Prevention and Control Options for Glyphosate-Resistant Kochia (*Kochia scoparia*)

by

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Abstract

Glyphosate resistant *Kochia scoparia* is a growing concern in Canada, and is increasing in incidence. Trials were conducted in naturally occurring glyphosate susceptible kochia populations or in areas seeded with glyphosate susceptible kochia seed. By looking at herbicides with different modes of action that can control kochia as effectively as glyphosate, as well as introducing a new herbicide application window, it may be possible to decrease the occurrence of glyphosate-resistant kochia biotypes from becoming the majority of kochia populations on the Canadian prairies. Fluroxypyr + MCPA ester (a group 4 herbicide), bromoxynil + 2,4D (a combination of group 6 and 4 herbicides), saflufenacil and carfentrazone-ethyl (group 14), pyrasulfotole + bromoxynil (a combination of group 27 and 6 herbicides) were as effective at controlling kochia as glyphosate when applied pre-seeding. It was determined that herbicide effectiveness varies according to location. Introducing a new window of herbicide application in the fall, post-harvest, was not effective at controlling kochia.

Preface

Included in this thesis is research conducted as part of an investigation into the emergence of glyphosate-resistant *Kochia scoparia* in southern Alberta, and a follow up survey to determine its distribution and frequency. Dr. Hugh Beckie and Dr. Robert Blackshaw of Agriculture and Agri-food Canada, along with Dr. Linda Hall, the lead collaborator at the University of Alberta, led the study.

The initial survey, appearing in appendix 1, was conducted by myself. Dr. Beckie screened all seed for herbicide resistance and wrote the manuscript, which was edited by the other authors. It has been published as H.J. Beckie, R.E. Blackshaw, R. Low, L.M. Hall, C.A. Sauder, S. Martin, R.N. Brandt, and S.W. Shirriff, “Glyphosate- and Acetolactate Synthase Inhibitor-Resistant Kochia (*Kochia scoparia*) in Western Canada,” *Weed Science (2013)*, vol. 61, 310-318.

Fifty percent of the survey appearing in appendix 2 was conducted by myself; the manuscript was written by Dr. Hall and edited by the other authors. It has been published as L.M. Hall, H.J. Beckie, R. Low, S.W. Shiriff, R.E. Blackshaw, N. Kimmel, and C. Neeser, “Survey of glyphosate-resistant kochia (*Kochia scoparia* L. Schrad.) in Alberta,” *Can. J. Plant Sci.* (2014), vol. 94, 127-130.

The literature review found in chapter 2, data collection and analysis of chapters 3 and 4, as well as concluding analysis found in chapter 5, are the candidates original work.

Dedication

This thesis is dedicated first to my grandparents, George, Helen, Harvey and Betty

who have inspired me throughout my life.

Second, to my parents Bob and Marg and my Aunt Fran

who have always been there with unconditional encouragement and support.

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I would like to acknowledge Dr. Linda Hall for all of her efforts in making this masters project come to fruition. Dr. Hall has supported me throughout my degree both in and out of the classroom, presenting me with opportunities to collaborate with other researchers, and encouraging me to push myself academically. I would not have reached this point without her. To that end I acknowledge my supervisory committee, Dr. Hugh Beckie and Dr. Edward Bork. Their comments and guidance were invaluable throughout the last four years.

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I would like to give special acknowledgement to Dr. Hugh Beckie and Dr. Bob Blackshaw for giving me the opportunity to be a part of the group that found the first glyphosate-resistant weeds in western Canada. It really was a once-in-a-career opportunity.

I have been very fortunate to have some incredible teachers in my academic life. It has been a long, windy road and they have helped me navigate it. As such I would like to thank Dr. Rong-Cai Yang, Hester Jiskoot, Jim Byrne, Ken Perl, Brett Jensen, Steve McRae, Edith Olson, Brian Meller, Jackie Martin and Peter Rilstone.

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List of Abbreviations

WAH	Weeks After Harvest
AB	Alberta
a.i.	Active Ingredient
ALS	Acetolactate Synthase
ANOVA	Analysis of Variance
C	Celsius
cm	Centimetre
CO_2	Carbon dioxide
dS	deciSiemens
EC	Electrical Conductivity
ESPS	5-enolypyruvoylshikimate 3-phosphate synthase enzyme
g	Grams
GDD	Growing Degree Days
GR	Glyphosate-Resistant
HR	Herbicide-Resistant
hr	Hour

ha	Hectare
IAH	Immediately After Harvest
ISSR	Inter-Simple Sequence Repeat
IWM	Integrated Weed Management
km	Kilometre
kPa	Kilo Pascal
m	Metre
MB	Manitoba
MCPA	2-methyl-4-chlorophenoxyacetic acid
MITE	Miniature Inverted Repeat Transposable Elements
mL	Millilitre
mm	Millimetre
PEP	Phosphoenolpyruvate
R	Resistant
RNAi	Ribonucleic Acid Interference
S	Susceptible
SK	Saskatchewan
SOA	Site-Of-Action

WAA	Weeks After Application

Chapter One: Introduction

1.1. Background

Kochia scoparia is one of the most rapidly increasing weed species in the Canadian prairies. Since the 1970s kochia has increased in abundance across the prairie provinces and is now listed as the 10th most abundant weed species in crop following herbicide application (Leeson *et al.* 2005). Kochia is an early emerging weed, surfacing in the spring after >50 growing degree days (GDD)(at a base temperature of 0°C)(Bullied *et al.* 2003; Schwinghamer and Van Acker 2008) and therefore is a frequent target for pre-seeding weed control with glyphosate. Under good conditions it produces large amounts of seed. It has been reported that between 2,000 and 30,000 seeds are produced per plant (Friesen *et al.* 2009). However, kochia has a relatively short seed bank persistence, so in the absence of annual seed return, population abundance can be reduced (Friesen *et al.* 2009; Schwinghamer and Van Acker 2008). In the fall, kochia flowers indeterminately, with seed production and maturity continuing after the harvest of the surrounding crop, which can result in injured/decapitated kochia plants returning seed to the seed bank.

Beckie *et al.* (2011) reported that 85% of western Canadian kochia populations were resistant to Group 2 acetolactate synthesis (ALS) inhibitors and resistance to dicamba has been reported (Preston *et al.* 2009) . There was great concern amongst the agriculture community in the United States when kochia was reported to have evolved resistance to the glyphosate molecule in 2006, originally identified in three fields in Kansas (Waite 2008). By the start of 2011, glyphosate-resistant (GR) kochia had been reported in Nebraska and South Dakota (Heap 2015). This project was undertaken to prevent or delay GR kochia incidence by proposing alternate chemical weed control techniques to reduce the use of glyphosate on kochia. With the discovery of GR kochia in southern Alberta in 2011, the project scope changed to include a survey of the range and extent in the province (Appendix 1 and 2). The experiments became more relevant as non-glyphosate control options also delay the selection of resistance. As of 2015, GR kochia has been reported in the American states of Kansas, South Dakota, North Dakota, Nebraska, Montana, Colorado, Oklahoma, and Montana and in the Canadian provinces of Alberta, Saskatchewan and Manitoba.

1.2. Research Objectives

1.2.1. Compare herbicide options for pre-seeding control of GR kochia including herbicide with a diversity of sites of action.

Since pre-seeding herbicide products have increased in scope and usage, a variety of commercially available herbicides were tested for their effectiveness at reducing kochia biomass and then compared to glyphosate-treated kochia. The research objective is investigated in Chapter 3 and the following hypothesis was made:

- Herbicides applied pre-seeding would limit kochia biomass at the beginning of the growing season equal to that displayed by glyphosate.

1.2.2. Investigate the utility of post-harvest application on kochia seed banks.

Utilization of post-harvest applications may control kochia seed production, and may be more efficient for producers. A variety of commercially available herbicides were tested for their effectiveness at reducing kochia biomass, seed production, and viable seed set at two post-harvest intervals, and the results compared to kochia treated with glyphosate. The research objective is investigated in Chapter 4 and the following hypotheses made:

- Herbicides applied post-harvest would reduce kochia biomass.

- Herbicides applied post-harvest would reduce seed biomass produced by kochia.

1.3. Literature Cited

Beckie, H. J., S. I. Warwick, C. A. Sauder, C. Lozinski, and S. Shirriff. 2011. Occurrence and molecular characterization of acetolactate synthase (ALS) inhibitor-resistant kochia (*Kochia scoparia*) in western Canada. Weed Technol. 25:170-175.

Bullied, W. J., A. M. Marginet, and R. C. Van Acker. 2003. Conventional-and conservation-tillage systems influence emergence periodicity of annual weed species in canola. Weed Sci. 51:886-897.

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. 2014. The International Survey of Herbicide Resistant Weeds. www.weedscience.org. Accessed: January 24, 2014.

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, Saskatchewan, Canada: Agriculture and Agri-Food Canada, Saskatoon Research Centre Weed Survey Series Publication 05-1CD :395 .

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

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

Waite J. C. 2008. Glyphosate Resistance in Kochia. Masters of Science dissertation. Manhattan Kansas: Kansas State University.

Chapter Two: Literature Review

2.1. Biology of Kochia

Kochia scoparia L. is a member of the Amaranthaceae family, which contains approximately 2,500 species (Friesen *et al.* 2009). Of the 13 to 16 recognized species of kochia found in the world, three species are found in North America: *K. scoparia*, *K. americana*, and *K. californica*. Of these, only *K. scoparia* is found in Canada, where it has become a significant weedy species in the western provinces. Kochia species are diploid organisms containing 18 chromosomes (Friesen *et al.* 2009). The three kochia species found in North America are found to be genetically distinct and there is no evidence to support hybridization or gene flow between the species (Lee *et al.* 2005).

Kochia is a facultative alkali halophyte (Khan *et al.* 2001). Kochia seeds are highly salt tolerant and germination occurs over a wide range of temperatures. It utilizes NADP-ME in the C_4 photosynthetic pathway, giving the plant an advantage in water-use efficiency and salinity tolerance as compared to C_3 species. In addition, kochia is adapted to dry, hot conditions with reduced leaves that are composed of a hairy under surface, inconspicuous apetalous flowers, a deep taproot, and an extensive lateral root system.

2.1.1. Distribution and Abundance of *Kochia scoparia*

Kochia is native to Eurasia and was likely introduced to North America in the mid-1800s as an ornamental (Friesen *et al.* 2009), and is now prevalent throughout the continent, occurring in 42 of the 48 contiguous United States (with the exception of Arkansas, Alabama, Georgia, Florida, North Carolina, and Maryland) (Heap 2015). In Canada, kochia is found in all the provinces with the exception of Newfoundland and Labrador. While considered rare in the prairie provinces of Alberta and Saskatchewan in 1948, by 2009 it had become the 10th most abundant weed in arable fields in the Canadian Prairies (Friesen *et al.* 2009). It is the 4th most abundant weed in the southern semiarid Grassland region (Leeson *et al.* 2005). In the 2000s residual weed surveys (weeds that were present following in-crop application of herbicides) reported that in fields where kochia occurred, densities averaged 4.5 plants m^{-2} but densities of >100 plants m^{-2} were reported.

Kochia can tolerate nearly all temperature ranges in Canada, although it has a defined northern expansion limit set by the length of the frost-free growing season (Friesen *et al.* 2009).. It is well adapted to arid and semi-arid regions of the prairies and to saline soils that limit or prohibit crop growth. Kochia is also tolerant of conditions associated with acidic soils, including aluminum and manganese toxicity that are normally toxic to other plants. Kochia is found in a wide range of habitats: as an in-crop weed common in direct seeded systems, especially where chemical fallow is practiced; as a ruderal weed in disturbed areas with low resource availability; and in saline or alkaline areas and in rangeland.

2.1.2. Morphology

Kochia seed is oval or nearly oval, from 1.5 to 2.0 mm long, flattened and grooved. It is enclosed in a papery envelope, formerly 5 winged sepals (Friesen *et al.* 2009). Seedling cotyledons are short, narrow, and bright pink on the under surface. True leaves are sessile, linear, and covered in dense hairs. The juvenile has alternatively arranged leaves, on an erect and much-branched stem. Leaves on the juvenile appear grayish-green. Flowers on mature plants are numerous and inconspicuous, born in the leaf axis. Pollen production is prolific, usually indicating an outcrossing species (see Population Biology, below). Kochia has a deep rooting system and lateral roots may extend to a horizontal distance of 7 m (Davis *et al.* 1967).

Morphologically, mature kochia is very diverse, with the environment playing a large part in its phenotypic characteristics with growth, height, and seed production influenced by both inter- and intraspecific competition within a field (Becker 1978; Friesen *et al.* 2009). When grown in the absence of competition, the growth form is nearly spherical, while in dense stands growth form can be erect, tall, and single-stemmed. Growth form and stand densities influence seed dispersal distances.

2.1.3. Life Cycle

Kochia is an annual broadleaf weedy species that emerges very early in the spring after >50 growing degree days (at a base temperature of 0°C) (Bullied *et al.* 2003; Schwinghamer and Van Acker 2008); however, additional germination can continue throughout the growing season (Friesen *et al.* 2009). Emergence begins prior to crop seeding, before many other common weedy species, and may provide kochia with a distinct survival advantage in cropping systems. Kochia emergence is influenced by vertical seed placement in soil; 74% of exposed kochia seeds on soil germinated compared to 52% of kochia seeds that germinated when planted at a depth of 3 mm. No seedlings germinated when kochia seed was planted at a depth in excess of 40 mm (Friesen *et al.* 2009).

Kochia is a short day plant and will typically start to flower between 8 and 10 weeks after emergence; but flowering, seed set, and maturation continues until a frost kills the plant (Eberlein and Fore 1984; Mickelson *et al.* 2004). A typical kochia plant may produce between 2,000 and 30,000 seeds per plant (Friesen *et al.* 2009), depending on plant density, environmental conditions, and resource availability.

Kochia disperses seed as it reaches maturity. The seeds drop below the plant, although kochia can also disperse its seed over a wide area as a tumbleweed (Baker *et al.* 2008). As kochia plants reach maturity an abscission zone forms at the base of the plant due to declining internal moisture levels. The tumbleweed mechanism occurs when the entire above ground portion of a mature plant breaks close to the soil surface and rolls from wind pressure, dropping seed along the way. It is reported that once the plant reaches maturity, wind speeds between 40 and 48 km hr^{-1} cause the abscission zone to break at the base of the plant (Becker 1978). Partially as a result of the long distance dispersal facilitated by tumbling, kochia has the highest rate of spread of any weed in western USA (Forcella 1985) and is one of the most abundant weeds in western Canada (Friesen *et al.* 2009).

2.1.4. Seed Banks

Freshly harvested kochia seed does not exhibit a high degree of dormancy, and germination can be very rapid, within two hours of receiving appropriate conditions (personal observation). Kochia seed is relatively short-lived in the seed bank (Schwinghamer and Van Acker 2008). After one spring and summer, the residual seed bank was reported to be less than 10% of the total kochia that had emerged over that time period. The relatively short seed bank life provides a mechanism to decrease kochia populations. Should seed deposition be prevented for a single year, populations are likely to decline.

2.1.5. Pollination Biology

Kochia is self-compatible, enabling a single plant to produce seed and facilitating colonization of new disturbed areas. However where kochia occurs in proximity, the frequency of pollen-mediated gene flow is relatively high. Kochia is a protogynous flowering species, where the stigma of the plant becomes receptive to pollen prior to the anther of the plant releasing pollen. While self-compatible it is believed that the emergence and deterioration of stigmas occur before anther maturity, reducing self-pollination within the same flower (Friesen *et al.* 2009), allowing the plant to be more receptive to foreign pollen and less dependent on self-pollination (Stallings *et al.* 1995). In a study using sulfonyurea resistance as a marker, Stallings *et al.* conducted field trials to measure pollen-mediated gene flow in kochia. Short distance (1.5 m) outcrossing was 13.1% and declined with distance to 1.4% 29 m away from the pollen source. Gene flow was correlated with prevailing wind direction, as expected for wind vectored pollen movement. Mulugeta *et al.* (1994) reported pollen to be 99.9% deposited within 154.4 m and pollen remained viable for <1 day to 12 days. Therefore, while kochia is primarily self-pollinated, there is "substantial outcrossing" potential for considerable distances (Friesen *et al.* 2009; Mulugeta *et al.* 1994; Stallings *et al.* 1995). Cross-pollination facilitates the ability for genes to move with pollen, the stacking of resistance genes, and genetic variability within populations.

2.1.6. Genetic Diversity

Genetic diversity is a measure of the variation found in heritable characteristics of a species that may vary within a population (in the case of outcrossing species) or between populations (in the case of self-pollinating species). Mengistu and Messersmith (2002) examined the diversity of 13 kochia populations using 45 ISSR loci and correlation with herbicide resistance status. They reported that kochia is a genetically diverse species, with most of the diversity found within populations and not among them; indicating "substantial levels of gene flow within and among populations" (Friesen *et al.* 2009).

2.1.7. Allelopathy

In laboratory settings, kochia has shown to have allelopathic effects on some crop species, including sorghum (*Sorghum bicolor* L.), soybean (*Glycine max*), cotton (*Gossypium hirsutum* L.), and sunflower (*Helianthus annuus*), and at least one native range species, blue grama (*Bouteloua gracilis*) (Friesen *et al.* 2009). Additionally, it is believed that kochia has autotoxicity; where a large kochia population within the geographical region will reduce vegetative and reproductive growth throughout the stand (Friesen *et al.* 2009). There was no direct evidence that alleleopathy plays a role under natural conditions.

2.1.8. A History of Herbicide Resistance in Kochia

Kochia resistant to the photosystem II herbicide triazine was first reported in 1986 (Salhoff and Martin 1986), to ALS inhibitor chlorsulfuron in 1990 (Primiani *et al.* 1990), and to dicamba in 1994 (Cranston *et al.* 2001) – all of which occurred first in the United Sates. The first ALS inhibitor resistance was cross-resistant to other sulfonylurea herbicides and the imidazolinone herbicide imazapyr. The mechanism of resistance to the ALS inhibitors is a genetic change to the gene encoding for the ALS enzyme, which reduces the sensitivity of the enzyme to herbicide inhibition (Saari *et al.* 1990). Subsequent research has shown that target site resistance in ALS inhibitors can be conferred at several different positions on the gene, and by several different amino acid substitutions (Tranel and Wright 2009; Warwick *et al.* 2008). The most common was a Trp574Leu mutation, followed by a Pro197 mutation by one of nine amino acids. Interestingly, 30 kochia plants (10% of samples) were identified with more than one ALS target site mutation. This suggests that mutations are occurring independently and that pollen gene flow is occurring. Later Beckie *et al.* (2011) reported that 85% of kochia populations were resistant to ALS inhibitors.

Kochia resistant to glyphosate and ALS inhibitors was reported in 2011 (Beckie *et al.* 2012; Hall *et al.* 2014) (see below).

2.1.9. Summary

Several aspects of the biology of kochia have influenced the selection of herbicide resistance. Firstly, abundance is very high. Although densities >100 plants m^{-2} have been reported in residual weed surveys (Leeson *et al.* 2005), kochia seedlings can form a dense carpet in early spring prior to seeding. This is due in part to the large number of small seeds produced and the lack of seed dormancy. In the absence of a seed bank, kochia populations turn over rapidly.

Kochia has a high genetic diversity from which traits can be selected due to pollen and seed mediated gene flow. Additionally, herbicide resistance traits can be moved long distances by seed-mediated wind dispersal from plants, short distances by dehiscence from the maternal plant, and short to moderate distances via pollen-mediated gene flow (at least to distances of 30 m).

2.2. Glyphosate and Glyphosate Resistance

2.2.1. Why is Glyphosate Such an Important Herbicide?

Glyphosate (N-(phosphonomethyl)glycine) is the world's most widely used herbicide due to its low toxicity, non-residual nature, and non-selective activity on both annual and perennial weeds. It is widely used in many applications, including agricultural, domestic, forestry, and industrial uses such as railway weed control and vegetation management on oilfield leases (Duke and Powles 2008).

As a herbicide it has many positive attributes and few negative drawbacks (Baylis 2000).

