG, Miller SD (1990) Kochia (*Kochia scoparia*) interference in sunflower (*Helianthus annuus*). Weed Technol 4:52–56.

Eberlein CV, Fore ZA (1984) Kochia biology. Weeds Today 15:5-6

Eberlein CV, Guttieri MJ, Fletcher FN (1993) Broadleaf weed control in potatoes (*Solanum tuberosum*) with postemergence directed herbicides. Weed Technol 7:298-303

Everitt JH, Alaniz MA, LEE JB (1983) Seed germination characteristics of kochia of *Kochia scoparia*. J Range Manag. 36:646–648

Evetts LL, Burnside OC (1972) Germination and seedling development of common milkweed and other species. Weed Sci 20:371–378

Fawcett RS, Slife FW (1978) Effects of 2, 4-D and dalapon on weed seed production and dormancy. Weed Sci 26:543–547

Fenster CR, Wicks GA (1982) Fallow systems for winter wheat in western Nebraska. Agron J 74:9-13

Field LM, Foster SP (2002) Amplified esterase genes and their relationship with other insecticide resistance mechanisms in English field populations of the aphid, *Myzus persicae* (Sulzer). Pest Manag Sci 58: 889–894

Forcella F (1985) Spread of kochia in the northwestern United States. Weeds Today 16:4–6

Franz JE, Mao MK, Sikorski JA (1997) Glyphosate: A unique global herbicide. In: Glyphosate's molecular mode of action. Washington, DC: American Chemical Society. pp 521–642

Friesen LF, Beckie HJ, Warwick SI, Van Acker RC (2009) The biology of Canadian weeds. 138. *Kochia scoparia* (L.) Schrad. Can J Plant Sci 89:141–167

Gaines TA, Zhang W, Wang D, Bukun B, Chisholm ST, Shaner DL, Nissen SJ, Patzoldt WL, Tranel PJ, Culpepper AS, Grey TL, Webster TM, Vencill WK, Sammons RD, Jiang JM, Preston C, Leach JE, Westra P (2010) Gene amplification confers glyphosate resistance in *Amaranthus palmeri*. Proc Natl Acad Sci 107:1029–1034

Gaines TA, Wright AA, Molin WM, Lorentz L, Riggins CW, Tranel PJ, Beffa R, Westra P, Powles SB (2013) Identification of genetic elements associated with EPSPS gene amplification. PLoS One.doi:101371/journalpone0065819

Giacomini D, Westra P, Ward SM (2014) Impact of genetic background in fitness cost studies: an example from glyphosate-resistant Palmer amaranth. Weed Sci 62: 29–37

Gulden RH, Shirtliffe SJ, Thomas AG (2003) Harvest losses of canola (*Brassica napus*) cause large seedbank inputs. Weed Sci 51:83–86

Gulden RH, Thomas AG, Shirtliffe SJ (2004) Secondary dormancy, temperature, and seed burial depth regulate seed bank dynamics in *B. napus*. Weed Sci 52:382–388

Hall LM, Topinka K, Huffman J, Davis L, Good A (2000) Pollen flow between herbicide-resistant *Brassica napus* is the cause of multiple-resistant *B. napus* volunteer. Weed Sci 48:688–694

Harker KN, Clayton GW, Blackshaw RE, O'Donovan JT, Johnson EN, Gan Y, Holm FA, Sapsford KL, Irvine RB, Van Acker RC (2006) Persistence of glyphosate-resistant canola in western Canadian cropping systems. Agron J 98:107–11

Heap I (2013) International Survey of Herbicide Resistant Weeds. www.weedscience.com Accessed: June 09, 2013

Heap I (2014) International Survey of Herbicide Resistant Weeds. http://www.weedscience.org Accessed: January 09, 2014

Heap I (2014) International Survey of Herbicide Resistant Weeds. http://www.weedscience.org Accessed: July 09, 2014

Heap IM (2014) International Survey of Herbicide Resistant Weeds. www.weedscience.org (accessed October 2014)

Heap I (2015) The International Survey of Herbicide Resistant Weeds. http://www.weedscience.org. Accessed: January 14, 2015

Heap I (2015) The International Survey of Herbicide Resistant Weeds. http://www.weedscience.org. Accessed: Mar 14, 2015

Hutchinson PJS, Hancock DM, Beutler BR (2005) Efficacy of reduced sulfentrazone rates applied preemergence with metribuzin in potato (*Solanum tuberosum*). Weed Technol 19:954-958