2.2.2. History of Glyphosate Use in Canada

Glyphosate was first introduced to Canada in 1974, two years after its first introduction to the United States, and marketed as a post-emergence non-selective herbicide (Duke and Powles 2008; Grossbard and Atkinson 1985). It was widely adopted in chemical fallow and pre-seeding in zero-tillage cropping systems to replace tillage as a non-selective weed control and for post-harvest control. Due to its non-selective nature, glyphosate use was initially limited in cereal and oilseed production to removing weeds prior to crop emergence or after the desired crop had been harvested (Duke and Powles 2008). It wasn't until the introduction of GR crops canola (*Brassica napus* L.), corn (*Zea mays* L.), soybean (*Glycine max* L. Merr.), and sugar beet (*Beta vulgaris* L.) that glyphosate was used as a selective herbicide in crop (Beckie *et al.* 2006; Duke and Powles 2008; Duke 2005).

Since the initial introduction of GR soybeans to the world market in 1995, five major GR crop species have emerged including soybean, corn, cotton and canola, which were planted to 74.9 million hectares worldwide in 2007. Canada is the fourth largest planter of GR crops in the world, and plants approximately 89% of the global GR canola (Dill *et al.* 2008).

The use of glyphosate in agricultural settings, both in Canada and around the world, has been consistently increasing. In 1995 there was a global demand for 51,078 tonne (Woodburn 2000); however, by 2012 the global demand had risen to 500,000 tonne (Székács and Darvas 2012), an increase that is largely attributed to the increased use of GR crops (Duke and Powles 2008). Another factor contributing to the rise in the use of glyphosate has been a reduction in the cost of the herbicide.

Glyphosate came off patent in 2000, allowing generic manufacturers to produce and sell the chemical, resulting in a decline in the price of glyphosate by 40% by 2006 (Duke and Powles 2009).

2.2.3. Use Patterns of Glyphosate

Glyphosate is used as a desiccant, as a fall applied herbicide for winter annual and perennial control, as a pre-seeding herbicide, and as an in-crop treatment. Since the introduction of GR crops there has been a shift towards in-crop weed control, attributed to the higher fuel and labour costs associated with fall and spring weed control techniques (Givens *et al.* 2009). Although the use of glyphosate has encouraged the adoption of zero-till and low-till farming practices, reduced the need for summer fallow, and has aided in soil and water conservation by Canadian growers (Holm and Johnson 2010), it has increased the number of applications of a single herbicide mode of action in a growing season (Givens *et al.* 2009).

2.2.4. Physio-chemical Properties

Glyphosate is a crystalline solid that is highly soluble in aqueous solutions, but generally insoluble in organic solvents as indicated by its low octanol/water partition coefficient (K_{ow}) of 0.0006-0.0017, and is considered to be hydrophilic. Glyphosate is a zwitterion, having four separate dissociation constants (pKa), dissociating its first phosphonic, carboxylate, second phosphonic and amine proton at pHs of 0.8, 2.3, 6.0, and 11.0 respectively (Franz *et al.* 1997). Glyphosate is considered a weak-acid herbicide.

2.2.5. Uptake and Translocation

Glyphosate is a systemic herbicide that enters the plant via diffusion through the leaf cuticle and is dependent on the plant surface intercepting the herbicide spray. The rate of uptake is modified considerably by the formulation and the surfactant system of the product (Nalewaja *et al.* 1996). The rate of uptake is variable between species and is believed to confer some differences in tolerance. Once inside the plant cells, glyphosate is rapidly translocated primarily in the phloem, with up to 70% of the absorbed chemical translocated out of the leaves and concentrating in meristematic tissue (Franz *et al.* 1997).

2.2.6. Mode of Action

Glyphosate is the only Group 9 herbicide, targeting the 5-enolpyruvoylshikimate 3-phosphate synthase enzyme (EPSPS), preventing the 6th step in the shikimic acid pathway (Franz *et al.* 1997). The shikimic pathway is a sequence of seven metabolic steps and is responsible for the metabolism of carbohydrates to chorismate, a precursor for the production of the aromatic amino acids phenylalanine, tyrosine, and tryptophan as well as aromatic secondary metabolites and pathway intermediates which produce branch point compounds that are substrates for other metabolic pathways (Franz *et al.* 1997; Herrmann and Weaver 1999). While present in bacteria, fungi, plants, and some protozoans, the pathway is absent in animals whose dietary requirement for aromatic amino acids derived from the shikimate pathway are obtained from consuming organisms possessing the pathway (Franz *et al.* 1997; Herrmann and Weaver 1999; Starcevic *et al.* 2008).

In plants, EPSPS is a nuclear encoded, monomeric, monofunctional protein located predominantly in the chloroplasts and root plastids (Franz *et al.* 1997). Its structure consists of two “distinct hemispherical globular domains” or sections that are connected by a “double-stranded hinge” (Franz *et al.* 1997). When the ligand phosphoenolpyruvate (PEP) binds to the enzyme the sections close and the enzyme catalyzes the transfer of the ligand to shikimate 3-phosphate. Glyphosate, however, acts as a competitive inhibitor to PEP, by binding to the EPSPS enzyme and thereby blocks the transfer step (Franz *et al.* 1997).

2.2.7. Environmental Profile

Glyphosate is considered to have one of the safest environmental profiles amongst herbicides in use today. The herbicide binds tightly to soil colloids, limiting its mobility from the point of contact with the soil, where it is rapidly degraded into CO_2 and aminomethylphosphonic acid by microorganisms found in the soil (Simonsen *et al.* 2008; Sprankle *et al.* 1975). Once the herbicide has come into contact with the soil, it is bound so tightly that there is virtually no soil activity; its rapid degradation ensures that there is no residual activity and due to its chemical structure the compound is not volatile, preventing atmospheric contamination (Duke and Powles 2008). There is no evidence to suggest adverse effects to the environment after the application of glyphosate (Duke and Powles 2008).

In terms of its impact on other organisms, studies have shown glyphosate and its decomposition products to have a very low level of toxicity in mammals, birds, and fish, attributed to these organisms' lack of a shikimate pathway, and is not retained in animal tissue; making the likelihood of bioaccumulation in the food chain low enough to be considered insignificant. The glyphosate molecule itself is considered to be non-toxic, non-mutagenic, non-carcinogenic, non-teratogenic, and not neurotoxic (Franz *et al.* 1997).

2.2.8. History of Glyphosate Resistance in Crops

The first commercially introduced GR crops were soybean and canola in 1995, followed by cotton in 1997, and maize in 1998 (Duke 2005) all of which were adopted over a short period of time. One of the greatest driving factors encouraging such rapid adoption of GR crops is the economic advantage it presents the grower.

Dill (2005) reported weed-management cost savings of \$25 ha^{-1} in GR soybean as opposed to non-GR soybeans. In addition to the monetary savings presented in chemical weed control, applying glyphosate to GR crops provided growers savings in terms of fuel, as they had to make fewer trips to the field to till their soil, which are reported as savings up to 53 L ha^{-1}. This in turn not only created benefits such as needing lower horse-powered equipment required in weed management, but also promoted soil conservation issues such as water use efficiency, reduction in top soil erosion, and maintenance of organic matter content (Dill 2005). While initially reported that herbicide-resistant (HR) crops resulted in a reduction in herbicide use, HR crops increased herbicide use in the U.S. by an estimated 239 million kgs (527 million pounds) between 1996-2011, primarily due to an increasing reliance on glyphosate (Benbrook 2012). Additionally, when glyphosate came off patent in 2000, it led to many generic glyphosate herbicides becoming available, prompting a decline in the price of glyphosate, making adoption of GR crops and increased use of glyphosate more attractive to growers from an economical standpoint (Brookes and Barfoot 2014; Duke 2005).

Farmers planted 0.55 billion hectares of HR corn, soybeans, and cotton from 1996 through 2011, with HR soybeans accounting for 60% of these hectares. Most of these hectares (ha) were GR crops (Benbrook 2012). This has led to the glyphosate chemical being used as the exclusive form of weed control in a wide area over multiple years.

2.2.9. History of Glyphosate Resistance in Weeds

Glyphosate-resistant *Lolium rigidum* (rigid ryegrass) was first identified in the state of Victoria, Australia in 1996, 20 years after the glyphosate herbicide was first used commercially (Pratley *et al.* 1999). The following year glyphosate-resistant *Eleusine indica* (Goosegrass) in Malaysia was reported (Lee and Ngim 2000), and by 2015 glyphosate-resistance is reported in 32 weed species across all six continents where agriculture is possible, with many species having evolved resistance to multiple herbicides (Heap 2015). Glyphosate resistance evolved as a result of high selection pressure from repetitive use of the glyphosate herbicide.

2.2.10. History of Glyphosate Resistance in Kochia

Glyphosate-resistant kochia was first identified in the state of Kansas, USA in 2007 (Waite 2008; Waite *et al.* 2013); the following year it was confirmed in South Dakota, and in 2011 it was confirmed in Nebraska and Alberta (Beckie *et al.* 2011). It now has been reported in seven states and three provinces of Canada (Beckie *et al.* 2015). Almost all populations were also resistant to ALS inhibitors tribenuron/thifensulfuron, but none were resistant to dicamba. Resistance level was considered low to moderate, with a resistance factor (the ratio of the rates required for 50% control of the resistant and susceptible populations) of 4 to 7. Most of the sites where GR kochia were identified were in or close to areas where chemical fallow was practiced and glyphosate may have been applied alone several times per season. Some Canadian populations were identified in waste areas or in cropped fields, but in Manitoba they were identified in GR soybean and corn crops.

2.2.11. Selection for Herbicide Resistance

Herbicide resistance is caused by selective pressures, which causes local populations of weeds to select for traits that allow them to survive and pass on the enhanced fitness to subsequent generations. It is defined to “describe a characteristic of species (as intact plants or plant cells in culture) to withstand substantially higher concentrations of a herbicide than the wild type of the same plant species” (Powles and Holtum 1994). It requires a heritable mutation in the gene or pathway that confers resistance to the herbicide, and is largely based on the genetic variation of the species, both within and outside a population. While this may lead to a change in the fitness of the resistant biotype as compared to the wild type, it “is directly related to the increase in the frequency of the resistance trait (phenotype) in the population” (Powles and Holtum 1994).

In populations that do not possess the allele(s) that confer resistance prior to the application of the herbicide, the probability of acquiring the resistant allele is based on the mutation frequency and size of the population being selected; however, if certain individuals within the population already possess the required mutation, it will be selected for in subsequent generations more rapidly (Powles and Holtum 1994). The time scale at which the development of resistance occurs is dependent on the genetic diversity within a population, the genetic diversity of any individuals entering the population from other populations, and the intensity of selection caused by the herbicide (Powles and Holtum 1994).

The rate of evolution, from susceptible biotype to resistant biotype within a population also depends on the mode of inheritance governing the resistant allele(s), the number of genes which confer resistance to the herbicide, the reproductive strategy of the weed (the degree to which a species is out-crossing or selfing), the influence of gene flow, and the fitness level resistant biotypes have compared to their wild type relatives (Powles and Holtum 1994).

2.2.12. Mechanisms of Glyphosate Resistance

Several mechanisms of glyphosate resistance have evolved (Powles and Preston 2006). It has been reported that the EPSPS target site resistance to glyphosate is caused by a change to the hydrophobic Pro 106 amino acid, which causes a structural change in the active site (Sammons and Gaines 2014). Moderate levels of resistance, between 2 to 15 times susceptible levels, is caused by this target site mutation. (Sammons and Gaines 2014; Lee and Ngim 2000).

Non-target site resistance was first reported in Australia in *Lolium ridgidum* (Pratley *et al.* 1999) and the mechanism subsequently investigated by Powles and Preston 2006. In *Lollium* and other weeds including *Conyz canadensis*, low levels of resistance are conferred by a reduction in glyphosate translocation from the treated leaves. The exact biochemical mechanism remains elusive but the trait is inherited as an incomplete dominant nuclear inherited trait.

In 2006 Palmer amaranth (*Amaranthus palmeri*) was identified in the USA by Culpepper *et al.* (2000) as resistant to between 6.2 and 8 times the rates of glyphosate needed to kill susceptible populations. The mechanism of glyphosate resistance was later determined to be gene amplification. Resistance was conferred by multiple duplications (60-100 fold) of the EPSPS gene on multiple chromosomes (Douglas Sammons and Gaines 2014; Gaines *et al.* 2010). Further research found this amplification was linked to “miniature inverted repeat transposable elements (MITEs)” next to the EPSPS gene copies in resistant individuals (Gaines *et al.* 2013). Vila-Aiub *et al.* (2014) reported that there was no fitness difference between resistant and susceptible plants.

In 2014, Jugulam *et al.* reported the mechanism and inheritance of glyphosate resistance in kochia. Glyphosate-resistant kochia was collected in Kansas in 2007, 2010, and 2012. The arrangement of EPSPS gene copies within the chromosome was determined. Plants collected in 2007 were shown to have an average of 9 EPSPS copies. By 2012 populations collected were shown to have between 12 and 16 copies, and a corresponding increase in tolerance of the glyphosate herbicide. Multiple copies of the gene encoding for EPSPS were located only on two chromosomes. This is different from the EPSPS amplification seen in *Amaranthus palmeri*, where EPSPS copies were distributed on many chromosomes across the genome (Gaines *et al.* 2010). Jugulam *et al.* (2014) reported that tandem amplification of a target gene is the basis for glyphosate resistance in kochia. They suggested that the mechanism of the EPSPS amplification was due to unequal crossover, as the EPSPS gene occurs at the telomere region of the chromosome. Sammons and Gaines (2014) reported that in several instances, when glyphosate selection was removed from subsequent plant lines, duplicate copies of EPSPS were not maintained; suggesting that the duplicated genes were either unstable or conferred a fitness penalty to the plant. Subsequent research into duplicate copies of EPSPS in *Palmer amaranth* by

Giacomini (2015) found no evidence of a fitness cost to the plant and, although there has been no research done to date on fitness penalties in kochia, it is reasonable to assume this would be true for any species exhibiting this type of gene amplification. The gradual development of resistance by multiple copies accumulating suggests that reduction in herbicide rates due to incomplete spray coverage and less-than-required spray volumes will result in subsequent generations becoming more resistant to glyphosate (Jugulam *et al.* 2014) and the long term-loss of glyphosate as a control option.

2.2.13. Summary

The selection of herbicide resistance to multiple herbicides including glyphosate has initiated research into alternative approaches to weed control, including new herbicides (reviewed in (Burton *et al.* 2014; Shaner and Beckie 2014), alternative time of application of herbicides, mechanical destruction of weed seeds (Walsh and Newman 2007; Walsh *et al.* 2013; Walsh and Powles 2014), RNAi technologies targeting specific herbicide resistant mechanisms (reviewed in (Shaner and Beckie 2014), and biological control. However, ultimately the appropriate use of the tools for chemical weed control is the responsibility of the grower. Unfortunately with widely dispersed weed such as kochia, the poor decisions of one grower can become the problem weed for the community.

2.3. Literature Cited

Baker, D. V., K. G. Beck, B. J. Bienkiewicz, and L. B. Bjostad. 2008. Forces necessary to initiate dispersal for three tumbleweeds. Invasive Plant Science and Management 1:59-65.

Baylis, A. D. 2000. Why glyphosate is a global herbicide: Strengths, weaknesses and prospects. Pest Manag. Sci. 56:299-308.

Becker, D. A. 1978. Stem abscission in tumbleweeds of the chenopodiaceae: Kochia. Am. J. Bot. 65:375-383.

Beckie, H., K. Harker, L. Hall, S. Warwick, A. Légère, P. Sikkema, G. Clayton, A. Thomas, J. Leeson, and G. Séguin-Swartz. 2006. A decade of herbicide-resistant crops in Canada. Can. J. Plant Sci. 86:1243-1264.

Beckie, H. J., R. E. Blackshaw, R. Low, L. M. Hall, C. A. Sauder, S. Martin, R. N. Brandt, and S. W. Shirriff. 2012. Glyphosate-and acetolactate synthase inhibitor-resistant kochia (*Kochia scoparia*) in western Canada. Weed Sci. 61:310-318.

Beckie, H. J., R. H. Gulden, N. Shaikh, E. N. Johnson, C. J. Willenborg, C. A. Brenzil, S. W. Shirriff, C. Lozinski, and G. Ford. 2015. Glyphosate-resistant kochia (*Kochia scoparia* L. Schrad.) in Saskatchewan and Manitoba. Can. J. Plant Sci. 95:1-5.

Beckie, H. J., S. I. Warwick, C. A. Sauder, C. Lozinski, and S. Shirriff. 2011. Occurrence and molecular characterization of acetolactate synthase (ALS) inhibitor-resistant kochia (*Kochia scoparia*) in western Canada. Weed Technol. 25:170-175.

Benbrook, C. M. 2012. Impacts of genetically engineered crops on pesticide use in the US-the first sixteen years. Environmental Sciences Europe 24:1-13.

Brookes, G. and P. Barfoot. 2014. Economic impact of GM crops: The global income and production effects 1996–2012. GM Crops & Food 5:65-75.

Bullied, W. J., A. M. Marginet, and R. C. Van Acker. 2003. Conventional- and conservation-tillage systems influence emergence periodicity of annual weed species in canola. Weed Sci. 51:886-897.

Burton, N., S. Shirriff, and H. Beckie. 2014. Response of glyphosate-resistant kochia (*Kochia scoparia* L. Schrad.) to alternative herbicides. Can. J. Plant Sci. 94(8):1407-1411.

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

Culpepper, A. S., A. C. York, R. B. Batts, and K. M. Jennings. 2000. Weed management in glufosinate-and glyphosate-resistant soybean (Glycine max). Weed Tech 14:77-88.

Davis, R., W. Johnson, and F. Wood. 1967. Weed root profiles. Agron. J. 59:555-556.

Dill, G. M., C. A. CaJacob, and S. R. Padgette. 2008. Glyphosate-resistant crops: Adoption, use and future considerations. Pest Manag. Sci. 64(4):326-331.

Dill, G. M. 2005. Glyphosate-resistant crops: History, status and future. Pest Manag. Sci. 61:219-224.

Duke, S. O. and S. B. Powles. 2008. Glyphosate: A once-in-a-century herbicide. Pest Manag. Sci. 64:319-325.

Duke, S. O. and S. B. Powles. 2009. Glyphosate-resistant crops and weeds: Now and in the future. AgBioForum 12:346-357.

Duke, S. O. 2005. Taking stock of herbicide-resistant crops ten years after introduction. Pest Manag. Sci. 61:211-218.

Eberlein, C. and Z. Fore. 1984. Kochia biology. Weeds Today. 15:5-7.

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

Franz J. E., M. K. Mao, and J. A. Sikorski. 1997. Glyphosate: A Unique Global Herbicide, ACS Monograph No. 189. Am. Chem. Soc.

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.

Gaines, T. A., A. A. Wright, W. T. Molin, L. Lorentz, C. W. Riggins, P. J. Tranel, R. Beffa, P. Westra, and S. B. Powles. 2013. Identification of genetic elements associated with EPSPS gene amplification. PLoS ONE 8:1-10.

Gaines, T. A., W. Zhang, D. Wang, B. Bukun, S. T. Chisholm, D. L. Shaner, S. J. Nissen, W. L. Patzoldt, P. J. Tranel, A. S. Culpepper, T. L. Grey, T. M. Webster, W. K. Vencill, R. D. Sammons, J. Jiang, C. Preston, J. E. Leach, and P. Westra. 2010. Gene amplification confers glyphosate resistance in *Amaranthus palmeri*. Proc. Natl. Acad. Sci. U. S. A. 107:1029-1034.

Giacomini, D. A. 2015. EPSPS Gene Duplication in *Palmer Amaranth:* Relative Fitness, Inheritance, and Duplication Mechanism of the Glyphosate Resistance Trait.Colorado Denver: Colorado State University.

Givens, W. A., D. R. Shaw, W. G. Johnson, S. C. Weller, B. G. Young, R. G. Wilson, M. D. Owen, and D. Jordan. 2009. A grower survey of herbicide use patterns in glyphosate-resistant cropping systems. Weed Technol. 23:156-161.