Isaacs MA, Murdock EC, Toler JE, Wallace SU (1989) Effects of late-season herbicide applications on sicklepod (*Cassia obtusifolia*) seed production and viability. Weed Sci 37: 761–765

Jacobson J, Jackson G, Jones C (2005) Fertilizer Guidelines for Montana Crops. Bozeman, MT: Montana State University Extension Service Publication EB 161. 25 p

Jenks BM, Willoughby GP, Markle DM (2006) Volunteer canola control in corn, soybean, sunflower, dry pea, and flax. Abstract 24-4 *in* Proceedings of the ASA-CSSA-SSSA International Annual Meetings. US Canola Association. Indianapolis, IN: American Society of Agronomy/Crop Science Society of Agronomy/Soil Science Society of Agronomy

Jha P, Norsworthy JK, Bridges W Jr., Riley MB (2008) Influence of glyphosate timing and row width on Palmer Amaranth (*Amaranthus palmeri*) and pusley (*Richardia* spp.) demographics in glyphosate-resistant soybean. Weed Sci 58:408–415

Jha P, Norsworthy JK (2012) Influence of late-season herbicide applications on control, fecundity, and progeny fitness of glyphosate-resistant palmer amaranth (*Amaranthus palmeri*) biotypes from Arkansas. Weed Technol 26:807–812

Jha P, Kumar V, Reichard N (2013) Preemergence residual herbicides: A valuable tool for weed control in glyphosate-resistant corn. Proc Weed Sci Soc Am 33

Jha P, Morishita DW, Felix J, Kumar V, Flenniken M (2015) Confirmation of glyphosate-resistant kochia in Idaho and Oregon. Proc Weed Sci Soc Am 104

Jugulam M, Niehues K, Godar AS, Koo D-H, Danilova T, Friebe B, Sehgal S, Varanasi VK, Wiersma A, Westra P, Stahlman PW, Gill BS (2014) Tandem amplification of a chromosomal segment harboring EPSPS locus confers glyphosate resistance in *Kochia scoparia*. Plant Physiol doi:10.1104/pp.1114.242826

Kemp NJ, Taylor EC, Renner KA (2009) Weed management in glyphosate- and glufosinate-resistant sugar beet. Weed Technol 23:416–424

Knezevic SZ, Weise SF, Swanton CL (1994) Interference of redroot pigweed (*Amaranthus retroflexus*) in corn (*Zea mays*). *Weed Sci* 42:568–573

Knezevic SZ, Streibig JC, Ritz C (2007) Utilizing R software package for dose-response studies: the concept and data analysis. Weed Technol 21:840–848

Knezevic SZ, Datta A, Scott J, Klein RN, Golus J (2009) Problem weed control in glyphosate-resistant soybean with glyphosate tank mixes and soil-applied herbicides. Weed Technol 23: 507-512

Kniss AR (2010) Comparison of conventional and glyphosate resistant sugar beet the year of commercial introduction in Wyoming. J Sugarbeet Res 47:127–134

Kniss AG, Sbatella GM, Wilson RG (2012) Volunteer glyphosate resistant corn interference and control in glyphosate resistant sugar beet. Weed Technol 26:348–355

Kumar V, Jha P, Reichard N (2014) Occurrence and characterization of kochia (*Kochia scoparia*) populations with resistance to glyphosate in Montana. Weed Technol 28:122-130

Kumar V, Jha P, Westra P, Westra E, Giacomini D, Vanhorn C (2014) EPSPS gene amplification confers glyphosate resistance in kochia (*Kochia scoparia*). Proc Weed Sci Soc Am 381 Available: http://wssaabstracts.com/public/22/ abstract-381.html

Kumar V, Jha P (2015a) Effective preemergence and postemergence herbicide programs for kochia control. Weed Technol 29:24–34

Kumar V, Jha P (2015b) Influence of herbicides applied postharvest in wheat stubble on control, fecundity, and progeny fitness of *Kochia scoparia* in the US Great Plains. Crop Prot 71:144–149

Leeson JY, Thomas AG, Hall LM, Brenzil CA, Andrews T, Brown KR, Van Acker RC (2005) Prairie Weed Surveys of Cereal, Oilseed and Pulse Crops from the 1970s to the 2000s. Saskatoon, Canada: Agriculture and Agri-Food Canada Weed Survey Series Publ. 05-1. 395p