Grossbard E. and D. Atkinson. 1985. The Herbicide Glyphosate. Butterworths & Co., London, UK, 490 pp.

Hall, L., H. Beckie, R. H. Low, S. Shirriff, N. Kimmel, C. Neeser, and R. Blackshaw. 2014. Short communication: Survey of glyphosate-resistant kochia (*Kochia scoparia* L. Schrad.) in Alberta. Can. J. Plant Sci. 94:127-130.

Heap I. 2015. The International Survey of Herbicide Resistant Weeds. www.weedscience.org. Accessed: January 24, 2015.

Herrmann, K. M. and L. M. Weaver. 1999. The shikimate pathway. Annual Review of Plant Biology. 50:473-503.

Holm F. A. and E. N. Johnson. 2010. A brief history of herbicide use in western Canada. Pages 77-79 *in* 17th Australasian Weeds Conference. New Frontiers in New Zealand: Together we can Beat the Weeds. Christchurch, New Zealand, 26-30 September, 2010. New Zealand Plant Protection Society.

Jugulam, M., K. Niehues, A. S. Godar, D. H. Koo, T. Danilova, B. Friebe, S. Sehgal, V. K. Varanasi, A. Wiersma, P. Westra, P. W. Stahlman, and B. S. Gill. 2014. Tandem amplification of a chromosomal segment harboring 5-enolpyruvylshikimate-3-phosphate synthase locus confers glyphosate resistance in *Kochia scoparia*. Plant Physiol. 166:1200-1207.

Khan, M. A., B. Gul, and D. J. Weber. 2001. Influence of salinity and temperature on the germination of *Kochia scoparia*. Wetlands Ecol. Manage. 9:483-489.

Lee, B., M. Kim, R. R. C. Wang, and B. Waldron. 2005. Relationships among 3 kochia species based on PCR-generated molecular sequences and molecular cytogenetics. Genome 48:1104-1115.

Lee, L. J. and J. Ngim. 2000. A first report of glyphosate-resistant goosegrass (*Eleusine indica* (L) Gaertn) in Malaysia. Pest Manag. Sci. 56:336-339.

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, Saskatchewan: Agriculture and Agri-Food Canada.

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

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.

Mulugeta, D., B. D. Maxwell, P. K. Fay, and W. E. Dyer. 1994. Kochia (*Kochia scoparia*) pollen dispersion, viability and germination. Weed Sci. 42:548-552.

Nalewaja, J., B. Devilliers, and R. Matysiak. 1996. Surfactant and salt affect glyphosate retention and absorption. Weed Res. 36:241-247.

Powles S. B. and J. A. Holtum. 1994. Herbicide Resistance in Plants: Biology and Biochemistry. Lewis Publishers Inc., Boca Raton, FL.

Powles, S. B. and C. Preston. 2006. Evolved glyphosate resistance in plants: Biochemical and genetic basis of resistance 1. Weed Technol. 20:282-289.

Powles, S. B., D. F. Lorraine-Colwill, J. J. Dellow, and C. Preston. 1998. Evolved resistance to glyphosate in rigid ryegrass (*Lolium rigidum*) in Australia. Weed Sci. 46:604-607.

Pratley, J., N. Urwin, R. Stanton, P. Baines, J. Broster, K. Cullis, D. Schafer, J. Bohn, and R. Krueger. 1999. Resistance to glyphosate in *Lolium rigidum*. I. Bioevaluation. Weed Sci. 405-411.

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

Saari, L. L., J. C. Cotterman, and M. M. Primiani. 1990. Mechanism of sulfonylurea herbicide resistance in the broadleaf weed, *Kochia scoparia.* Plant Physiol. 93:55-61.

Salhoff, C. R. and A. R. Martin. 1986. *Kochia scoparia* growth response to triazine herbicides. Weed Sci. 40-42.

Sammons, D.R. and T. A. Gaines. 2014. Glyphosate resistance: State of knowledge. Pest Manag. Sci. 70(9):1367:1377.

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

Shaner, D. L. and H. J. Beckie. 2014. The future for weed control and technology. Pest Manag. Sci. 70:1329-1339.

Simonsen, L., I. S. Fomsgaard, B. Svensmark, and N. H. Spliid. 2008. Fate and availability of glyphosate and AMPA in agricultural soil. Journal of Environmental Science and Health Part B 43:365-375.

Sprankle, P., W. Meggitt, and D. Penner. 1975. Adsorption, mobility, and microbial degradation of glyphosate in the soil. Weed Sci. 229-234.

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

Starcevic, A., S. Akthar, W. C. Dunlap, J. M. Shick, D. Hranueli, J. Cullum, and P. F. Long. 2008. Enzymes of the shikimic acid pathway encoded in the genome of a basal metazoan, nematostella vectensis, have microbial origins. Proceedings of the National Academy of Sciences 105:2533-2537.

Székács, A. and B. Darvas. 2012. Forty years with glyphosate. Herbicides–properties, Synthesis and Control of Weeds. Ed.Hasaneen, MNAE-G., InTech, Croatia 247-284.

Tranel, P. J. and T. R. Wright. 2009. Resistance of weeds to ALS-inhibiting herbicides: What have we learned? Weed Sci. 50:700-712.

Vila-Aiub, M.M. 2014. No fitness cost of glyphosate resistance endowed by massive EPSPS gene amplification in Amaranthus palmeri. Planta. 239(4):793-801.

Waite J. C. 2008. Glyphosate Resistance in Kochia. Masters of Science dissertation. Manhattan Kansas: Kansas State University.

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

Walsh, M. J. and S. B. Powles. 2014. High seed retention at maturity of annual weeds infesting crop fields highlights the potential for harvest weed seed control. Weed Technol. 28:486-493.

Walsh, M. and P. Newman. 2007. Burning narrow windrows for weed seed destruction. Field Crops Res. 104:24-30.

Walsh, M., P. Newman, and S. Powles. 2013. Targeting weed seeds in-crop: A new weed control paradigm for global agriculture. Weed Technol. 27:431-436.

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

Woodburn, A. T. 2000. Glyphosate: Production, pricing and use worldwide. Pest Manag. Sci. 56:309-312.

Chapter Three: A comparison of pre-seeding herbicides for control of *Kochia scoparia*

3.1. Introduction

Kochia scoparia is the 10th most abundant weed found on arable fields in the Canadian prairies. It is well adapted to arid and semi-arid regions, saline soils that limit crop growth, and is tolerant of acidic soils (Friesen *et al.* 2009). Kochia is a weed of agricultural fields, ruderal areas, and rangeland, facilitated by tumbleweed seed dispersal. As the plant reaches maturity, an abscission zone forms at the base of the plant. Wind speeds of between 40 and 48 km h^{-1} cause the abscission to break at the base of the stem, allowing the mature kochia to deposit its seeds across large distances as a tumbleweed (Becker 1978).

A typical kochia plant may produce between 2,000 and 30,000 seeds per plant (Friesen *et al.* 2009). Kochia seed has low innate seed dormancy, with seed viability decreasing significantly after 4 months in the soil (Zorner *et al.* 1984). Kochia is an early emerging plant, germinating after 50 GDD (Bullied *et al.* 2003; Schwinghamer and Van Acker 2008), with additional flushes of new seedlings continuing throughout the growing season. Abundance, early emergence and a lack of seed dormancy result in large numbers of kochia seedlings prior to crop emergence. In the absence of control by glyphosate and to reduce the selection of GR kochia, alternative pre-seed herbicides are required to provide control of kochia in cereal, pulse, and oilseed crops.

Glyphosate-resistant kochia was identified in Kansas in 2007 (Waite 2008), and by 2015 was reported in eight U.S. states (Heap 2015). The first reported incidence of kochia with resistance to multiple herbicide modes of action (ALS-inhibitors and glyphosate) in Canada was in southern Alberta in 2011. Three populations were identified on chem-fallow fields, with an additional seven populations identified during a survey later that year within a 20-km radius of the initial sites (Appendix 1). A subsequent survey was conducted in southern Alberta in 2012 to determine the distribution and abundance of the GR biotype that was identified in 13 additional populations (4% of fields surveyed) (Appendix 2). Further delineation was required to determine the geographical extent of GR kochia in Canada, and concurrent surveys were conducted in Saskatchewan and Manitoba in 2013. Seventeen of the 342 populations sampled in Saskatchewan and 2 of the 283 populations sampled in Manitoba were found to be glyphosate resistant (Beckie *et al.* 2015). In areas where kochia is common and glyphosate has been used repetitively over many years, glyphosate resistance should be a key consideration for growers making decisions on herbicides for kochia control.

3.2. Materials and Methods

3.2.1. Site Description

Six trials were conducted in Saskatchewan and Alberta in 2011 and 2012 to determine if herbicides applied pre-seeding would have a similar effect as that of glyphosate on kochia biomass. In 2011, trials were established in a naturally occurring kochia population at the Agriculture and Agri-food Canada research station in Scott, SK (52°21’38”N 108°50’15”W) and in a manually established trial in Ellerslie, AB (53°25’37”N 113°32’45”W). In 2012 trials were established in a naturally occurring kochia infestation near Lethbridge, AB (49°45’28”N 112°55’26”W) and manually established in trials near Ellerslie, AB (53°25’13”N 113°32’29”W), Olds, AB (51°45’59”N 114°00’56”W) and St. Albert, AB (53°41’34”N 113°37’16”W). All trials were conducted in fields that had crop stubble from previous years. Ten composite soil samples from a depth of 0 to 6 inches were taken from each trial using a hand soil auger and submitted for soil analysis.

In trials where naturally occurring populations of kochia were not present, kochia was seeded manually by hand, spreading the seed evenly across the entire trial area. Manually seeded kochia was obtained from a natural population near Brooks, Alberta in 2010. Although this seed was not tested for glyphosate resistance as resistance at this time had not been reported. The lack of resistance was confirmed in trials as the glyphosate treatment alone was effective. Herbicides were applied uniformly at all sites when kochia seedlings were at the 3-leaf stage (BBCH scale 13-15), when they were large enough to intercept the herbicide spray. One week after herbicide application, spring wheat (Stettler) was direct seeded using a Wintersteiger Plotseed XXL with no-till openers at a rate of 200 seeds m^{-2} using a drill seeder with 6 inch row spacing and a depth of 2 inches.

Trials were designed as a randomized complete block design with four replicates. Plot size was 2 m by 7 m. Three 0.25 m^2 quadrats were randomly established in each plot prior to herbicide application, at the front, middle and back of each plot. Locations, soil properties, planting date, herbicide application date, and density at time of application are listed in Table 3-1.

3.2.2. Herbicide Treatments

Herbicide treatments were selected from contact or residual herbicides that could be applied pre-seeding: pyrasulfotole + bromoxynil, 2,4D 700 ester, saflufenacil, carfentrazone-ethyl, fluroxypyr + MCPA, bromoxynil + 2,4D 700 ester, diquat, glufosinate ammonium, dicamba and diflufenzopyr + dicamba, and were compared to glyphosate (Tables 3-2). Clethodim (0.125 L ha^{-1}) was applied as a maintenance herbicide in Lethbridge to control wild oats.

All herbicide treatments were applied using a backpack sprayer with CO_2 calibrated to deliver 100 L ha^{-1} water volume at 275 kPa. The boom was 1.5 m long and equipped with four Air Bubble Jet low drift 80015VS nozzles. Herbicides were applied 38 cm above the plant canopy.

3.2.3. Effect of Herbicide Treatments

Kochia control was visually assessed on a scale of 0 (no control) to 100% (plant death) at 1, 2, and 4 weeks after application (WAA). Four WAA all kochia vegetation from within each quadrat was clipped at the soil surface, and was placed into cloth bags. The bags containing the kochia vegetation was dried at 50° C in an air oven for 60 hours, and weighed to determine the biomass.

3.2.4. Statistical Analysis

Data was analyzed in SAS software (ver. 9.3 SAS Institute, Inc, Cary, NC). Biomass and visual data were analyzed using the PROC MIXED procedure for ANOVA analysis. Variances were divided into random replication (block, plot, and quadrat) and fixed effects (herbicide treatment). Data was tested for homogeneity of variance, although the data was not normal. Arcsine, logarithmic and square root transformations of the data were explored in an attempt to normalize the data; however, in the absence of normal data, untransformed data was used. The Z-test was used to test the significance of the random interactions and the F-test was used to test the significance of fixed effects. Plant count data was analyzed using the PROC GENMOD, with treatment as a continuous variable. Data was tested for homogeneity of variance, although the data had negative binomial distribution. Arcsine, logarithmic and square root transformations of the data were explored in an attempt to normalize the data; however, in the absence of normal data, untransformed data was used. Means were separated using a Dunnett-Hsu adjustment at $P<0.05$.

3.3. Results

Sites varied in soil parameters including organic matter, pH and EC, and herbicides varied in their soil residual activity (Table 3-1). The average monthly temperatures of the sites were similar but the amount of rainfall sites received varied widely (Table 3-2), and may have influenced the available soil moisture and possibly the activity of soil active herbicides. Because soil organic matter and soil moisture may have influenced the activity of pyrasulfotole + bromoxynil, saflufencacil, and to a lesser extent the auxinic herbicides, sites were analysed separately. Glyphosate, carfentrazone-ethyl, diquat and glufosinate ammonium have no soil residual activity and thus are less like to be affected by soil parameters. Kochia susceptibility to herbicides may have been influenced by weather, including humidity and soil moisture.

Sites also varied in the density and origin of kochia populations; at Scott, and Lethbridge, natural populations were present but populations were manually established by seeding in Ellerslie (2011 and 2012), Olds (2012), and St. Albert (2012). Natural stands were more spatially variable but manually seeded stands were slower to emerge and resulted in delayed herbicide application (Table 3-1).

At Scott in 2011, soil organic matter was low (2.9%) (Table 3-1), and substantial rainfall was received in June (307-fold the long term average) (Table 3-2). Visually 2-4D, 700 ester, saflufenacil, and bromoxynil + 2,4D – 700 ester performed as well as glyphosate (Table 3-4). In untreated controls, kochia biomass averaged 54 g m^{-2} 4 WAA while averaging 11 g m^{-2} 4 WAA in plots treated with glyphosate. No herbicide significantly reduced kochia biomass (Table 3-5). This is possibly because of the recovery of kochia in response to rainfall (Table 3-2).

Ellerslie had high soil organic matter (12 and 11.2% in 2011 and 2012, respectively) (Table 3-1) and in June and July of 2011 received rainfall 147- and 158-fold the long-term average (Table 3-2). At Ellerslie in 2011, kochia densities prior to application averaged 99 plants m^{-2} in untreated controls at the time of application and had increased to 111 plants m^{-2} 4 WAA (Table 3-3). Kochia biomass 4 WAA was 36 g m^{-2} in the untreated checks (Table 3-6). Visually, pyrasulfotole + bromoxynil, carfentrazone-ethyl and fluroxypyr + MCPA ester performed as well as glyphosate (Table 3-4). Most herbicides, with the exception of dicamba, diquat, and glufosinate ammonium, had similar reductions in biomass as the glyphosate treatment (Table 3-6).

At Ellerslie in 2012, rainfall was lower than normal (Table 3-2). Kochia densities at the time of application averaged 46 plants m^{-2} in the untreated checks. However, by 4 WAA densities had increased to 276 plants m^{-2} (Table 3-3), suggesting that kochia populations were sprayed early relative to peak emergence. Kochia biomass in untreated checks averaged 92 g m^{-2}, while plots treated with glyphosate averaged 9 g m^{-2} 4 WAA (Table 3-7). At this site all herbicides, with the exception of diquat, were as effective as glyphosate in reducing kochia biomass (Table 3-7).

Similar to Ellerslie, the St. Albert 2012 site had high organic matter (11.9%) and below normal rainfall that may have influenced herbicide efficacy. Kochia densities at the time of herbicide application averaged 102 plants m^{-2} and had densities of 109 plants m^{-2} at the end of assessments, 4 WAA (Table 3-3). In untreated checks kochia biomass averaged 53 g m^{-2} while averaging 17 g m^{-2} 4WAA in plots treated with glyphosate. Pyrasulfotole + bromoxynil, 2,4D - 700 ester, saflufencacil, carfentrazone-ethyl, fluroxypyr + MCPA Ester, and bromoxynil + 2,4D - 700 ester all provided similar control to glyphosate (Table 3-8).

In Olds soil organic matter was 8.1% and rainfall near historical averages. Kochia densities in the untreated checks averaged 187 plants m^{-2} at the time of herbicide application and increased to 309 plants m^{-2} 4 WAA (Table 3-3), suggesting kochia populations were sprayed early relative to peak emergence. Kochia biomass exceeded 273g m^{-2} 4 WAA in untreated checks (Table 3-9). In this site only fluroxpyr + MCPA provided similar control as glyphosate (Table 3-9). Pyrasulfotole + bromoxynil, saflufencacil, carfentrazone-ethyl, bromoxynil + 2,4D, diquat and glufosinate ammonium provided similar efficacy to each other and 2,4-D, dicamba and diflufenzopyr + dicamba were similar to the untreated checks (Table 3-9).

In Lethbridge in 2012 soil organic matter was also low (3%). Kochia densities in the untreated checks averaged 191 plants m^{-2} at the time of herbicide application and increased to 701 plants m^{-2} (Table 3-3), suggesting kochia populations were sprayed early relative to peak emergence. Kochia biomass averaged 28 g m^{-2} in the untreated check 4 WAA. No herbicides were effective at this site (Table 3-10).

3.4. Discussion

Pre-seeding herbicides are limited to products that either have no residual activity or are safe to the crop. In addition to products currently registered for use pre-seeding (carfentrazone, 2,4-D, bromoxynil), we included products with kochia efficacy that are generally applied in crop (fluroxypyr, diquat, glufosinate, dicamba and diflufenzapyr + dicamba). Group 10 and 22 (glufosinate and diquat) have no residual activity and can be used for vegetation control in the absence of a planted crop. Group 4 and 19 products (fluroxypyr, dicamba and diflufenzapyr + dicamba) were included because of the limited resistance to this mode of action. However, auxinic herbicides may affect cereal crop when applied pre-seeding or early in crop. Further research on crop tolerance should be considered for fluroxypyr applied pre-seeding.

Treatment effects across all sites were variable; no herbicide was effective at every trial in the study and, in the case of the trials in Lethbridge and Scott, no herbicide was effective at reducing kochia biomass compared to the untreated check. Lack of control could not be explained by rainfall following herbicide application. It is possible that the high precipitation in Scott in 2011 allowed the kochia to overcome the herbicide effects over the course of the study, while at Lethbridge the high populations of kochia, and subsequent flush of weeds after the herbicide application may have masked herbicidal effects (Figure 3-1).

Of the other four trials in the study, fluroxypyr was as effective at controlling kochia biomass as glyphosate in all trials; bromoxynil + 2,4D, saflufenacil, carfentrazone-ethyl, and pyrasulfotole + bromoxynil controlled kochia biomass as effectively as glyphosate in 3 trials, diflufenzopyr + dicamba controlled kochia biomass as effectively as glyphosate in 2 trials, and glufosinate and dicamba were as effective at controlling kochia biomass as effectively as glyphosate in 1 trial each. These results differ from a greenhouse study looking at the response of GR kochia, which reported dicamba as the least effective herbicide at suppressing kochia biomass (Burton *et al.* 2014).

It is likely the differences between sites, such as soil organic matter, rainfall, and the emergence timing and flushes of kochia compared to the spray timing, influenced herbicide control of kochia. Effective tank mixes (a combination of two or more herbicides which can both control kochia and possess different sites of action (Wrubel and Gressel 1994) will have the greatest influence on the control of kochia populations and seed production, slowing the selection of GR kochia. While not consistently effective, tank mixes of glyphosate and bromoxynil + 2,4D, saflufenacil, carfentrazone-ethyl, and pyrasulfotole + bromoxynil should be considered for pre-seeding control of kochia in areas where glyphosate resistance has not been identified.