Légère A, Simard MJ, Johnson E, Stevenson EC, Beckie H, Blackshaw RE (2006) Control of volunteer canola with herbicides; effects of plant growth stage and cold acclimation. Weed Technol 20:485–493

Légère A, Stevenson FC, Beckie HJ, Warwick SI, Johnson EN, Hrynewich B, Lozinski C (2013) Growth characterization of kochia (*Kochia scoparia*) with substitutions at Pro197 or Trp574 conferring resistance to acetolactate synthase–inhibiting herbicides. Weed Sci 61:267–276

Lehnhoff EA, Keith BK, Dyer WE, Menalled FD (2013) Impact of biotic and abiotic stresses on the competitive ability of multiple herbicide resistant wild oats (*Avena fatua*). PLoS ONE doi: 10.1371/journal.pone.0064478

Lenssen AW, Waddell J, Johnson GD, Carlson GR (2007) Diversified cropping systems in semiarid Montana: Nitrogen use during drought. Soil Tillage Res 94:362–375

Lloyd KL, Johnson JM, Gover AE, Sellmer JC (2011) Preemergence and postemergence suppression of kochia on rights-of-way. Weed Technol 25:292–297

Lorentz L, Gaines TA, Nissen SJ, Westra P, Strek HJ, Dehne HW, Ruiz-Santaella JP, Beffa R (2014) Characterization of glyphosate resistance in *Amaranthus tuberculatus* populations. J Agric Food Chem 62:8134–8142

Manthey FA, Hareland GA, Zollinger RK, Huseby DJ (1996) Kochia interference with oat (*Avena sativa*). Weed Technol 10:522–525

Marquardt PT, Johnson WG (2013) Influence of clethodim application timing on control of volunteer corn in soybean. Weed Technol 27:645–648

Massinga RF, Currie RS, Horak MJ, Boyer J Jr. (2001) Interference of Palmer amaranth in corn. Weed Sci 49:202–208

Menalled FD, Smith RG (2007) Competitiveness of herbicide-resistant and herbicide-susceptible kochia (*Kochia scoparia* [L.] Schrad.) under contrasting management practices. Weed Biol Manage 7:115–119

Mengistu LW, Messersmith CG (2002) Genetic diversity of kochia. Weed Sci 50:498–503

Mesbah, A, Miller SD, Fornstrom KJ, Legg DE (1994) Kochia (*Kochia scoparia*) and green foxtail (*Setaria viridis*) interference in sugar beets (*Beta vulgaris*). Weed Technol 8:754-759

Mickelson JA, Bussan AJ, Davis ES, Hulting AG, Dyer WE (2004) Postharvest kochia (*Kochia scoparia*) management with herbicides in small grains. Weed Technol 18:426-431

Mohseni-Moghadam M, Schroeder J, Ashigh J (2013) Mechanism of resistance and inheritance in glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) populations from New Mexico, USA. Weed Sci 61:517–525

Moyer JR, Roman ES, Lindwall CW, Blackshaw RE (1994) Weed management in conservation tillage systems for wheat production in North and South America. Crop Prot 4:243–259

Nandula VK, Manthey FA (2002) Response of kochia (*Kochia scoparia*) inbreds to 2,4-D and dicamba. Weed Technol 16:50-54

Nandula VK, Reddy KN, Poston DH, Rimando AM, Duke SO (2008) Glyphosate tolerance mechanism in Italian ryegrass (*Lolium multiflorum*) from Mississippi. Weed Sci 56:344–349

Nandula VK (Ed) (2010) Glyphosate Resistance in Crops and Weeds. Wiley Inc.

Nandula VK, Reddy KN, Koger CH, Poston DH, Rimando AM, Duke SO, Bond JA, Ribeiro DN (2012) Multiple resistance to glyphosate and pyrithiobac in Palmer amaranth (*Amaranthus palmeri*) from Mississippi and response to flumiclorac. Weed Sci 60:179–188

Nandula VK, Wright A, Molin W, Ray J, Bond J, Eubank T (2014) *EPSPS* amplification in glyphosate-resistant spiny amaranth (*Amaranthus spinosus*): a case of gene transfer via interspecific hybridization from glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*). Pest Manag Sci 70:1902–1909

Norsworthy JK, Oliver LR (2001) Effect of seeding rate of drilled glyphosate-resistant soybean (*Glycine max*) on seed yield and gross profit margin. Weed Technol 15:284–292

Norsworthy JK, Jha P, Bridges W Jr. (2007) Sicklepod survival and fecundity in wide- and narrow-row glyphosate-resistant soybean (Glycine max). Weed Sci 55:252–259

Norsworthy JK, Smith KL, Scott RC, Gbur EE (2007) Consultant perspectives on weed management needs in Arkansas cotton. Weed Technol 21:825-831

Norsworthy, JK, Ward SM, Shaw DR, Llewellyn RS, Nichols RL, Webster TM, Bradley KW, Frisvold G, Powles SB, Burgos NR, Witt WW, Barrett M (2012) Reducing the risks of herbicide resistance: Best management practices and recommendations. Weed Sci 60:31-62

O'Donovan JT, Harker KN, Dew DA (2008) Effect of density and time of removal of volunteer canola (*Brassica rapa* L.) on yield loss of wheat (*Triticum aestivum* L.). Can J Plant Sci 88:839–842

Ogg AG Jr, Parker R (2000) Control of Volunteer Crop Plants. Pullman, WA: Washington State University Extension Bull. EB1523:1–4

Pederson BP, Neve P, Andreasen C, Powles SB (2007) Ecological fitness of a glyphosate-resistant Lolium rigidum population: growth and seed production along a competition gradient. Basic Appl Ecol 8:258–268

Powles SB (2010) Gene amplification delivers glyphosate-resistant weed evolution. Proc Natl Acad Sci 107:955–956

Preston C, Belles DS, Westra PH, Nissen SJ, Ward SM (2009) Inheritance of resistance to the auxinic herbicide dicamba in kochia (*Kochia scoparia*). Weed Sci 57:43–47

Primiani MM, Cotterman JC, Saari LL (1990) Resistance of *Kochia scoparia* to sulfonylurea and imidazolinone herbicides. Weed Technol 4:169–172

Rainbolt CR, Thill DC, Young FL (2004) Control of volunteer herbicide-resistant wheat and canola. Weed Technol 18:711–718

Rakow G, Woods D (1987) Out-crossing in rape and mustard under Saskatchewan prairie conditions. Can J Plant Sci 67:147–151

Raybould AF, Gray A (1994) Will hybrids of genetically modified crops invade natural communities? Trends Ecol Evol 9:85–89

Reddy SS, Stahlman PW, Geier PW, Thompson CR (2012) Weed control and crop safety with premixed s-metolachlor and sulfentrazone in sunflower. Am J Plant Sci 3:1625–1631

Ribeiro DN, Pan Z, Duke SO, Nandula VK, Baldwin BS, Shaw DR, Dayan FE (2014) Involvement of facultative apomixis in inheritance of *EPSPS* gene amplification in glyphosate-resistant *Amaranthus palmeri*. Planta 239:199–212

Saari LL, Cotterman JC, Primiani MM (1990) Mechanism of sulfonylurea herbicide resistance in the broadleaf weed, *Kochia scoparia.* Plant Physiol 93:55–61

Salas RA, Dayan FE, Pan Z, Watson SB, Dickson JW, Scott RC, Burgos NR (2012) *EPSPS* gene amplification in glyphosate-resistant Italian ryegrass (*Lolium perenne* ssp. multiflorum) from Arkansas. Pest Manag Sci 68:1223–1230

Sammons DR, Gaines TA (2014) Glyphosate resistance: state of knowledge. Pest Manag Sci 70:1367–1377

Sathasivan K, Haughn GW, Murai N (1990) Nucleotide sequence of a mutant acetolactate synthase gene from imidazolinone-resistant *Arabidopsis thaliana* var. Columbia. Nucleic Acids Res 18:2188

Sbatella GM, Wilson RG (2010) Isoxaflutole shifts kochia (*Kochia scoparia*) populations in continuous corn. Weed Technol 24: 392−396

Schwinghamer TD, Van Acker RC (2008) Emergence timing and persistence of kochia (*Kochia scoparia*). Weed Sci. 56:37-41

Scott RK, Wilcockson SJ (1976) Weed biology and the growth of sugar beet. Ann Appl Bio 83:331–335

Seefeldt SS, Jensen JE, Fuerst EP (1995) Log-logistic analysis of herbicide dose-response relationships. Weed Technol 19:218-227

Shaner DL, Nadler-Hassar T, Henry WB, Koger CH (2005) A rapid *in vivo* shikimate accumulation assay with excised leaf discs. Weed Sci 53, 769–774