These results are similar to those found by Kumar and Jha (2015) who found that herbicides applied pre-seeding could serve as a foundation for kochia control. In their experiment, fourteen treatments containing a total of a combination of 18 herbicides were evaluated for visual kochia efficacy at 8, 10, and 12 weeks after treatment. Although the treatments and visual assessments cannot be directly compared, the study supports the idea that effective tank mixes will have the greatest influence on kochia populations that emerged early in the growing season.

Table 3-1. Location, soil characteristics, spray date and density for pre-seeding studies in 2011 and 2012.

Location	Year	Soil texture	Soil OM	Soil pH	EC	Application date	Kochia density
			%		dS m^{-1}		# m^{-2}
Scott, SK[b]	2011	Loam	2.9	5	n/a	June 1	32
Ellerslie, AB[a]	2011	Clay loam	12	6.3	0.46	June 13	99
Ellerslie, AB[a]	2012	Clayloam	11.2	71	0.44	June 14	46
St. Albert, AB[a]	2012	Clay	11.9	7	0.30	June 12	102
Olds, AB[a]	2012	Loam	8.1	7.6	2.04	June 11	187
Lethbridge, AB[b]	2012	Sandy clay	3	8.1	0.62	May 18	191

[a] Kochia seeded into trials

[b] Trials seeded into natural kochia populations

Table 3-2. Mean monthly air temperatures and precipitation during May, June and July at trial locations for pre-seeding studies in 2011 and 2012.

Location	Year	Air Temperature			Precipitation		
		---------C (Long term average)---------			----- mm (% of Long term average) -----		
		May	June	July	May	June	July
Scott, SK[b]	2011	10.1 (10.8)	14.4 (15.3)	17.0 (17.1)	30.8 (84.0)	190.2 (307.0)	76.2 (95.0
Ellerslie, AB[a]	2011	11.0 (10.6)	15.1 (14.6)	17.2 (17.0)	15.6 (31.0)	128.2 (147.0)	150.4 (158.
Ellerslie, AB[a]	2012	11.1 (10.6)	15.1 (14.6)	17.2 (17.0)	37.7 (76.0)	72.4 (83.0)	104.8 (110.
St. Albert, AB[a]	2012	10.9 (10.6)	14.8 (14.6)	16.9 (17.0)	43.3 (86.0)	75.7 (87.0)	90.2 (95.0
Olds, AB[a]	2012	9.3 (9.4)	13.3 (13.3)	15.7 (15.4)	57.7 (54.6)	91.6 (89.6)	81.4 (87)
Lethbridge, AB[b]	2012	11.0 (11.3)	15.2 (15.5)	18.1 (18.0)	50.9 (49.4)	78.4 (63.0)	39.0 (47.5

[a] Kochia was seeded into trials

[b] Trials took place in natural kochia populations

Weather data was obtained from Alberta Agriculture and Forestry's Current and Historical Alberta Weather Station Data Viewer located at: http://agriculture.alberta.ca/acis/alberta-weather-data-viewer.jsp

Table 3-3. Kochia plant densities, at time of herbicide application and 4 weeks after application for all study sites.

Treatment	Rate	Ellerslie / 2011			Ellerslie / 2012			Lethbridge / 2012			Olds / 2012			St. Albert / 2012		
	g ai ha^{-1}	W 0	W 4	Pr>|z|	W 0	W 4	Pr>|z|	W 0	W 4	Pr>|z|	W 0	W 4	Pr>|z|	W 0	W 4	Pr>|z|
		# m^{-2}			# m^{-2}			# m^{-2}			# m^{-2}			# m^{-2}		
Untreated Check		99	111	0.9434	46	276	<0.0001	191	701	<0.0001	187	309	<0.0001	102	109	0.4473
Glyphosate control	900	98	5	<0.0001	42	5	<0.0001	86	102	0.7248	125	9	<0.0001	115	1	<0.0001
Pyrasulfotole + bromoxynil[a]	205	86	3	<0.0001	47	24	<0.0001	170	377	<0.0001	197	114	<0.0001	44	7	<0.0001
2,4D - 700 ester	560	90	16	<0.0001	44	118	<0.0001	146	571	<0.0001	177	330	<0.0001	38	14	<0.0001
Saflufenacil	18	108	1	<0.0001	64	29	<0.0001	109	192	<0.0001	234	89	<0.0001	55	4	0.0008
Carfentrazone-ethyl	8.9	97	14	<0.0001	52	97	<0.0001	146	259	0.0003	285	360	0.1188	108	80	0.0001
Fluroxypyr + MCPA ester	107 + 556	167	10	<0.0001	48	61	0.3148	178	318	0.0005	118	137	0.4483	86	23	<0.0001
Bromoxynil + 2,4D - 700 ester	280.3 + 280.3	143	6	<0.0001	52	106	0.0002	90	230	<0.0001	135	147	0.9661	47	8	<0.0001
Diquat[b]	637	72	1	<0.0001	58	15	0.8254	177	56	0.7027	200	23	<0.0001	75	15	0.0349
Glufosinate ammonium[c]	300	27	24	<0.0001	8	14	0.0004	43	221	0.5027	47	26	<0.0001	22	53	<0.0001
Dicamba	139	107	24	0.083	50	14	<0.0001	130	221	0.5893	217	26	<0.0001	42	53	0.8076
Diflufenzopyr + dicamba[a]	28.6 + 71.4	185	112	0.0008	36	233	<0.0001	88	171	0.0181	174	232	0.0437	58	55	0.5865

W 0 = plant counts prior to herbicide application

W 4 = plant counts at 4 WAA

[a] Merge (0.5% vol/vol)

[b] AMS (1.25% vol/vol)

[c] Agsurf (0.1% vol/vol)

Table 3-4. Visual estimates of kochia control at 4 WAA for all study sites.

Treatment	Rate		Scott/2011		Ellerslie/2011		Ellerslie/2012		St. Albert/2012		Lethbridge/2012		Olds/2012
	g ai ha^{-1}	WAA	VA	S.E.	VA	S.E.	VA	S.E.	VA	S.E.	VA	S.E.	VA
			-----	-----	-----	-----	-----	-----	%	-----	-----	-----	-----
		1	0^{a}	7.1	0^{a}	7.1	0^{a}	7.1	0^{a}	7.1	0^{a}	7.1	0^{a}
Untreated check		2	0^{a}	14.1	0^{a}	14.1	0^{a}	14.1	0^{a}	14.1	0^{a}	14.1	0^{a}
		4	0^{a}	0.0	0^{a}	0.0	0^{a}	0.0	0^{a}	0.0	0^{a}	0.0	0^{a}
		1	30^{b}	7.1	30^{b}	7.1	94^{c}	7.1	94^{c}	7.1	40^{b}	7.1	83^{c}
Glyphosate control	900	2	100^{c}	14.1	100^{c}	14.1	94^{c}	14.1	94^{c}	14.1	83^{c}	14.1	99^{c}
		4	83^{c}	0.0	100^{c}	0.0	98^{c}	0.6	99^{c}	0.0	92^{c}	3.0	95^{c}
		1	75^{b}	7.1	75^{b}	7.1	85^{c}	7.1	89^{c}	7.1	52^{b}	7.1	90^{c}
Pyrasulfotole + bromoxynil[e]	205	2	98^{c}	14.1	96^{c}	14.1	93^{c}	14.1	95^{c}	14.1	59^{b}	14.1	90^{c}
		4	46^{b}	6.4	96^{c}	6.4	81^{c}	6.4	88^{c}	6.4	56^{b}	6.4	61^{b}
		1	18^{b}	7.1	18^{b}	7.1	45^{b}	7.1	50^{b}	7.1	39^{b}	7.1	68^{b}
2,4D - 700 ester	560	2	85^{c}	14.1	79^{b}	14.1	71^{b}	14.1	51^{b}	14.1	26^{b}	14.1	82^{b}
		4	86^{c}	6.4	73^{b}	6.4	50^{b}	6.4	73^{b}	6.4	39^{b}	6.4	50^{b}
		1	100^{c}	7.1	100^{c}	7.1	98^{c}	7.1	99^{c}	7.1	73^{b}	7.1	98^{c}
Saflufencacil	18	2	95^{c}	14.1	98^{c}	14.1	98^{c}	14.1	99^{c}	14.1	83^{c}	14.1	99^{c}
		4	86^{c}	6.4	83^{b}	6.4	92^{c}	6.4	99^{c}	6.4	64^{b}	6.4	84^{c}
		1	79^{b}	7.1	79^{b}	7.1	71^{b}	7.1	53^{b}	7.1	86^{c}	7.1	80^{b}
Carfentrazone-ethyl	8.9	2	65^{b}	14.1	98^{c}	14.1	78^{b}	14.1	58^{b}	14.1	79^{b}	14.1	91^{c}
		4	58^{b}	6.4	98^{c}	6.4	73^{b}	6.4	83^{b}	6.4	73^{b}	6.4	63^{b}
		1	26^{b}	7.1	25^{b}	7.1	60^{b}	7.1	73^{b}	7.1	50^{b}	7.1	81^{b}
Fluroxypyr + MCPA ester	107 + 556	2	65^{b}	14.1	92^{c}	14.1	80^{b}	14.1	83^{b}	14.1	78^{b}	14.1	96^{c}
		4	75^{b}	6.4	97^{c}	6.4	79^{b}	6.4	93^{c}	6.4	54^{b}	6.4	91^{c}
		1	86^{c}	7.1	87^{c}	7.1	78^{b}	7.1	65^{b}	7.1	38^{b}	7.1	81^{b}
Bromoxynil + 2,4D - 700 ester	280.3 + 280.3	2	78^{b}	14.1	67^{b}	14.1	76^{b}	14.1	70^{b}	14.1	61^{b}	14.1	90^{c}
		4	92^{c}	6.4	37^{a}	6.4	76^{b}	6.4	93^{c}	6.4	56^{b}	6.4	50^{b}
		1	94^{c}	7.1	93^{c}	7.1	40^{b}	7.1	20^{b}	7.1	58^{b}	7.1	90^{c}
Diquat[f]	637	2	91^{c}	14.1	90^{c}	14.1	40^{b}	14.1	23^{b}	14.1	76^{b}	14.1	91^{c}
		4	73^{b}	6.4	79^{b}	6.4	48^{b}	6.4	63^{b}	6.4	60^{b}	6.4	49^{b}
		1	45^{b}	7.1	42^{b}	7.1	46^{b}	7.1	50^{b}	7.1	80^{b}	7.1	88^{c}
Glufosinate ammonium[g]	300	2	80^{b}	14.1	73^{b}	14.1	68^{b}	14.1	53^{b}	14.1	87^{c}	14.1	95^{c}
		4	79^{b}	6.4	50^{b}	6.4	89^{c}	6.4	78^{b}	6.4	91^{c}	6.4	84^{c}
		1	5^{a}	7.1	16^{a}	7.1	40^{b}	7.1	35^{b}	7.1	30^{b}	7.1	25^{a}
Dicamba	139	2	50^{b}	14.1	40^{b}	14.1	45^{b}	14.1	43^{b}	14.1	79^{b}	14.1	43^{b}
		4	53^{b}	6.4	45^{b}	6.4	45^{b}	6.4	58^{b}	6.4	54^{b}	6.4	25^{a}
		1	18^{b}	7.1	23^{b}	7.1	39^{b}	7.1	45^{b}	7.1	60^{b}	7.1	69^{b}
Diflufenzopyr + dicamba[e]	28.6 + 71.4	2	71^{b}	14.1	70^{b}	14.1	65^{b}	14.1	46^{b}	14.1	79^{b}	14.1	69^{b}
		4	72^{b}	6.4	81^{b}	6.4	70^{b}	6.4	65^{b}	6.4	74^{b}	6.4	53^{b}

Means followed by the same letter are not significantly different to visual ratings of the same location and week of observation according to a Dunnett-HSU adjustment where $P < 0.05$

[e] Merge (0.5% vol/vol)

[f] AMS (1.25% vol/vol)

[g] Agsurf (0.1% vol/vol)

Table 3-5. Kochia biomass per m^{-2} at 4 WAA in Scott, Saskatchewan – 2011.

Treatment	Rate	Kochia biomass	S.E.
	g ai ha^{-1}	g m^{-2}	
Untreated check		54.17[a]	18.80
Glyphosate control	900	11.72[a]	18.80
Pyrasulfotole + bromoxynil[c]	205	19.94[a]	18.80
2,4D - 700 ester	560	42.21[a]	18.80
Saflufenacil	18	16.35[a]	18.80
Carfentrazone-ethyl	8.9	19.61[a]	18.80
Fluroxypyr + MCPA ester	107 + 556	20.51[a]	18.80
Bromoxynil + 2,4D - 700 ester	280.3 + 280.3	21.48[a]	18.80
Diquat[d]	637	11.45[a]	18.80
Glufosinate ammonium[e]	300	28.20[a]	18.80
Dicamba	139	18.99[a]	18.80
Diflufenzopyr + dicamba[c]	28.6 + 71.4	17.54[a]	18.80

Means followed by the same letter are not significantly different according to a Dunnett-HSU adjustment where $P < 0.05$

[c] Merge (0.5% vol/vol)

[d] AMS (1.25% vol/vol)

[e] Agsurf (0.1% vol/vol)

Table 3-6. Kochia biomass per m^{-2} at 4 WAA in Ellerslie, Alberta – 2011.

Treatment	Rate	Kochia biomass	S.E.
	g ai ha^{-1}	g m^{-2}	
Untreated check		36.72[a]	5.53
Glyphosate control	900	11.02[b]	5.53
Pyrasulfotole + bromoxynil[c]	205	10.27[b]	5.53
2,4D - 700 ester	560	11.00[b]	5.53
Saflufenacil	18	10.93[b]	5.53
Carfentrazone-ethyl	8.9	8.16[b]	5.53
Fluroxypyr + MCPA ester	107 + 556	8.54[b]	5.53
Bromoxynil + 2,4D - 700 ester	280.3 + 280.3	10.50[b]	5.53
Diquat[d]	637	17.17[ab]	5.53
Glufosinate ammonium[e]	300	13.28[ab]	5.53
Dicamba	139	20.89[a]	5.53
Diflufenzopyr + dicamba[c]	28.6 + 71.4	7.70[b]	5.53

Means followed by the same letter are not significantly different according to a Dunnett-HSU adjustment where $P < 0.05$

[c] Merge (0.5% vol/vol)

[d] AMS (1.25% vol/vol)

[e] Agsurf (0.1% vol/vol)

Table 3-7. Kochia biomass per m^{-2} at 4 WAA in Ellerslie, Alberta – 2012.

Treatment	Rate	Kochia biomass	S.E.
	g ai ha^{-1}	g m^{-2}	
Untreated check		92.06[a]	11.83
Glyphosate control	900	9.32[b]	11.83
Pyrasulfotole + bromoxynil[c]	205	24.62[b]	11.83
2,4D - 700 ester	560	22.57[b]	11.83
Saflufenacil	18	16.37[b]	11.83
Carfentrazone-ethyl	8.9	27.05[b]	11.83
Fluroxypyr + MCPA ester	107 + 556	7.01[b]	11.83
Bromoxynil + 2,4D - 700 ester	280.3 + 280.3	21.41[b]	11.83
Diquat[d]	637	47.25[a]	11.83
Glufosinate ammonium[e]	300	13.79[b]	11.83
Dicamba	139	15.72[b]	11.83
Diflufenzopyr + dicamba[c]	28.6 + 71.4	26.65[b]	11.83

Means followed by the same letter are not significantly different according to a Dunnett-HSU adjustment where $P < 0.05$

[c] Merge (0.5% vol/vol)

[d] AMS (1.25% vol/vol)

[e] Agsurf (0.1% vol/vol)

Table 3-8. Kochia biomass per m^{-2} at 4 WAA in St. Albert, Alberta – 2012.

Treatment	Rate	Kochia biomass	S.E.
	g ai ha^{-1}	g m^{-2}	
Untreated check		53.50[a]	10.37
Glyphosate control	900	17.42[b]	10.37
Pyrasulfotole + bromoxynil[c]	205	18.10[b]	10.37
2,4D - 700 ester	560	19.02[b]	10.37
Saflufenacil	18	16.08[b]	10.37
Carfentrazone-ethyl	8.9	3.98[b]	10.37
Fluroxypyr + MCPA ester	107 + 556	7.24[b]	10.37
Bromoxynil + 2,4D - 700 ester	280.3 + 280.3	17.32[b]	10.37
Diquat[d]	637	30.15[ab]	10.37
Glufosinate ammonium[e]	300	22.64[ab]	10.37
Dicamba	139	44.13[ab]	10.37
Diflufenzopyr + dicamba[c]	28.6 + 71.4	46.17[ab]	10.37

Means followed by the same letter are not significantly different according to a Dunnett-HSU adjustment where $P < 0.05$

[c] Merge (0.5% vol/vol)
[d] AMS (1.25% vol/vol)
[e] Agsurf (0.1% vol/vol)

Table 3-9. Kochia biomass per m^{-2} at 4 WAA in Olds, Alberta – 2012.

Treatment	Rate	Kochia biomass	S.E.
	g ai ha^{-1}	g m^{-2}	
Untreated check		273.86[a]	40.60
Glyphosate control	900	15.33[c]	40.60
Pyrasulfotole + bromoxynil[c]	205	48.37[b]	40.60
2,4D - 700 Ester	560	134.41[a]	40.60
Saflufenacil	18	32.69[b]	40.60
Carfentrazone-ethyl	8.9	96.62[b]	40.60
Fluroxypyr + MCPA ester	107 + 556	19.82[c]	40.60
Bromoxynil + 2,4D - 700 ester	280.3 + 280.3	31.05[b]	40.60
Diquat[d]	637	47.78[b]	40.60
Glufosinate ammonium[e]	300	46.29[b]	40.60
Dicamba	139	161.97[a]	40.60
Diflufenzopyr + dicamba[c]	28.6 + 71.4	205.95[a]	40.60

Means followed by the same letter are not significantly different according to a Dunnett-HSU adjustment where P < 0.05

[c] Merge (0.5% vol/vol)
[d] AMS (1.25% vol/vol)
[e] Agsurf (0.1% vol/vol)

Table 3-10. Kochia biomass per m^{-2} at 4 WAA in Lethbridge, Alberta – 2012.

Treatment	Rate	Kochia biomass	S.E.
	g ai ha^{-1}	g m^{-2}	
Untreated check		28.39[a]	9.83
Glyphosate control	900	19.97[a]	9.83
Pyrasulfotole + bromoxynil[c]	205	12.79[a]	9.83
2,4D - 700 ester	560	34.34[a]	9.83
Saflufenacil	18	12.57[a]	9.83
Carfentrazone-ethyl	8.9	16.23[a]	9.83
Fluroxpyr + MCPA ester	107 + 556	28.90[a]	9.83
Bromoxynil + 2,4D - 700 ester	280.3 + 280.3	43.73[a]	9.83
Diquat[d]	637	14.37[a]	9.83
Glufosinate ammonium[e]	300	9.82[a]	9.83
Dicamba	139	28.81[a]	9.83
Diflufenzopyr + dicamba[c]	28.6 + 71.4	17.55[a]	9.83

Means followed by the same letter are not significantly different according to a Dunnett-HSU adjustment where $P < 0.05$

[c] Merge (0.5% vol/vol)
[d] AMS (1.25% vol/vol)
[e] Agsurf (0.1% vol/vol)

Figure 3-1. Herbicide symptomology 2 WAA. A: Untreated; B: Glyphosate control; C: Pyrasulfotole + bromoxynil; D: 2,4D – 700 ester; E: Saflufenacil; F: Carfentrazone-ethyl; G: Fluroxypyr + MCPA ester; H: Bromoxynil + 2,4D – 700 ester; I: Diquat; J: Glufosinate ammonium; K: Dicamba; L: Diflufenzopyr + Dicamba.

3.5. Literature Cited

Becker, D. A. 1978. Stem abscission in tumbleweeds of the chenopodiaceae: Kochia. Am. J. Bot. 65:375-383.

Beckie, H. J., R. H. Gulden, N. Shaikh, E. N. Johnson, C. J. Willenborg, C. A. Brenzil, S. W. Shirriff, C. Lozinski, and G. Ford. 2015. Glyphosate-resistant kochia (K*ochia scoparia* L. Schrad.) in Saskatchewan and Manitoba. Can J. Plant Sci. 95:1-5.

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

Beckie, H. J., S. I. Warwick, C. A. Sauder, C. Lozinski, and S. Shirriff. 2011. Occurrence and molecular characterization of acetolactate synthase (ALS) inhibitor-resistant kochia (*Kochia scoparia*) in western Canada. Weed Technol. 25:170-175.

Bullied, W. J., A. M. Marginet, and R. C. Van Acker. 2003. Conventional-and conservation-tillage systems influence emergence periodicity of annual weed species in canola. Weed Sci. 51:886-897.

Burton, N., S. Shirriff, and H. Beckie. 2014. Response of glyphosate-resistant kochia (K*ochia scoparia* L. Schrad.) to alternative herbicides. Can. J. Plant Sci. 94(8), 1407-1411.

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.

Hall, L., H. Beckie, R. H. Low, S. Shirriff, N. Kimmel, C. Neeser, and R. Blackshaw. 2013. Short communication: Survey of glyphosate-resistant kochia (*Kochia scoparia* L. Schrad.) in Alberta. Can. J. Plant Sci. 94(1), 127-130.

Heap I. 2015. The International Survey of Herbicide Resistant Weeds. www.weedscience.org. Accessed: January 24, 2014.

Kumar, V. and P. Jha. 2015. Effective preemergence and postemergence herbicide programs for kochia control. Weed Technol. 29(1):24-34.

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

Waite J. C. 2008. Glyphosate Resistance in Kochia. Masters of Science dissertation. Manhattan Kansas: Kansas State University.

Wrubel, R. P. and J. Gressel. 1994. Are herbicide mixtures useful for delaying the rapid evolution of resistance? A case study. Weed Technol. 635-648.

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. 602-607.

Chapter Four: Post-harvest applied herbicides do not reduce kochia seed production or viability.

4.1. Introduction

Herbicide-resistant weeds are ubiquitous in major crops in western Canada, infesting 29% of western Canadian farmlands (Beckie *et al.* 2011). The reliance on non-selective herbicide in agricultural and vegetation management systems and the lack of cropping system diversity, including frequent use of GR crops, has selected for GR weeds in many parts of the world (Powles 2008). *Kochia scoparia* was the first weed identified as GR in western Canada (Beckie *et al.* 2013; Hall *et al.* 2013). Because 85% of kochia populations in western Canada were resistant to ALS inhibitors, many of the identified populations were also cross-resistant with ALS inhibitors (Beckie *et al.* 2013; Warwick *et al.* 2008). Glyphosate-resistant kochia populations from these studieswere associated with areas where chemical fallow was practiced. In chem-fallow, glyphosate may be used alone and repeatedly within a growing season to control relatively mature kochia. In addition, glyphosate may be used in the cropping phase as a pre-seeding herbicide for kochia control or, if GR crops are grown, as an in-crop herbicide.

Kochia is the 10th most abundant weed in western Canadian agricultural fields, common in ruderal areas and industrial sites in arid to semiarid regions (Leeson *et al.* 2005). Seedlings are numerous and emerge early (Friesen *et al.* 2009; Schwinghamer and Van Acker 2008), often before other weed seedlings and the planted crop. Plant morphology is variable, influenced by density, and varies from erect single stemmed plants grown in high density or competitive crops to spherical shapes. Kochia flowering is photoperiod controlled and is indeterminate, with seed set and maturation continuing until the plant is killed by frost. Although kochia is primarily self-pollinating, it has sufficient pollen-mediated gene flow to acquire multiple resistance genes or alleles (Warwick *et al.* 2008) and spread resistance over short distances (Mulugeta *et al.* 1994; Stallings *et al.* 1995). Kochia may produce between 2,000 to 30,000 seeds per plant (Stallings *et al.* 1995). At maturity an abscission layer forms at the plant base that permits it to break in the wind and tumble (Becker 1978). Seed dispersal can occur over long distances when spherical plants tumble across the landscape, whereas erect plants are less likely to be widely dispersed. Kochia seed has little dormancy, with most germinating in the year following dispersal. Seed banks and kochia populations may be rapidly reduced in abundance if seed production could be reduced or eliminated.

While the seedling stage of kochia has been the primary target for herbicide intervention, the late maturation of kochia opens the option of later herbicide intervention, targeting the reduction of seed production (Mickelson *et al.* 2004; Young and Whitesides 1987). Harvest of cereal and pulse crops often decapitates kochia plants, removing immature seeds, although flowering and seed set continue on the remaining portion of the plant (personal observation).

The effects of pre- and post-harvest herbicide application on seed set and viability have been examined for some herbicides and weed combinations. Seed set of wild oat has been reduced by flamprop-m-methyl application at plant maturity (Medd *et al.* 1992). Young and Whitesides (1987) reported that seed germination of Russian thistle (*Salsola iberica)* was reduced when glyphosate, paraquat, or chlorsulfuron were applied post-harvest. Mickelson *et al.* (2004) reported that glyphosate and paraquat effectively reduced seed production in kochia, although effects were dependent on the time of application. It is believed the time of application, leaf area, phloem mobility of the herbicide, seed sink strength relative to seed maturity, and the climate may all influence the effectiveness of post-harvest herbicides.

In anticipation of the spread of GR kochia, we explored the option to apply herbicides post harvest to reduce kochia seed set and viability. Experiments were conducted at 4 sites in 2011 and 2012 in southern and central Alberta, using a range of herbicides.

4.2. Materials and Methods

4.2.1. Site Description

Trials were conducted on naturally occurring kochia populations in wheat fields near Huzzar, AB (50°57’28”N 112°50’30”W) and Cluny, AB (50°55’18”N 112°51’59”W) in 2011. The Hazzar and Cluny sites were chosen for uniform kochia populations with 20, and 100 plants m^{-2}, respectively. Fields were seeded to wheat by the landowners. In 2012, to improve kochia population uniformity, kochia seed was spread by hand, spreading the seed evenly across the entire trial area, in the spring after the planting of CDC Patrick peas near Lethbridge, AB (49°45’28”N 112°55’26”W) and the University of Alberta St. Albert research station (53°41’34”N 113°37’16”W). Manually seeded kochia was obtained from a natural population near Brooks, Alberta in 2010. Although this seed was not tested for glyphosate resistance as resistance at this time had not been reported. The lack of resistance was confirmed in trials as the glyphosate treatment alone was effective. Peas were direct seeded using a Wintersteiger Plotseed XXL with no-till openers at a rate of 25 plants m^{-2} using a drill seeder with 12 inch row spacing and a depth of 1 inch.

All trials were conducted in fields that had crop stubble from previous years. No in-crop herbicides were applied to wheat or pea crops, with the exception of clethodim (0.125 L ha^{-1}) applied uniformly to all plots at Lethbridge.

Ten soil samples were taken from each trial and composited to assess the kochia seed bank and soil properties (Table 1). To simulate harvest, plants were cut to 15 cm by swathing and plots (2 m by 7 m) were established after harvest. Three 0.25 m^2 quadrats were randomly established in each plot prior to herbicide application, at the front, middle and back of each plot and initial kochia densities quantified prior to herbicide application.

4.2.2. Herbicide Treatments

Herbicide treatments were chosen from PRE and POST herbicides with known efficacy on kochia: pyrasulfotole + bromoxynil at 205 g a.i. ha^{-1}; dicamba at 139 g a.i. ha^{-1}, saflufenacil at 18g a.i. ha^{-1}; carfentrazone-ethyl at 8.9 g a.i. ha^{-1}; fluroxypyr + MCPA at 107 + 556 g a.i. ha^{-1}, glufosinate ammonium at 300 g a.i. ha^{-1}; diquat at 410 g a.i. ha^{-1} and diflufenozopyr + dicamba at 21.3 and 55 g a.i. ha^{-1} were compared to glyphosate at 900 g a.i. ha^{-1} and an untreated control. Treatments were applied immediately after harvest (IAH) and three weeks after harvest (WAH) using a backpack sprayer with pressurized CO_2 calibrated to deliver 100 L ha^{-1} water volume at 275 kPa. The boom was 1.5 m long and equipped with four Air Bubble Jet low drift 80015VS nozzles, and herbicides applied 38 cm above the plant canopy.

4.2.3. Assessments of Herbicide Activity

Kochia control was visually assessed on a scale of 0 (no control) to 100% (plant death) at 1, 2, and 4 WAH for the herbicide applied IAH. The same scale was used to assess kochia control at 3, 4, and 5 WAH for applications made 3 WAH. Seven WAH, kochia from within each quadrat was harvested, dried at 50° C for 60 hours and weighed. Dried kochia was hand threshed and sieved to 2.00 mm using a Fisher Scientific Number 10 Testing Sieve. In 2012 at Lethbridge and St. Albert, seed and soil in the 0.25 m^2 quadrats was collected using a shop vacuum and placed into cloth bags, dried at 50° C for 60 hours, and sieved to 2.00 mm using a Fisher Scientific Number 10 Testing Sieve.

4.2.4. Post Harvest Fecundity

Viability of seed on the remaining harvested vegetation was assessed using a subsampling strategy in which 3, 1 g samples of seed from each quadrat were germinated. Where seed from the quadrat was <1.0 g, all seed was tested. Seed was placed in a transparent germination box on a single sheet of Blue Blotter paper, produced by Hoffman Manufacturing, Inc., moistened with 30 mL of a 0.02% Helix solution and placed in the dark at 24 C. Seedlings were counted and removed every 2 days for 18 days, after which seeds had stopped germinating. Seeds were considered germinated when the seed had uncoiled and emergence of the white radicle had occurred. Seedlings were removed after counting. Seeds that did not germinate were considered non-viable.

4.2.5. Soil Seed Bank

Kochia seed bank densities were determined prior to and following herbicide application by sampling 10 bulked, 7x7x1 cm soil samples per trial taken prior to 7 WAA.

To quantify the seed loss to soil in 2012, seed on the soil was collected by vacuum from the quadrats and quantified to estimate the total seed production.

Soil samples were spread evenly over the bottom of a germination box and moistened with 30 mL of water. Germination boxes were placed in ambient light, and germinated seeds were recorded and removed every two days for 18 days after which germination had ceased. Seeds were considered germinated when the seed had uncoiled and emergence of the white radicle was observed. Seeds that did not germinate were considered non-viable.

4.2.6. Statistical Analysis

Trials were designed as randomized complete block design (RCBD) with four replicates, and two application timings. Data was analyzed in SAS software (ver. 9.3 SAS Institute, Inc, Cary, NC) using the PROC MIXED procedure for ANOVA analysis. Variances were divided into random replication (block, plot, and quadrat) and fixed effects (herbicide treatment). Data was tested for homogeneity of variance, although the data was not normal. Arcsine, logarithmic and square root transformations of the data were explored in an attempt to normalize the data; however, in the absence of normal data, untransformed data was used. The Z-test was used to test the significance of the random interactions and the F-test was used to test the significance of fixed effects. Data was tested for homogeneity of variance, although the data had negative binomial distribution. Arcsine, logarithmic and square root transformations of the data were explored in an attempt to normalize the data; however, in the absence of normal data, untransformed data was used. Means were separated using a Dunnett-Hsu adjustment at $P<0.05$.

4.3. Results

Soil organic matter varied from 3.0 to 12.0% and soil pH was high (>8.0) at the three southern Alberta sites.

Cluny soil EC was 11.0 dS m^{-2}, sufficient to limit wheat growth (Table 4-1) and to reduce the speed and germination of kochia (Steppuhn and Wall 1993).

There were no significant differences in the relative effects of herbicides between sites or years for all measured parameters, and therefore data from all sites were combined for analysis.

4.3.1. Visual Estimates of Kochia Control

After swathing to 15 cm, kochia basal leaves remained green and flowering. Only green (immature) seeds were present. Treatments applied IAH caused visual injury compared to untreated controls (Table 4-2) but during visual evaluations, herbicide control was difficult to separate from natural senescence of the plants. Both naturally occurring and seeded kochia populations were susceptible to glyphosate. At 1 WAA both diquat and glufosinate application resulted in rapid necrosis of leaves.

By 2 WAH, in mid-September, kochia plants were beginning to senesce, seed was maturing, and there were no significant differences between herbicides in visual injury. There was no evidence of re-growth from the apical meristem or leaf axis in any of the treatments, including the untreated control. Seed set was not the result of new flower initiation. Because of the maturity of the kochia plants and the relatively cool temperatures, it was expected that plants would not recover from cutting injury at harvest.

Herbicides applied 3 WAH were applied to kochia plants that had begun to senesce, lose leaves, and undergo seed maturation. No regrowth was observed and therefore the biomass was not expected to change. Visual symptom differences were apparent between herbicides, however the effects of the herbicide were difficult to differentiate from the senescence (Table 4-3, Figure 4-1).

4.3.2. Plant Biomass from Applied Herbicides

Kochia plant biomass was highly variable, averaging 35.02 m^{-2} in untreated controls 7 weeks after harvest. Herbicides applied IAH or 3 WAH did not significantly reduce kochia biomass (Table 4-5). Because kochia injured by harvest did not regrow, herbicides had no impact on biomass.

4.3.3. Seed Production of Kochia

Kochia had high and variable seed production. In untreated control an average of 5.65 g m^{-2} of hulled seed was separated from plant residue (Table 4-6). Kochia seeds are not easily removed from the hulls but we estimate that this is equivalent to 4,707 seeds using the calibration reported by Liebman and Sunberg (2006). Herbicides applied either IAH or 3 WAH did not reduce the kochia seed remaining on the plant.

4.3.4. Soil Seed Bank

Bulked soil samples of the seed bank taken from each trial demonstrated to have, on average, 671 seeds m^{-2} (Table 4-7). Herbicides applied either IAH or 3 WAH did not reduce the kochia seed found below the plants on the soil's surface.

4.4. Discussion

Most of the herbicides applied post-harvest have efficacy on kochia when applied to immature plants. Glyphosate, carfentrazone and suflufenacil are registered for pre-seeding control. Dicamba, fluroxypyr/2,4-D and pyrasulfotole/bromoxynil, and glufosinate are registered for kochia control in crop (with size restrictions). Burton *et al.* 2014 reported that dicamba/fluroxypyr, MCPA/bromoxynil and glufosinate were effective on GR kochia under greenhouse conditions.

In this experiment, crop harvest reduced the seed production potential of kochia because the vegetation removed at harvest is unable to produce viable seed. Decapitated kochia 15 cm in height have reduced lateral branching, and are unlikely to move in wind because of their height and lack of spherical shape (Figure 4-1). Soil in the uncontrolled check had an average of 671 seeds m^{-2} at the time of harvest, while 4,707 seeds m^{-2} remained on the plant, indicating that even the small kochia plants found in the trials would be able to regenerate the population in subsequent growing seasons. The treatments included in this thesis, despite exhibiting visual vegetative damage, had no significant difference on seed viability compared to the untreated check. It is believed that due to the late spray application window, visual assessments were not a good measure of control.

Seed production of kochia following harvest is similar to a study conducted by Mickelson *et al.* 2004, who found an average of 4,100 seeds per plant were produced between harvest and late September, although their study indicated that treatments of glyphosate and paraquat applied in early August or September were able to reduce kochia seed production. This is likely due to the differences in spray timings of the post-harvest applied herbicides in Montana.

Additionally, Kumar and Jha conducted two similar studies in 2015 (2015a; 2015b), which found that multiple herbicides applied in the fall were effective at controlling kochia biomass as compared to the untreated check. Furthermore, they found that several herbicides, when used in combination, were effective at reducing kochia seed production. This is likely due to the difference in spray timings of the post-harvest applied herbicides, occurring in June and early September respectively.

Due to geographical location, it is believed that post-harvest applied herbicides are not an effective tool in western Canada for the reduction of seed production of kochia. In contrast to Kumar and Jha, the kochia populations in this study had stopped vegetative growth by this application window and abundant viable seeds mature and are returned to the soil bank following harvest, which are sufficient to maintain the populations.

Table 4-1. Location, soil characteristics, spray date and density for post-harvest studies in 2011 and 2012.

Location	Year	Soil texture	Soil OM	Soil pH	EC	Planting date	IAH Application Date	3WAH Application Date	Kochia density
			%		dS m^{-1}				# m^{-2}
Cluny, AB[a]	2011	Clay loam	3.4	8.2	11	-	August 25	September 18	20
Hazzar, AB[a]	2011	Clay	4.0	8.5	1.22	-	August 25	-	41
Lethbridge, AB[b]	2012	Sandy clay	3.0	8.1	0.62	May 1	August 14	September 4	84
St. Albert, AB[b]	2012	Silty clay	12.0	6.9	0.27	May 17	August 23	September 13	76

[a] Trials seeded into natural kochia populations

[b] Kochia seeded into trials

Table 4-2. Temperature and precipitation data for post-harvest studies in 2011 and 2012.

Location	Year	Air Temperature						Precipitation					
		-----------------------C (Long term average)-----------------------						---------------------- mm (% of Long term average) --------------------					
		May	June	July	August	September	October	May	June	July	August	September	October
Cluny[a]	2011	9.0 (10.4)	13.0 (14.5)	16.0 (17.0)	16.0 (16.6)	14.0 (11.1)	6.0 (5.2)	41.7 (69)	34.0 (75)	34.3 (60)	27.1 (61)	27.7 (64)	8.1 (72)
Hazzar, AB[a]	2011	9.0 (10.4)	13.0 (14.5)	16.0 (17.0)	16.0 (16.6)	14.0 (11.1)	6.0 (5.2)	41.7 (69)	34.0 (75)	34.3 (60)	27.1 (61)	27.7 (64)	8.1 (72)
Lethbridge, AB[b]	2012	10.0 (11.1)	14.0 (15.2)	19.0 (18.2)	16.0 (17.7)	12.0 (12.6)	0 (6.6)	40.2 (68)	81.4 (87)	23.5 (58)	17.3 (46)	23.6 (57)	6.1 (30)
St. Albert, AB[b]	2012	9.0 (10.2)	14.0 (14.1)	17.0 (16.2)	16.0 (15.2)	12.0 (10.2)	0 (3.8)	23.1 (226)	48.0 (66)	86.1 (90)	73.4 (133)	19.8 (50)	15.2 (67)

[a] Trials seeded into natural kochia populations

[b] Kochia seeded into trials

Table 4-3. Visual estimates of kochia control, when herbicides were applied IAH for post-harvest studies in 2011 and 2012.

Treatment	Rate	Week 0		Week 1		Week 2		Week 3	
	g ai h^{-1}	%	S.E.	%	S.E.	%	S.E.	%	S.E.
Untreated check		0	0.0	0[a]	13.3	0[a]	0.0	19[a]	12.5
Glyphosate control	900	0	0.0	38[a]	13.3	68[b]	12.6	81[b]	12.5
Pyrasulfotole + bromoxynil	205	0	0.0	33[a]	13.3	55[b]	12.6	69[b]	12.5
Dicamba	139	0	0.0	32[a]	13.3	49[b]	12.6	70[b]	12.5
Saflufenacil[c]	18	0	0.0	42[b]	13.3	67[b]	12.6	69[b]	12.5
Carfentrazone-ethyl	8.9	0	0.0	29[a]	13.3	46[b]	12.6	76[b]	12.5
Fluroxypyr + MCPA ester	107 + 556	0	0.0	49[b]	13.3	50[b]	12.6	70[b]	12.5
Glufosinate ammonium[d]	300	0	0.0	78[b]	13.3	83[b]	12.6	86[b]	12.5
Diquat[e]	410	0	0.0	68[b]	13.3	71[b]	12.6	82[b]	12.5
Diflufenzopyr + dicamba[d]	21.3 + 55	0	0.0	35[a]	13.3	55[b]	12.6	79[b]	12.5

Means followed by the same letter are not significantly different according to a Dunnett-HSU adjustment where $P < 0.05$

[d] Merge (0.5% vol/vol)

[e] AMS (1.25% vol/vol)

[f] Agsurf (0.1% vol/vol)

Table 4-4. Visual estimates of kochia control, when herbicides were applied 3 WAH for post-harvest studies in 2011 and 2012.