Simard MJ, Légère A, Pageau D, Lajeunesse J, Warwick S (2002) The frequency and persistence of volunteer canola (*Brassica napus*) in Québec cropping systems. Weed Technol 16:433–439

Singh BJ, Shaner DL (1998) Rapid determination of glyphosate injury to plants and identification of glyphosate-resistant plants. Weed Technol 12:527–530

Sivakumaran K, Mulugeta D, Fay PK, Dyer WE (1993) Differential herbicide response among sulfonylurea-resistant *Kochia scoparia* L. accessions. Weed Sci 41:159–165

Spangler AJ, Sprague CL, Steinke K (2014) Impact of Nitrogen and Weeds on Glyphosate-Resistant Sugarbeet Yield and Quality. Weed Technol 28:189–199

Stahlman PW, Olson BLS, Thompson, CR, Zollinger RK (2010) Pyroxasulfone (KIH-485) for weed control in sunflower. Pages 1-7 *in* Proc of the 1st Australian Summer Grains Conference. Gold Coast, Australia

Stallings GP, Thill DC, Mallory-Smith CA, Shafii B (1995) Pollen-mediated gene flow of sulfonylurea-resistant kochia (*Kochia scoparia*). Weed Sci 43:95–102

Steadman KJ, Eaton DM, Plummer JA, Ferris DJ, Powles SB (2006) Late-season non-selective herbicide applications reduce Lolium rigidum seed numbers, seed viability, and seedling fitness. Aust J Agric Res 57:133–141

Steckel LE, Sprague CL (2004) Late-season common waterhemp (Amaranthus rudis) interference in narrow- and wide-row soybean. Weed Technol 18:947–952

Stephenson DO IV, Bond JA, Landry RL, Edwards HM (2013) Effect of coapplied glyphosate, pyrithiobac, pendimethalin, or *S*-metolachlor on cotton injury, growth, and yield. Weed Technol 27:305-309

Stoebel DM, Dean AM, Dykhuizen DE (2008) The cost of expression of *Escherichia coli* lac operon proteins is in the process, not in the products. Genetics 178: 1653–1660

Taylor SE, Oliver LR (1997) Sicklepod (*Senna obtusifolia*) seed production and viability as influenced by late-season postemergence herbicide applications. Weed Sci 45:497–501

Thomas WE, Pline-Srnic WP, Viator RA, Wilcut JW (2005) Effects of glyphosate application timing and rate on sicklepod (*Senna obtusifolia*) fecundity. Weed Technol 19:55–61

Tonks DJ, Westra P (1997) Control of sulfonylurea-resistant kochia (*Kochia scoparia*). Weed Technol 11:270-276

Tranel PJ, Wright TR (2002) Resistance of weeds to ALS-inhibiting herbicides: what have we learned? Weed Sci 50:700–712

Tranel PJ, Riggins CW, Bell MS, Hager AG (2011) Herbicide resistance in *Amaranthus tuberculatus*: a call for new options. Pest Manag Sci 59:5808–5812

Tranel PJ, Wright TR, Heap IM (2015) Mutations in herbicide-resistant weeds to ALS inhibitors. http://www.weedscience.org. Accessed: January 7, 2015

Udeigwe TK, Teboh JM, Eze PN, Stietiya MH, Kumar V, Hendrix J, Mascagni HJ, Ying T, Kandakji T (2015) Implications of leading crop production practices on environmental quality and human health. J Environ Manage 151: 267–279

[USDA-ERS] United States Department of Agriculture–Economic Research Service (2012) Canola Production. http://www.ers.usda.gov/topics/crops/soybeans-oil-crops/canola.aspx#canolaprod. Accessed June 7, 2014

[USDA-NASS] United States Department of Agriculture– National Agricultural Statistics Service (2013) Montana's Rank in the Nation's Agriculture. http://www.nass.usda.gov/Statistics_by_State/Montana/Publications/Annual_Statistical_Bulletin/2013/RankMTRank.pdf. Accessed Feb 6, 2015

Van horn CR, Westra P (2014) Investigating the mechanism of resistance in nineteen giant ragweed accessions. Proc Weed Sci Soc Am 92

Vila-Aiub MM, Neve P, Powles SB (2009) Fitness costs associated with evolved herbicide resistance alleles in plants. New Phytol 184: 751–767