Treatment	Rate	Week 3		Week 4		Week 5	
	g ai h^{-1}	%	S.E.	%	S.E.	%	S.E.
Untreated check		0	0.0	42[a]	4.6	59[a]	4.1
Glyphosate control	900	0	0.0	87[b]	4.6	96[b]	4.1
Pyrasulfotole + bromoxynil	205	0	0.0	69[b]	4.6	88[b]	4.1
Dicamba	139	0	0.0	82[b]	4.6	92[b]	4.1
Saflufenacil[c]	18	0	0.0	83[b]	4.6	91[b]	4.1
Carfentrazone-ethyl	8.9	0	0.0	80[b]	4.6	93[b]	4.1
Fluroxypyr + MCPA ester	107 + 556	0	0.0	76[b]	4.6	94[b]	4.1
Glufosinate ammonium[d]	300	0	0.0	89[b]	4.6	96[b]	4.1
Diquat[e]	410	0	0.0	90[b]	4.6	96[b]	4.1
Diflufenzopyr + dicamba[d]	21.3 + 55	0	0.0	76[b]	4.6	94[b]	4.1

Means followed by the same letter are not significantly different according to a Dunnett-HSU adjustment where P < 0.05

[d] Merge (0.5% vol/vol)
[e] AMS (1.25% vol/vol)
[f] Agsurf (0.1% vol/vol)

Table 4-5. Dry weight biomass of kochia when herbicide was applied IAH and 3 WAH for post-harvest studies in 2011 and 2012.

Treatment	Rate	Immediately After Harvest		Three Weeks after Harvest	
	g ai ha^{-1}	g m^{-2}	S.E.	g m^{-2}	S.E.
Untreated check		44.32[a]	10.49	25.73[a]	9.67
Glyphosate control	900	42.08[a]	10.49	25.00[a]	9.67
Pyrasulfotole + bromoxynil	205	20.98[a]	10.49	33.60[a]	9.67
Dicamba	139	32.05[a]	10.49	42.81[a]	9.67
Saflufenacil[c]	18	44.03[a]	10.49	42.10[a]	9.67
Carfentrazone-ethyl	8.9	39.91[a]	10.49	39.37[a]	9.67
Fluroxypyr + MCPA ester	107 + 556	36.31[a]	10.49	41.73[a]	9.67
Glufosinate ammonium[d]	300	36.15[a]	10.49	35.38[a]	9.67
Diquat[e]	410	39.69[a]	10.49	32.95[a]	9.67
Diflufenzopyr + dicamba[d]	21.3 + 55	36.43[a]	10.49	42.21[a]	9.67

Means followed by the same letter are not significantly different according to a Dunnett-HSU adjustment where $P < 0.05$

[c] Merge (0.5% vol/vol)

[d] AMS (1.25% vol/vol)

[e] Agsurf (0.1% vol/vol)

Table 4-6. Seed biomass remaining on kochia plants when herbicide was applied IAH and 3 WAH for post-harvest studies in 2011 and 2012.

Treatment	Rate	Immediately After Harvest		Three Weeks after Harvest	
	g ai ha^{-1}	g m^{-2}	S.E.	g m^{-2}	S.E.
Untreated check		6.61[a]	1.23	4.69[a]	1.83
Glyphosate control	900	3.87[a]	1.23	4.89[a]	1.83
Pyrasulfotole + bromoxyni	205	2.78[a]	1.23	5.40[a]	1.83
Dicamba	139	6.32[a]	1.23	5.85[a]	1.83
Saflufenacil[c]	18	9.27[a]	1.23	5.17[a]	1.83
Carfentrazone-ethyl	8.9	7.58[a]	1.23	6.34[a]	1.83
Fluroxypyr + MCPA ester	107 + 556	5.08[a]	1.23	9.09[a]	1.83
Glufosinate ammonium[d]	300	5.74[a]	1.23	5.86[a]	1.83
Diquat[e]	410	5.82[a]	1.23	5.62[a]	1.83
Diflufenzopyr + dicamba[d]	21.3 + 55	4.71[a]	1.23	6.59[a]	1.83

Means followed by the same letter are not significantly different according to a Dunnett-HSU adjustment where $P < 0.05$

[c] Merge (0.5% vol/vol)

[d] AMS (1.25% vol/vol)

[e] Agsurf (0.1% vol/vol)

Table 4-7. Number of kochia seeds from the seed bank that germinated over a period of 18 days from post-harvest studies in 2012.

Treatment	Rate	Immediately After Harvest		Three Weeks after Harves	
	g ai ha^{-1}	# of germinated seeds per m^{-2}	S.E.	# of germinated seeds per m^{-2}	S.E.
Untreated check		531[a]	125.6	810[a]	223.4
Glyphosate control	900	372[a]	125.6	527[a]	223.4
Pyrasulfotole + bromoxyni	205	518[a]	125.6	1036[a]	223.4
Dicamba	139	793[a]	125.6	1216[a]	223.4
Saflufenacil[c]	18	724[a]	125.6	1360[a]	223.4
Carfentrazone-ethyl	8.9	630[a]	125.6	1293[a]	223.4
Fluroxypyr + MCPA ester	107 + 556	440[a]	125.6	972[a]	223.4
Glufosinate ammonium[d]	300	577[a]	125.6	741[a]	223.4
Diquat[e]	410	781[a]	125.6	888[a]	223.4
Diflufenzopyr + dicamba[d]	21.3 + 55	510[a]	125.6	949[a]	223.4

Means followed by the same letter are not significantly different according to a Dunnett-HSU adjustment where $P < 0.05$

[c] Merge (0.5% vol/vol)

[d] AMS (1.25% vol/vol)

[e] Agsurf (0.1% vol/vol)

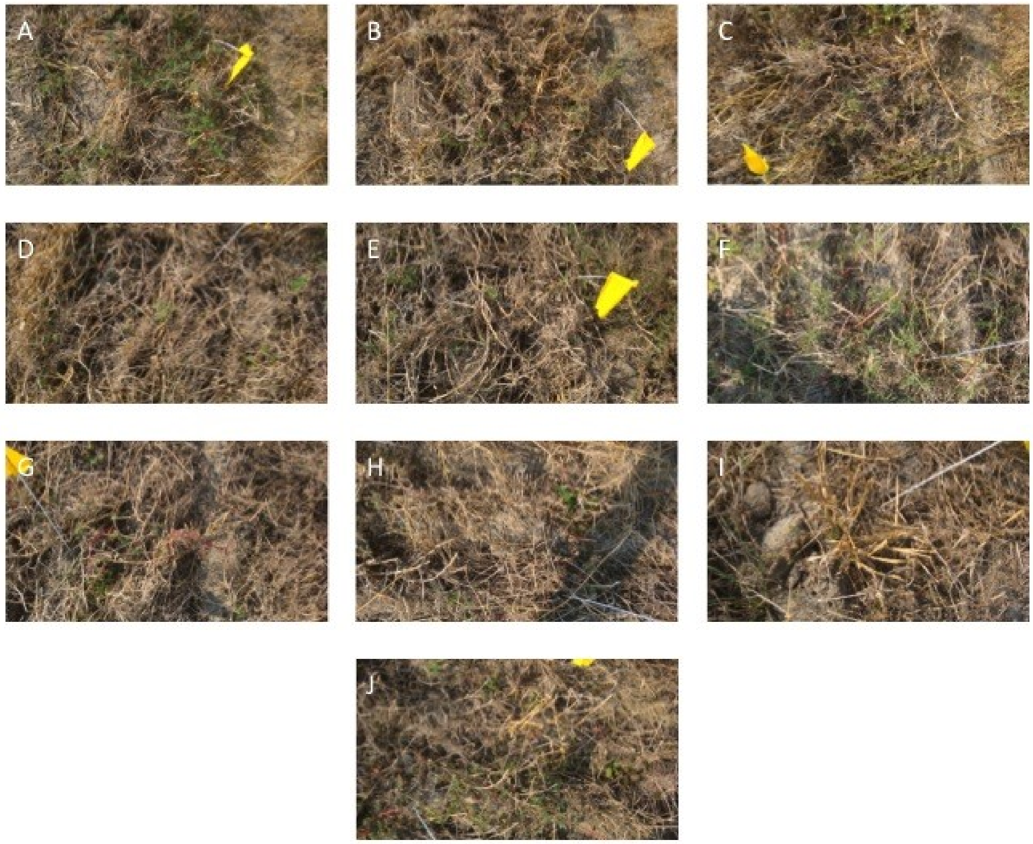

Figure 4-1. Herbicide symptomology 1 WAA when sprayed IAH. A: Untreated; B: Glyphosate control; C: Pyrasulfotole + bromoxynil; D: Dicamba; E: Saflufenacil; F: Carfentrazone-ethyl; G: Fluroxypyr + MCPA ester; H: Glufosinate ammonium; I: Diquat; J: Diflufenzopyr + Dicamba.

4.5. Literature Cited

Becker, D. A. 1978. Stem abscission in tumbleweeds of the chenopodiaceae: Kochia. Am. J. Bot. 65:375-383.

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

Beckie, H. J., S. I. Warwick, C. A. Sauder, C. Lozinski, and S. Shirriff. 2011. Occurrence and molecular characterization of acetolactate synthase (ALS) inhibitor-resistant kochia (*Kochia scoparia*) in western Canada. Weed Technol. 25:170-175.

Burton, N., S. Shirriff, and H. Beckie. 2014. Response of glyphosate-resistant kochia (*Kochia scoparia* L. Schrad.) to alternative herbicides. Can. J. Plant Sci. 94(8):1407-1411.

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

Hall, L., H. Beckie, R. H. Low, S. Shirriff, N. Kimmel, C. Neeser, and R. Blackshaw. 2013. Short communication: Survey of glyphosate-resistant kochia (*Kochia scoparia* L. Schrad.) in Alberta. Can. J. Plant Sci. 94:127-130.

Kumar, V. and P. Jha. 2015a. Effective preemergence and postemergence herbicide programs for kochia control. Weed Technol. 29(1):24-34.

Kumar, V. and P. Jha. 2015b. Influence of herbicides applied postharvest in wheat stubble on control, fecundity, and progeny fitness of *Kochia scoparia* in the US great plains. Crop Protection 71:144-149.

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, Saskatchewan, Canada: Agriculture and Agri-Food Canada, Saskatoon Research Centre Weed Survey Series Publication 05-1CD :395.

Liebman, M. and D.N. Sundberg. 2006. Seed mass affects the susceptibility of weed and crop species to phytotoxins extracted from red clover shoots. Weed Sci. 54:340-345.

Medd, R., M. McMillan, and A. Cook. 1992. Spray-topping of wild oats (*Avena* spp.) in wheat with selective herbicides. Plant Prot. 7:62-65.

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.

Mulugeta, D., B. D. Maxwell, P. K. Fay, and W. E. Dyer. 1994. Kochia (*Kochia scoparia*) pollen dispersion, viability and germination. Weed Sci. 42:548-552.

Powles, S. B. 2008. Evolution in action: Glyphosate-resistant weeds threaten world crops. Outlooks on Pest Management December:256-259.

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

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

Steppuhn, H. and K. Wall. 1993. *Kochia scoparia* emergence from saline soil under various water regimes. J. Range Manage. 533-538.

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

Young, F. L. and R. E. Whitesides. 1987. Efficacy of postharvest herbicides on Russian thistle (*Salsola iberica*) control and seed germination. Weed Sci. 554-559.

Chapter Five: General Discussion and Conclusions

5.1. Biology of *Kochia scoparia*

Kochia is a pernicious weed with the potential to become a driving influence on the cropping systems of the Canadian prairies. It is a facultative halophyte with C_4 metabolism that allows it to withstand hot and dry conditions, often where crop competition can be limited. It has a plastic growth habit that allows adaptation to agricultural, ruderal, and disturbed native environments. Ruderal locations become refuges for kochia, allowing for re-infestation of crop areas. It has the potential for high fecundity as well as seed dispersal at both short and long distances. Seed dormancy is low and large numbers of seedlings emerge early in the spring where populations were present in the previous year. It has been selected for resistance to many common herbicides, including ALS inhibitors and glyphosate. Integrated weed control options are limited because early emergence and late seed production occur outside of the temporal range of crop competition. Researchers have repeatedly turned to new herbicides, and herbicide mixtures, to control kochia. This thesis examined alternative herbicide modes of action applied pre-seeding and the use of alternative herbicide timings.

Research presented in this thesis supports the use of multiple herbicides with different modes of action to be used to control *Kochia scoparia* at early pre-seeding application timings; however, the herbicides included in this thesis, when applied post-harvest, were not an effective tool in the reduction of seed production of kochia following harvest.

5.2. Results Summarized by Research Objective

5.2.1 Develop herbicide recommendations for pre-seeding application of herbicides to reduce kochia seed production

The over use of glyphosate as a pre-emergent herbicide has been detailed in Chapter Three. This overutilization has selected for GR kochia populations across Western Canada and the United States. Six split plot trials were established at 5 locations over 2 years using a pre-emergent application timing consisting of 11 herbicides. Control of kochia was evaluated through visual assessments and kochia weights. An ANOVA was used to determine the effect of herbicide efficacy based on fresh weight biomass by location. Results indicate that different herbicides controlled kochia at varying rates depending on the location of the trial; however, there are herbicides that can provide kochia control equal to that of glyphosate.

5.2.2 Investigate the utility of post-harvest application on kochia seed banks and seedling establishment

Effectiveness of post-harvest applications was investigated and compared in the trials described and discussed in Chapter Four. It was determined that herbicides included in this study applied post-harvest were not an effective tool in western Canada for the reduction of kochia seed production.

5.3. Future Research

Many stakeholders will need to work together in the near future to tackle the growing problem of herbicide resistant weeds, including growers, agrochemical companies, governments and educational institutions. While the use of multiple herbicides for kochia control will continue to be developed as a short term solution for control in-crop, weed control based on alternatives to existing herbicides need to be developed for pernicious weeds such as kochia. Some of these ideas were reviewed by Shaner and Beckie (2014) and include:

- Commit more resources to research and educate growers on IWM techniques which include mechanical, cultural and biological, and does not focus exclusively on chemicals;
    - Ensure this research is published and becomes available to growers;
- Continue to develop herbicides with new sites-of-action (SOA), and novel herbicides such as RNAi technology to manage weeds that have developed resistance to existing modes of action;
- Advocate and educate growers on the need for pre-emergence herbicides, and to provide data to show their effectiveness at controlling weed establishment to reduce the need for multiple in-crop herbicide applications; and,
- Research the effectiveness of site-specific weed management in which the distribution of weed populations within a field can be recorded and tracked so that a variety of IWM techniques (such as mechanical weed control and targeted herbicide application) can be used at multiple periods of time over the growing season to control undesirable species prior to them setting seed in the fall.

However, effective strategies to manage and control kochia will be difficult to develop until the biology of kochia and the ecological niche it occupies is more fully understood. Further areas of research that will aid in the understanding of this weed include:

- Investigate the ecological relationship between kochia and domesticated crops that may act to reduce, and are competitive against, kochia populations. This research could be conducted by establishing long-term research sites using different crop rotations and seeding rates;

- Conduct long-term studies specifically looking at the management of kochia seed in the seed bank;
- Model the distance viable kochia seed can travel on a tumbleweed under various wind conditions to give a better understanding of the range at which gene-flow may occur between separate kochia populations; and,
- Investigate kochia population genetics to determine the rate of mutation for resistance, and if resistance comes from a single or multiple plants within a population.

5.5. Literature Cited

Belvins, R.L. and W.W. Frye. 1993. Conservation tillage: an ecological approach to soil management. Advances in agronomy. 51:34-77.

Shaner, D. L. and H. J. Beckie. 2014. The future for weed control and technology. Pest Manag. Sci. 70:1329-1339.

Triplett, G.B. and W.A. Dick. 2008. No-tillage crop production: a revolution in agriculture! Agronomy Journal. 100:153

Wicks, G.A. and B.R. Somerhalder. 1971. Effect of seedbed preparation for corn on distribution of weed seed. Weed Science. 666-668

Bibliography

Baker, D. V., K. G. Beck, B. J. Bienkiewicz, and L. B. Bjostad. 2008. Forces necessary to initiate dispersal for three tumbleweeds. Invasive Plant Science and Management 1:59-65.

Baylis, A. D. 2000. Why glyphosate is a global herbicide: Strengths, weaknesses and prospects. Pest Manag. Sci. 56:299-308.

Becker, D. A. 1978. Stem abscission in tumbleweeds of the chenopodiaceae: Kochia. Am. J. Bot. 65:375-383.

Beckie, H., K. Harker, L. Hall, S. Warwick, A. Légère, P. Sikkema, G. Clayton, A. Thomas, J. Leeson, and G. Séguin-Swartz. 2006. A decade of herbicide-resistant crops in Canada. Can. J. Plant Sci. 86:1243-1264.

Beckie, H. J., R. E. Blackshaw, R. Low, L. M. Hall, C. A. Sauder, S. Martin, R. N. Brandt, and S. W. Shirriff. 2012. Glyphosate-and acetolactate synthase inhibitor-resistant kochia (*Kochia scoparia*) in western Canada. Weed Sci. 61:310-318.

Beckie, H. J., R. H. Gulden, N. Shaikh, E. N. Johnson, C. J. Willenborg, C. A. Brenzil, S. W. Shirriff, C. Lozinski, and G. Ford. 2015. Glyphosate-resistant kochia (*Kochia scoparia* L. Schrad.) in Saskatchewan and Manitoba. Can. J. Plant Sci. 95:1-5.

Beckie, H. J., S. I. Warwick, C. A. Sauder, C. Lozinski, and S. Shirriff. 2011. Occurrence and molecular characterization of acetolactate synthase (ALS) inhibitor-resistant kochia (*Kochia scoparia*) in western Canada. Weed Technol. 25:170-175.

Belvins, R.L. and W.W. Frye. 1993. Conservation tillage: an ecological approach to soil management. Advances in agronomy. 51:34-77.

Benbrook, C. M. 2012. Impacts of genetically engineered crops on pesticide use in the US-the first sixteen years. Environmental Sciences Europe 24:1-13.

Brookes, G. and P. Barfoot. 2014. Economic impact of GM crops: The global income and production effects 1996–2012. GM Crops & Food 5:65-75.

Bullied, W. J., A. M. Marginet, and R. C. Van Acker. 2003. Conventional-and conservation-tillage systems influence emergence periodicity of annual weed species in canola. Weed Sci. 51:886-897.

Burton, N., S. Shirriff, and H. Beckie. 2014. Response of glyphosate-resistant kochia (*Kochia scoparia* L. Schrad.) to alternative herbicides. Can. J. Plant Sci. 94(8):1407-1411.

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

Culpepper, A. S., A. C. York, R. B. Batts, and K. M. Jennings. 2000. Weed management in glufosinate-and glyphosate-resistant soybean (Glycine max). Weed Tech 14:77-88.

Davis, R., W. Johnson, and F. Wood. 1967. Weed root profiles. Agron. J. 59:555-556.

Dill, G. M., C. A. CaJacob, and S. R. Padgette. 2008. Glyphosate-resistant crops: Adoption, use and future considerations. Pest Manag. Sci. 64(4):326-331.

Dill, G. M. 2005. Glyphosate-resistant crops: History, status and future. Pest Manag. Sci. 61:219-224.

Duke, S. O. and S. B. Powles. 2008. Glyphosate: A once-in-a-century herbicide. Pest Manag. Sci. 64:319-325.

Duke, S. O. and S. B. Powles. 2009. Glyphosate-resistant crops and weeds: Now and in the future. AgBioForum 12:346-357.

Duke, S. O. 2005. Taking stock of herbicide-resistant crops ten years after introduction. Pest Manag. Sci. 61:211-218.

Eberlein, C. and Z. Fore. 1984. Kochia biology. Weeds Today. 15:5-7.

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

Franz J. E., M. K. Mao, and J. A. Sikorski. 1997. Glyphosate: A Unique Global Herbicide, ACS Monograph No. 189. Am. Chem. Soc.

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.

Gaines, T. A., A. A. Wright, W. T. Molin, L. Lorentz, C. W. Riggins, P. J. Tranel, R. Beffa, P. Westra, and S. B. Powles. 2013. Identification of genetic elements associated with EPSPS gene amplification. PLoS ONE 8:1-10.

Gaines, T. A., W. Zhang, D. Wang, B. Bukun, S. T. Chisholm, D. L. Shaner, S. J. Nissen, W. L. Patzoldt, P. J. Tranel, A. S. Culpepper, T. L. Grey, T. M. Webster, W. K. Vencill, R. D. Sammons, J. Jiang, C. Preston, J. E. Leach, and P. Westra. 2010. Gene amplification confers glyphosate resistance in amaranthus palmeri. Proc. Natl. Acad. Sci. U. S. A. 107:1029-1034.

Giacomini, D. A. 2015. EPSPS Gene Duplication in *Palmer Amaranth:* Relative Fitness, Inheritance, and Duplication Mechanism of the Glyphosate Resistance Trait.Colorado Denver: Colorado State University.

Givens, W. A., D. R. Shaw, W. G. Johnson, S. C. Weller, B. G. Young, R. G. Wilson, M. D. Owen, and D. Jordan. 2009. A grower survey of herbicide use patterns in glyphosate-resistant cropping systems. Weed Technol. 23:156-161.

Grossbard E. and D. Atkinson. 1985. The Herbicide Glyphosate. Butterworths & Co., London, UK, 490 pp.

Hall, L., H. Beckie, R. H. Low, S. Shirriff, N. Kimmel, C. Neeser, and R. Blackshaw. 2014. Short communication: Survey of glyphosate-resistant kochia (*Kochia scoparia* L. Schrad.) in Alberta. Can. J. Plant Sci. 94:127-130.

Heap I. 2014. The International Survey of Herbicide Resistant Weeds. www.weedscience.org. Accessed: January 24, 2014.

Herrmann, K. M. and L. M. Weaver. 1999. The shikimate pathway. Annual Review of Plant Biology. 50:473-503.

Holm F. A. and E. N. Johnson. 2010. A brief history of herbicide use in western Canada. Pages 77-79 *in* 17th Australasian Weeds Conference. New Frontiers in New Zealand: Together we can Beat the Weeds. Christchurch, New Zealand, 26-30 September, 2010. New Zealand Plant Protection Society.

Jugulam, M., K. Niehues, A. S. Godar, D. H. Koo, T. Danilova, B. Friebe, S. Sehgal, V. K. Varanasi, A. Wiersma, P. Westra, P. W. Stahlman, and B. S. Gill. 2014. Tandem amplification of a chromosomal segment harboring 5-enolpyruvylshikimate-3-phosphate synthase locus confers glyphosate resistance in *Kochia scoparia*. Plant Physiol. 166:1200-1207.

Khan, M. A., B. Gul, and D. J. Weber. 2001. Influence of salinity and temperature on the germination of *Kochia scoparia*. Wetlands Ecol. Manage. 9:483-489.

Kumar, V. and P. Jha. 2015a. Effective preemergence and postemergence herbicide programs for kochia control. Weed Technol. 29(1):24-34.

Kumar, V. and P. Jha. 2015b. Influence of herbicides applied postharvest in wheat stubble on control, fecundity, and progeny fitness of *Kochia scoparia* in the US great plains. Crop Protection 71:144-149.

Lee, B., M. Kim, R. R. C. Wang, and B. Waldron. 2005. Relationships among 3 kochia species based on PCR-generated molecular sequences and molecular cytogenetics. Genome 48:1104-1115.

Lee, L. J. and J. Ngim. 2000. A first report of glyphosate-resistant goosegrass (*Eleusine indica* (L) Gaertn) in Malaysia. Pest Manag. Sci. 56:336-339.

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, Saskatchewan, Canada: Agriculture and

Agri-Food Canada, Saskatoon Research Centre Weed Survey Series Publication 05-1CD :395 .

Liebman, M. and D.N. Sundberg. 2006. Seed mass affects the susceptibility of weed and crop species to phytotoxins extracted from red clover shoots. Weed Sci. 54:340-345.

Medd, R., M. McMillan, and A. Cook. 1992. Spray-topping of wild oats (*Avena* spp.) in wheat with selective herbicides. Plant Prot. 7:62-65.

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

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.

Mulugeta, D., B. D. Maxwell, P. K. Fay, and W. E. Dyer. 1994. Kochia (*Kochia scoparia*) pollen dispersion, viability and germination. Weed Sci. 42:548-552.

Nalewaja, J., B. Devilliers, and R. Matysiak. 1996. Surfactant and salt affect glyphosate retention and absorption. Weed Res. 36:241-247.

Powles, S. B. 2008. Evolution in action: Glyphosate-resistant weeds threaten world crops. Outlooks on Pest Management December:256-259.

Powles S. B. and J. A. Holtum. 1994. Herbicide Resistance in Plants: Biology and Biochemistry. Lewis Publishers Inc., Boca Raton, FL.

Powles, S. B. and C. Preston. 2006. Evolved glyphosate resistance in plants: Biochemical and genetic basis of resistance 1. Weed Technol. 20:282-289.

Powles, S. B., D. F. Lorraine-Colwill, J. J. Dellow, and C. Preston. 1998. Evolved resistance to glyphosate in rigid ryegrass (*Lolium rigidum*) in Australia. Weed Sci. 46:604-607.

Pratley, J., N. Urwin, R. Stanton, P. Baines, J. Broster, K. Cullis, D. Schafer, J. Bohn, and R. Krueger. 1999. Resistance to glyphosate in *Lolium rigidum*. I. Bioevaluation. Weed Sci. 405-411.

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

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

Saari, L. L., J. C. Cotterman, and M. M. Primiani. 1990. Mechanism of sulfonylurea herbicide resistance in the broadleaf weed, *Kochia scoparia.* Plant Physiol. 93:55-61.

Salhoff, C. R. and A. R. Martin. 1986. *Kochia scoparia* growth response to triazine herbicides. Weed Sci. 40-42.

Sammons, D. R. and T. A. Gaines. 2014. Glyphosate resistance: State of knowledge. Pest Manag. Sci. 70(9):1367:1377.

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

Shaner, D. L. and H. J. Beckie. 2014. The future for weed control and technology. Pest Manag. Sci. 70:1329-1339.

Simonsen, L., I. S. Fomsgaard, B. Svensmark, and N. H. Spliid. 2008. Fate and availability of glyphosate and AMPA in agricultural soil. Journal of Environmental Science and Health Part B 43:365-375.

Sprankle, P., W. Meggitt, and D. Penner. 1975. Adsorption, mobility, and microbial degradation of glyphosate in the soil. Weed Sci. 229-234.

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

Starcevic, A., S. Akthar, W. C. Dunlap, J. M. Shick, D. Hranueli, J. Cullum, and P. F. Long. 2008. Enzymes of the shikimic acid pathway encoded in the genome of a basal metazoan, nematostella vectensis, have microbial origins. Proceedings of the National Academy of Sciences 105:2533-2537.

Steppuhn, H. and K. Wall. 1993. *Kochia scoparia* emergence from saline soil under various water regimes. J. Range Manage. 533-538.

Székács, A. and B. Darvas. 2012. Forty years with glyphosate. Herbicides–properties, Synthesis and Control of Weeds. Ed.Hasaneen, MNAE-G., InTech, Croatia 247-284.

Tranel, P. J. and T. R. Wright. 2009. Resistance of weeds to ALS-inhibiting herbicides: What have we learned? Weed Sci. 50:700-712.

Triplett, G.B. and W.A. Dick. 2008. No-tillage crop production: a revolution in agriculture! Agronomy Journal. 100:153

Vila-Aiub, M.M. 2014. No fitness cost of glyphosate resistance endowed by massive EPSPS gene amplification in Amaranthus palmeri. Planta. 239(4):793-801.

Waite J. C. 2008. Glyphosate Resistance in Kochia. Masters of Science dissertation. Manhattan Kansas: Kansas State University.

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

Walsh, M. J. and S. B. Powles. 2014. High seed retention at maturity of annual weeds infesting crop fields highlights the potential for harvest weed seed control. Weed Technol. 28:486-493.

Walsh, M. and P. Newman. 2007. Burning narrow windrows for weed seed destruction. Field Crops Res. 104:24-30.

Walsh, M., P. Newman, and S. Powles. 2013. Targeting weed seeds in-crop: A new weed control paradigm for global agriculture. Weed Technol. 27:431-436.

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

Wicks, G.A. and B.R. Somerhalder. 1971. Effect of seedbed preparation for corn on distribution of weed seed. Weed Science. 666-668

Woodburn, A. T. 2000. Glyphosate: Production, pricing and use worldwide. Pest Manag. Sci. 56:309-312.

Wrubel, R. P. and J. Gressel. 1994. Are herbicide mixtures useful for delaying the rapid evolution of resistance? A case study. Weed Technol. 635-648.

Young, F. L. and R. E. Whitesides. 1987. Efficacy of postharvest herbicides on Russian thistle (*Salsola iberica*) control and seed germination. Weed Sci. 554-559.

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. 602-607.

Appendix 1: Glyphosate and Acetolactate Synthase Inhibitor – Resistant Kochia (*Kochia scoparia*) in Western Canada

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Glyphosate- and Acetolactate Synthase Inhibitor–Resistant Kochia (*Kochia scoparia*) in Western Canada

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In summer, 2011, we investigated suspected glyphosate-resistant (GR) kochia in three chem-fallow fields (designated F1, F2, F3, each farmed by a different grower) in southern Alberta. This study characterizes glyphosate resistance in those populations, based on data from dose–response experiments. In a greenhouse experiment, the three populations exhibited a resistance factor ranging from 4 to 6 based on shoot biomass response (GR_{50} ratios), or 5 to 7 based on survival response (LD_{50} ratios). Similar results were found in a field dose–response experiment at Lethbridge, AB, in spring 2012 using the F2 kochia population. In fall 2011, we surveyed 46 fields within a 20-km radius of the three chem-fallow fields for GR kochia. In the greenhouse, populations were screened with glyphosate at 900 g ae ha^{-1}. Seven populations were confirmed as GR, the farthest site located about 13 km from the three originally confirmed populations. An additional GR population more than 100 km away was later confirmed. Populations were screened for acetolactate synthase (ALS)–inhibitor (thifensulfuron : tribenuron) and dicamba resistance in the greenhouse, with molecular characterization of ALS-inhibitor resistance in the F1, F2, and F3 populations. All GR populations were resistant to the ALS-inhibiting herbicide, but susceptible to dicamba. ALS-inhibitor resistance in kochia was conferred by Pro_{197}, Asp_{376}, or Trp_{574} amino acid substitutions. Based upon a simple empirical model with a parameter for selection pressure, calculated from weed relative abundance and glyphosate efficacy, and a parameter for seedbank longevity, kochia, wild oat, and green foxtail were the top three weeds, respectively, predicted at risk of selection for glyphosate resistance in the semiarid Grassland region of the Canadian prairies; wild oat, green foxtail, and cleavers species were predicted at greatest risk in the subhumid Parkland region. This study confirms the first occurrence of a GR weed in western Canada. Future research on GR kochia will include monitoring, biology and ecology, fitness, mechanism of resistance, and best management practices.

Nomenclature: Dicamba; glyphosate; thifensulfuron; tribenuron; cleavers: false cleavers, *Galium spurium* L. or catchweed bedstraw, *Galium aparine* L.; green foxtail, *Setaria viridis* (L.) Beauv.; kochia, *Kochia scoparia* (L.) Schrad. KCHSC, synonym: *Bassia scoparia* (L.) A.J. Scott.; wild oat, *Avena fatua* L.
Key words: ALS-inhibitor resistance, glyphosate resistance, herbicide resistance, multiple resistance, target-site mutation.

Kochia is an annual broadleaf weed species native to Eurasia, and introduced as an ornamental to the Americas in the mid- to late 1800s (reviewed in Friesen et al. 2009). This naturalized species is a common and economically important weed in crop production systems and ruderal (noncrop disturbed) areas in semiarid to arid regions of North America. It is one of the top 10 most abundant agricultural weeds in the Canadian prairies (Leeson et al. 2005). Kochia is reported to have the highest rate of spread among 40 alien weed species in the northwestern United States (Forcella 1985), and has expanded northward in the Canadian prairies during the past 40 yr (Beckie et al. 2012; Thomas and Leeson 2007). Kochia, a C_4 species, is highly competitive in cropping systems because of its ability to germinate at low soil temperatures and emerge early; grow rapidly; tolerate heat, drought, and salinity; and exert allelopathic effects on neighboring species (Friesen et al. 2009).

In cereal or pulse (annual legume) crops in western Canada, ALS-inhibiting herbicides or synthetic auxins (e.g., dicamba) are commonly used to control kochia (Saskatchewan Ministry of Agriculture 2012). ALS-inhibitor herbicide-resistant (HR) populations of the species were first reported in the United States in 1987 (Primiani et al. 1990; Saari et al. 1990; Thompson et al. 1994), and in Canada (prairies) in 1988 (Morrison and Devine 1994). Target-site mutations at Pro_{197} with Thr, Ser, Arg, Leu, Glu, and Ala substitutions were reported for chlorsulfuron-HR kochia populations from Kansas (Guttieri et al. 1992, 1995). A Trp_{574}Leu mutation of the *ALS* gene was identified in ALS-inhibitor–HR populations from Illinois (Foes et al. 1999) and the Czech Republic (Salava et al. 2004). A field survey in Manitoba, Canada, in 2004 found widespread, broad cross-resistance (across ALS-inhibitor classes) in 102 of 114 kochia populations collected (B. Murray and L. Friesen, unpublished data). The broad cross-resistance in those populations suggested a target mutation, such as Trp_{574}Leu, which imparts plants with high-level, broad-spectrum resistance (Beckie and Tardif 2012).

The molecular basis for ALS-inhibitor resistance was determined for 24 HR kochia populations from western Canada (Warwick et al. 2008). *ALS* gene sequences revealed three target-site mutations (Pro_{197}, Asp_{376}, and Trp_{574}). The Trp_{574}Leu mutation was found in 70% of plants, whereas the remaining plants had the highly variable residue Pro_{197}, with substitution by one of nine amino acids, or Asp_{376}Glu and Trp_{574}Arg substitutions. This study also reported the first field-selected presence of two *ALS* target-site mutations in individual kochia plants. These included combinations Pro_{197} + Trp_{574} (23 HR plants) and Pro_{197} + Asp_{376} (7 HR plants). Kochia is predominantly selfed but capable of outcrossing, with pollen-mediated gene flow documented to a distance of 29 m (Stallings et al. 1995). The detection of Pro_{197}, Asp_{376},

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and Trp_{574} mutations, as well as both combinations, from geographically separate regions suggested multiple origins of these mutations. A more recent survey of 109 fields was conducted across western Canada to determine the extent of ALS-inhibitor and dicamba resistance in kochia (Beckie et al. 2009, 2011b). All kochia populations were susceptible to dicamba. ALS-inhibitor–HR kochia was found in 85% of the fields surveyed in western Canada. *ALS* sequence data (Pro_{197} and Asp_{376} mutations) and genotyping data (Trp_{574} mutation) confirmed the presence of all three target-site mutations as well as two mutational combinations (Pro_{197} + Trp_{574}, Asp_{376} + Trp_{574}) in HR individuals.

From 1974 to 1995 in Canada, glyphosate was commonly applied preseeding (burndown treatment), preharvest (primarily in cereals and pulses), or to a lesser extent, postharvest. With the introduction of GR crops beginning in 1996, glyphosate usage increased markedly (Beckie et al. 2011a). In 2011 in Canada, GR canola (*Brassica napus* L.), soybean [*Glycine max* (L.) Merr.], and corn (*Zea mays* L.) comprised 47, 72, and 90% of the respective crop area (R. Ripley, B. Senft, M. Reidy, personal communication). Western Canada accounts for 99% (8.5 million ha) of the nation's canola area, 20% of soybean area (344,000 ha), and 9% of grain corn area (122,000 ha) (Statistics Canada 2012). In western Canada, soybean and corn are grown mainly in southern Manitoba because of sufficient heat units (i.e., growing degree-days, GDD). In Canada, the first report of a GR weed was giant ragweed (*Ambrosia trifida* L.) in 2008 in GR soybean in eastern Canada (southwestern Ontario); a survey conducted in 2009 and 2010 documented the HR biotype in 47 new locations in three counties in the province (Vink et al. 2012). In 2010, GR horseweed (referred as Canada fleabane in Canada; *Conyza canadensis* Cronq.) was documented in the same region (Sikkema et al. 2013).

Worldwide, GR kochia was first reported in Kansas in 2007, followed by South Dakota in 2009, and Nebraska in 2011; these HR populations were selected primarily in GR corn and soybean fields (Heap 2012). In August 2011, we investigated suspected GR kochia in three chem-fallow fields (each farmed by a different grower) in southern Alberta. This study characterizes glyphosate resistance in those populations, based on data from dose–response experiments. We also describe the results of a GR kochia survey comprising 46 fields within a 20-km radius of those three chem-fallow fields, which was conducted in the fall of 2011. Confirmed GR kochia populations were screened for ALS-inhibitor and dicamba resistance in the greenhouse, with molecular characterization of ALS-inhibitor resistance in populations in the three chem-fallow fields. Looking ahead, we estimate the GR risk for the most abundant weed species in the semiarid Grassland and subhumid Parkland regions of the Canadian prairies (Agriculture and Agri-Food Canada 2003) using a simple empirical model.

Materials and Methods

Plant Material. In August 2011, 15 kochia plants (vegetative stage) were randomly selected throughout each of three chem-fallow fields (surveyed using a "W" pattern; fields labeled F1, F2, F3), which were located within 3.5 km of each other in the County of Warner in southern Alberta. The no-till cropping systems used in the fields were wheat (*Triticum aestivum* L.) or mustard [*Sinapis alba* L. or *Brassica juncea* (L.) Czern.] alternating with chem-fallow. For the latter, glyphosate (alone) was applied periodically over the 2011 growing season at 1.5 to two times the recommended (1×) rate (450 g ae ha^{-1}). GR crops had not been grown in the three fields. Plants were transplanted into 10-L pots, watered, and transported to Saskatoon, SK. In the greenhouse, plants were covered with pollen bags (Chantler Packaging, Mississauga, ON) and grown to maturity, at which time the selfed seeds were harvested over a 3-wk period. For the dose–response experiments, seeds from each plant collected in a field were combined into a composite sample (total of three samples or populations F1, F2, and F3).

Greenhouse Dose–Response Experiment. The dose–response experiment was conducted in the greenhouse at Saskatoon, SK, in November 2011, and repeated the following month. The experiment was arranged in a completely randomized design with four replications (one pot per replicate) per treatment. In addition to the suspected Alberta populations—F1, F2, and F3—two known herbicide-susceptible (HS) populations from Hanley, SK, and Hays, KS, and three known GR populations from Phillip, Scott, and Russell, KS, were included in the experiment. Five seeds were planted 1 cm deep in 10-cm square pots containing a mixture of soil, peat, vermiculite, and sand (3 : 2 : 2 : 2 by volume) plus a controlled-release fertilizer (15–9–12, 150 g 75 L^{-1}; Scotts Osmocote PLUS, Mississauga, ON). The experiment was conducted under a 20/16 C day/night temperature regime with a 16-h photoperiod supplemented with 230 µmol m^{-2} s^{-1} illumination. Pots were watered daily to field capacity.

Glyphosate (Roundup WeatherMax, K+ salt 540 g ae L^{-1} formulation, Monsanto Canada Inc., Winnipeg, MB) was applied to seedlings when 7 to 8 cm tall. The herbicide was applied using a moving-nozzle cabinet sprayer equipped with a flat-fan nozzle tip (TeeJet 8002VS, Spraying Systems Co., Wheaton, IL) calibrated to deliver 200 L ha^{-1} of spray solution at 275 kPa in a single pass over the foliage. Glyphosate was applied at one-eighth (0.125×), one-quarter, one-half, one (450 g ae ha^{-1}), two, three, four, and five times the field-recommended rate, plus a nontreated control. Three weeks after treatment (WAT), plant response to herbicide application was visually scored as HS: dead (0) or nearly dead (1); or GR: some injury but new growth (level 2) or no injury (level 3). Assessments were made relative to herbicide-treated and -untreated HS and GR check populations. Although the scoring system is likely related to dose, rating levels were distinct when visually evaluating plants in the greenhouse. Following herbicide injury rating, shoot biomass was harvested. Harvested biomass was immediately weighed (fresh weight), dried at 60 C for 3 d, then weighed again.