Vila-Aiub MM, Goh SS, Gaines TA, Han H, Busi R, Yu Q, Powles SB (2014) No fitness cost of glyphosate resistance endowed by massive EPSPS gene amplification in *Amaranthus palmeri*. Planta. 239:793–801

Vila-Aiub MM, Gundel PE, Preston C (2015) Experimental methods for estimation of plant fitness costs associated with herbicide-resistance genes. Weed Sci SI: 203–216

Waite J, Thompson CR, Peterson DE, Currie RS, Olson BLS, Stahlman PW, Khatib KA (2013) Differential kochia (*Kochia scoparia*) populations response to glyphosate. Weed Sci 61:193–200

Walker ER, Oliver LR (2008) Weed seed production as influenced by glyphosate applications at flowering across a weed complex. Weed Technol 22: 318–325Wagner A (2005) Energy constraints on the evolution of gene expression. Mol Biol Evol 22: 1365–1374

Wang W, Xia H, Yang X, Xu T, Si HJ, Cai XX, Wang F, Su J, Snow AA, Lu BR (2013) A novel 5-enolpyruvoylshikimate-3-phosphate (EPSP) synthase transgene for glyphosate resistance stimulates growth and fecundity in weedy rice (Oryza sativa) without herbicide. New Phytol 202: 679–688

Warwick SI, Xu R, Sauder C, Beckie HJ (2008) Acetolactate synthase target-site mutations and single nucleotide polymorphism genotyping in ALS-resistant kochia (*Kochia scoparia*). Weed Sci 56:797–806

Weatherspoon DM, Schweizer EE (1969) Competition between kochia and sugarbeets. Weed Sci 17:464–467

Weatherspoon DM and Schweizer EE (1971) Competition between sugarbeets and five densities of kochia. Weed Sci 19:125-128.

Westhoven AM, Kruger GR, Gerber CK, Stachler JM, Loux MM, Johnson WG (2008) Characterization of selected common lambsquarters (*Chenopodium album*) biotypes with tolerance to glyphosate. Weed Sci 56:685–691

Westra P, Wiersma A, Leach J, Reddy A (2013) Regional whole plant and molecular response of kochia to glyphosate. Proc. Weed Sci Soc Am 53:316

Wicks GA, Martin AR, Mahnken GW (1993) Control of triazine-resistant kochia (*Kochia scoparia*) in conservation tillage corn (*Zea mays*). Weed Sci 41:225–231

Wicks GA, Martin AR, Haack AE, Mahnken GW (1994) Control of triazine-resistant kochia (*Kochia scoparia*) in sorghum (*Sorghum bicolor*). Weed Technol 8:748–753

Wiersma AT, Gaines TA, Preston C, Hamilton JP, Giacomini D, Buell CR, Leach JE, Westra P (2015) Gene amplification of 5-enol-pyruvylshikimate-3-phosphate synthase in glyphosate-resistant *Kochia scoparia*. Planta 241:463–474

Williams, MM, Boydston RA (2006) Volunteer potato interference in carrot. Weed Sci 54:94–99

Wilson RG, Yonts CD, Smith JA (2002) Influence of glyphosate and glufosinate on weed control and sugar beet (*Beta vulgaris*) yield in herbicide tolerant sugar beet. Weed Technol 16:66–73

Wolf R, Clay SA, Wrage LJ (2000) Herbicide strategies for managing kochia (*Kochia scoparia*) resistant to ALS-inhibiting herbicides in wheat (*Triticum aestivum*) and soybean (*Glycine max*). Weed Technol 14:268-273

York AC, Beam JB, Culpepper AS (2005) Control of volunteer glyphosate-resistant soybean in cotton. J Cotton Sci 9:102–109

Young JA, Evans RA, Stevens R, Everett RL (1981) Germination of *Kochia prostrata* seed. Agron J 73:957–961

Yu Q, Jalaludin A, Han H, Chen M, Sammons RD, Powles SB (2015) Evolution of double amino acid substitution in the EPSP synthase in *Eleusine indica* conferring high level glyphosate resistance. Plant Physiol doi: http://dx.doi.org/10.1104/pp.15.00146

Zimdahl RL (2004) Weed-Crop competition. A Review, second ed. Blackwell publishing Ltd., Oxford, UK, p 220

Zorner PS, Zimdahl RL, Schweizer EE (1984) Effect of depth and duration of seed burial on kochia (*Kochia scoparia*). Weed Sci. 32:602-607