GR Kochia Survey. A field survey was conducted in late September and October 2011 in the County of Warner, Alberta, in an area within a 20-km radius of fields F1, F2, and F3. An approximately similar number of sites (total of 46) in each of the cardinal directions were surveyed (Figure 1). Fields with kochia were randomly selected when driving along primary or secondary roads. Between 15 and 20 mature kochia plants (i.e., target of 1,000 viable seeds) were collected from each field, bulked in a cotton bag, and air-dried. Plants from a site were hand-threshed to avoid sample cross-contamination.

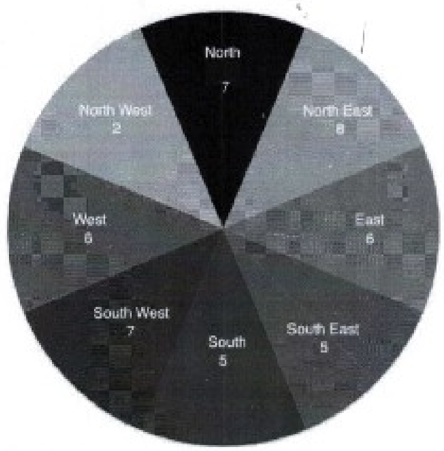

Figure 1. Number of fields sampled for kochia, by cardinal direction sector, in a survey conducted in fall 2011 in the County of Warner, Alberta, in an area within a 20-km radius of fields F1, F2, and F3 (total of 46 fields).

In February 2012, seeds were planted in 52 by 26 by 5–cm flats containing a potting mixture as described previously. A minimum total of 100 F_1 seedlings (3 to 5 cm tall) per population (three flats or replicates per experiment run and repeated) were sprayed with glyphosate at 900 g ae ha^{-1} according to procedures described previously. Plants were visually rated for survival 3 WAT using the above-mentioned scoring system. Known GR and HS populations served as positive and negative controls, respectively.

ALS-Inhibitor Resistance in GR Kochia Populations and Molecular Characterization. All kochia populations (F1, F2, F3, plus those surveyed) confirmed as GR were screened in March 2012 for resistance to a synthetic auxin herbicide, dicamba, and an ALS-inhibiting herbicide, thifensulfuron : - tribenuron premixture, using procedures described previously for the surveyed populations. Herbicides were sprayed to plants 3 to 5 cm tall using Banvel II (480 g L^{-1} dicamba, BASF Canada, Mississauga, ON) at 140 g ai ha^{-1} or Refine SG (thifensulfuron at 10 g ai ha^{-1} and tribenuron at 5 g ai ha^{-1}, E.I. duPont, Mississauga, ON) plus a nonionic surfactant (Agral 90, Norac Concepts Inc., Ottawa, ON) at 0.2% v/v. Known HR and HS controls were included in both experiment runs.

ALS Gene Sequencing. Sequence data of the entire *ALS* gene were generated for 20 kochia parental-generation plants of Alberta populations F1, F2, F3, and Kansas GR population Phillip (plus 10 plants of HS population Hanley). DNA was extracted from freeze-dried leaf tissue (10 to 20 mg) of glyphosate-treated (F1, F2, F3, Phillip) or untreated (Hanley) plants using Fast DNA SPIN kit (QBioGen, MP Biomedicals, Solon, OH) following the manufacturer's instructions.

Two sets of primers were used to amplify the entire *ALS* gene (Table 1). Polymerase chain reaction (PCR) amplifications of the *ALS* gene were performed using Ready-To-Go (GE Healthcare UK Limited, Little Chalfont, Buckinghamshire, UK) PCR beads with approximately 25 ng of genomic DNA and 400 nM of each primer in a total of 25 ml. PCR was performed under the following conditions: 2 min incubation at 95 C; 40 cycles of 30 s at 94 C, 90 s at 58 C, 90 s at 68 C; followed by 5 min at 72 C. PCR fragments were purified using the E-Gel iBase Power System with 0.8% CloneWell SYBR Safe gels (Invitrogen Corporation, Carlsbad, CA). Sequencing used ABI BigDye Terminator Reaction Mix (v. 3.1; PE Corporation, PE Biosystems, Foster City, CA). Primers used for sequencing were the same as those used for PCR amplification, and a further two internal primers were used to ensure complete coverage (Table 1). Polymorphisms or nucleotide heterozygosity was based on the appearance of two peaks at a single nucleotide position. Amino acid and nucleotide positions are numbered based on the amino acid sequence of *ALS* from *Arabidopsis* (Sathasivan et al. 1990).

Risk Assessment of Glyphosate Resistance in Canadian Prairie Weeds. We wanted to look ahead to try to identify other Canadian prairie weed species that may be at greatest risk to evolve glyphosate resistance. Because we have an extensive and unique weed survey database of thousands of prairie cropland fields spanning 40 yr, we utilized this data set in conjunction with an empirical model to estimate the top three weed species in the semiarid (southern) Grassland and subhumid (northern) Parkland regions of the prairies at potential risk of glyphosate resistance.

A classic model of herbicide resistance evolution was described by Gressel and Segel (1982):

$$N_n = N_o[1 + (f \times a/B)]^n \qquad [1]$$

where N_n is the proportion of HR individuals in the population after n herbicide applications, N_o is the initial

Table 1. Primers for amplifying and sequencing kochia *ALS* gene fragments.

Primers	5′–3′	Region of homology
b1-F[a]	ATGGCGTCTACTGTGCAAATCCC	1–23
KGenFor[b]	CGGGCCGTGTTGGTGTCTG	449–467
RuTh-F-2b[c]	GAAGAATAAGCAACCCCATGTGTC	1194–1217
5-F[a]	AATTACTCTAGCTGGAGGG	1294–1312
RuTh-R[c]	GACACATGGGGTTGCTTATT TTC	1194–1217
RuTh-R-3[c]	AACTTGTTCTTCCATCACCTTCG	1974–1995
e-rev[a]	GAAATCTTTCAACAATATAGGAAGATC	2227–2200

[a] Foes et al. 1999.
[b] Warwick et al. 2008.
[c] Warwick et al. 2010.

frequency of HR plants prior to herbicide use, f is the relative fitness of HR vs. HS biotypes, B is the average weed seedbank longevity, and a is the selection pressure. Little is known about how N_e and f vary by weed species resistant to glyphosate; selection pressure and seedbank longevity can be better estimated.

Glyphosate selection pressure was estimated for the top 10 most abundant prairie weed species [excluding Canada thistle, *Cirsium arvense* (L.) Scop., but including wild mustard, *Sinapis arvensis* L.] in two main agroecological regions of the prairies (Agriculture and Agri-Food Canada 2003). Selection pressure was quantified as the product of (1) relative abundance of a weed in each region; (2) proportional weed emergence as a function of soil GDD base 0 C under conservation tillage (Bullied et al. 2003; Schwinghamer and Van Acker 2008) at glyphosate application at preseeding (early May, 250 GDD), twice in-crop (early June, 650 GDD; late June, 850 GDD), and postharvest (September, > 1,000 GDD); and (3) glyphosate efficacy for each weed based on expert opinion. Relative abundance of a weed is a composite index based on field frequency, field uniformity, and weed density; these data are collected in July and August after all herbicide treatments have been applied (Leeson et al. 2005). Total selection pressure is simply relative abundance multiplied by efficacy, since weed emergence in a growing season totals 1.0. Because of the spaced-out germination of weeds, glyphosate resistance risk rating for each species was calculated as total selection pressure divided by seedbank longevity (Equation 1) using data from Van Acker (2009).

Dose–Response Data Analysis. Greenhouse data were combined across runs upon confirmation of homogeneity of variances (Steel and Torrie 1980). Statistical analysis of shoot biomass (fresh or dry weight) dose–response curves followed the procedure detailed by Seefeldt et al. (1995). Data were fitted to the log-logistic model [Equation 2]:

$$y = c + (d - c)/(1 + \exp(b(\ln(x) - \ln(GR_{50})))) \qquad [2]$$

where y = shoot fresh or dry weight (percentage of nontreated control), x = glyphosate dose (g ha^{-1}; a value of 1.0 was added to each dose to calculate natural logarithms, ln), c = lower limit (asymptote) of the response curve, d = upper limit, b = slope, and GR_{50} = dose (g ha^{-1}) of herbicide that reduced shoot fresh or dry weight by 50% relative to the nontreated control. However, the survival dose–response curves were best described using the quadratic (suspected or known GR populations) [Equation 3] or exponential decay model (HS populations) [Equation 4]:

$$y = cx^2 + bx + d \qquad [3]$$

where y = survival (percentage of control), x = glyphosate dose (g ha^{-1}), d = intercept, b = linear coefficient, and c = curvilinear coefficient;

$$y = de^{-bx} \qquad [4]$$

where y = survival (percentage of control), x = glyphosate dose (g ha^{-1}), d = intercept, and db = initial slope.

Data were fitted to the models using a derivative-free nonlinear regression procedure, provided with PROC NLIN (SAS 1999). Regression analyses were performed on treatment means averaged over replications as recommended by Gomez and Gomez (1984). Coefficients of determination (R^2) were

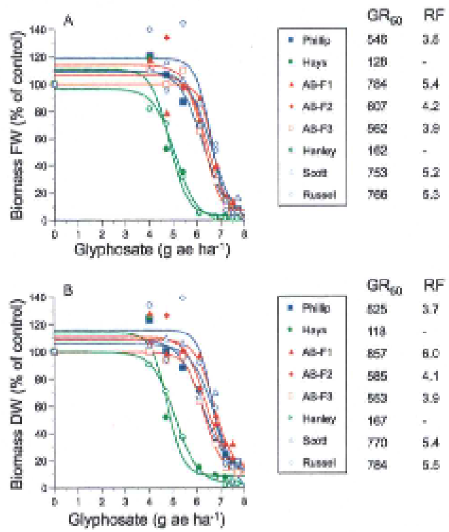

Figure 2. Kochia shoot biomass (fresh weight, FW [A] and dry weight, DW [B]) response to increasing dose of glyphosate in a greenhouse experiment: susceptible populations, Hanley and Hays; glyphosate-resistant (GR) populations from Kansas, Phillip, Scott, and Russell; and suspected GR populations from southern Alberta, F1, F2, and F3. Other abbreviations: GR_{50}, glyphosate dose resulting in a 50% reduction in biomass; RF, resistance factor, calculated as GR_{50} of a GR population divided by average GR_{50} of the susceptible populations. See text for regression equation and Table 2 for parameter estimates.

calculated as described by Kvalseth (1985) using the residual sum of squares value from the SAS output. Standard errors of the parameter estimates were calculated. Parameter estimates are considered significant at the 0.05 level if the standard error is less than one-half the value of the estimate (Koutsoyiannis 1977). Individual response curves were systematically compared for common parameters using the lack-of-fit F test at the 0.05 level of significance, as outlined by Seefeldt et al. (1995). The resistance index or factor (RF) was calculated as GR_{50} (biomass) or LD_{50} (survival) of a suspected or known GR population divided by average GR_{50} or LD_{50} of the two HS populations, where GR_{50} and LD_{50} is the dose resulting in a 50% reduction in aboveground biomass and survival, respectively, relative to the nontreated control.

Results and Discussion

Glyphosate Dose–Response Experiments. Based on shoot biomass (fresh weight) response to increasing doses of glyphosate, the three GR Kansas populations exhibited a RF of 4 to 5 (Figure 2A; Table 2). The three Alberta populations—F1, F2, and F3—responded to glyphosate similarly as the Kansas populations: RF of 4 to 5. Therefore, these three Alberta populations can be considered to have a low level of resistance to glyphosate, i.e., RF ≤ 5 (RF categorization detailed in Beckie and Tardif 2012). Similar results were obtained when shoot dry weight was regressed against

Table 2. Parameter estimates (standard errors in parentheses) of regression equations for shoot fresh and dry weight and plant survival response (% of nontreated control) of eight kochia populations (glyphosate-susceptible: Hays, Hanley; glyphosate-resistant: Phillip, Scott, Russell; suspected glyphosate-resistant: AB-F1, AB-F2, AB-F3) to increasing doses of glyphosate under greenhouse conditions. Refer to equations 2 to 4 in text for description of regression parameters.

Population	*d*	*c*	*b*	ED_{50}[a]	R^2	RF[b]
Fresh weight						
Hays, KS	110.6 (11.8)	2.6 (6.5)	2.64 (1.15)	128 (25)	0.95	—
Hanley, SK	96.3 (3.7)	0.1 (2.3)	2.22 (0.35)	162 (13)	0.99	—
Phillip, KS	109.6 (5.6)	0.2 (1.1)	2.01 (0.64)	546 (100)	0.98	3.8
Scott, KS	109.1 (7.5)	0.2 (3.4)	2.04 (1.05)	755 (241)	0.95	5.2
Russell, KS	116.5 (10.8)	1.0 (5.2)	2.94 (2.05)	766 (217)	0.90	5.3
AB-F1	99.5 (6.4)	5.1 (2.5)	3.65 (2.08)	784 (138)	0.95	5.4
AB-F2	113.7 (8.1)	0.1 (1.1)	2.30 (1.11)	607 (152)	0.95	4.2
AB-F3	105.8 (4.0)	2.8 (6.6)	2.93 (0.82)	562 (62)	0.99	3.9
Dry weight						
Hays, KS	113.5 (13.8)	8.7 (7.1)	3.06 (1.79)	118 (25)	0.93	—
Hanley, SK	99.5 (1.5)	2.5 (1.0)	2.11 (0.18)	167 (6)	0.99	—
Phillip, KS	108.9 (6.5)	10.3 (3.2)	1.95 (0.79)	525 (125)	0.97	3.7
Scott, KS	105.4 (9.5)	0.5 (4.2)	1.91 (1.39)	770 (227)	0.93	5.4
Russell, KS	115.4 (10.4)	11.3 (7.8)	3.57 (2.71)	784 (214)	0.90	5.5
AB-F1	108.5 (6.8)	10.8 (3.1)	2.51 (1.47)	857 (244)	0.94	6.0
AB-F2	110.4 (6.4)	14.9 (1.1)	2.39 (1.10)	585 (132)	0.95	4.1
AB-F3	99.2 (2.2)	9.5 (3.9)	2.63 (0.45)	553 (41)	0.99	3.9
Survival						
Hays, KS	101.8 (7.9)		−0.00221 (0.00050)	310	0.99	—
Hanley, SK	104.5 (6.8)		−0.00205 (0.00041)	340	0.99	—
Phillip, KS	101.9 (7.5)	−8.1E-6 (9.6E-7)	−0.012 (0.0021)	1900	0.85	5.8
Scott, KS	105.4 (4.6)	−2.4E-6 (6.0E-6)	−0.041 (0.015)	1260	0.97	3.9
Russell, KS	106.2 (7.0)	−4.9E-6 (9.1E-7)	−0.038 (0.020)	1280	0.94	3.9
AB-F1	100.5 (1.7)	−1.0E-5 (2.1E-6)	0.00013 (0.00007)	2250	0.99	6.9
AB-F2	101.5 (7.8)	−5.6E-6 (1.0E-7)	−0.020 (0.022)	1720	0.84	5.3
AB-F3	103.7 (7.5)	−1.0E-5 (9.8E-7)	−0.014 (0.0021)	1740	0.91	5.4

[a] Abbreviations: AB, Alberta; ED_{50}, effective dose reducing growth or survival by 50% compared with the nontreated control; KS, Kansas; RF, resistance factor (index); SK, Saskatchewan.

[b] ED_{50} of resistant population divided by ED_{50} mean of susceptible populations; standard error not available for survival regression models.

glyphosate dose (Figure 2B; Table 2). Both the Kansas GR populations and Alberta populations had a RF ranging from 4 to 6 (a low to moderate level of resistance). Based on survival response to increasing doses of glyphosate, RF for the three Kansas populations ranged from 4 to 6, whereas RF for the three Alberta populations ranged from 5 to 7 (Figure 3; Table 2). Seedlings of the three Alberta populations survived 900 g ha^{-1}, whereas there were no survivors from the two HS populations at that dose. Therefore, the three Alberta populations are indeed GR, responding similarly as the Kansas populations to increasing dose of glyphosate.

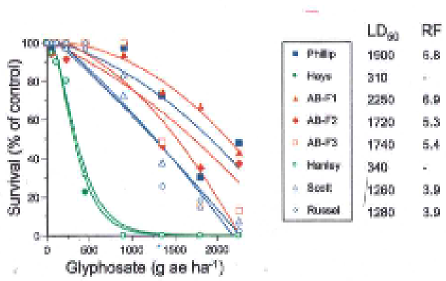

Figure 3. Kochia survival as a function of increasing dose of glyphosate in a greenhouse experiment: susceptible populations, Hanley and Hays; glyphosate-resistant (GR) populations from Kansas, Phillip, Scott, and Russell; and suspected GR populations from southern Alberta, F1, F2, and F3. Other abbreviation: RF, resistance factor, calculated as LD_{50} of a GR population divided by average LD_{50} of the susceptible populations). See text for regression equations and Table 2 for parameter estimates.

For other GR weed species, RF values generally are ≤ 10 (Beckie 2012). Target-site mutation generally confers a lower level of resistance to glyphosate than physiological processes resulting in reduced translocation, although levels can be similar in rigid ryegrass (*Lolium rigidum* Gaudin) (Preston et al. 2009). Regardless, the three Alberta populations would not be controlled in the field by realistic glyphosate application rates. When sampled in August 2011, it was apparent that the kochia populations in the glyphosate-treated chem-fallow fields were likely GR, with linear strips of surviving plants oriented in a southwest to northeast direction (prevailing winds from the southwest) (Figure 4). Kochia was the only weed species present, and it was common to observe live plants next to dead plants throughout the three fields. The three Alberta populations may indeed be a single genotype, with the tumbleweed dispersing seeds across the open landscape as observed previously with ALS-inhibitor–HR kochia. The relative roles of GR kochia seed immigration (gene flow) vs. evolution through glyphosate selection in each of the three fields is presently unknown.

To examine the response of GR kochia to increasing rates of glyphosate under field conditions, a dose–response trial was established in spring 2012 at Lethbridge, AB (the same trial at a site in Saskatchewan was terminated because of poor seedling emergence due to flooding). The trial was arranged in a split-block design with four replications, with glyphosate rate as main plot factor—the same rates as those in the greenhouse experiment plus 2,700 (6×) and 3,150 g ha^{-1} (7×), applied to seedlings 4 cm tall—and kochia population

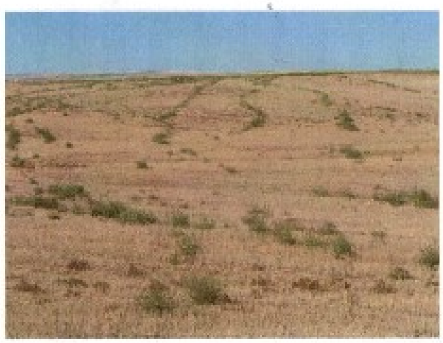

Figure 4. Southern Alberta chem-fallow field in August 2011, where the F1 population was sampled; glyphosate had been applied earlier in the growing season at 570 g ae ha^{-1}.

(GR-F2 and a non-GR population) as split-block factor. The kochia populations were planted May 15 into fallow land at a 0.5-cm depth using a small-plot seeder. The exponential decay model best described the response of aboveground biomass of the two kochia populations to glyphosate (vegetative plants harvested 8 wk after planting). The RF equaled 6.2 based on aboveground biomass dose response of the F2 population (GR_{50} = 330 and 53 g ha^{-1} for GR and non-GR kochia populations, respectively). Although the trial was not successfully repeated in the field, the computed RF was similar to that determined in the greenhouse experiment (RF = 4.1).

GR Kochia Survey. Of the 46 populations screened for glyphosate resistance (900 g ha^{-1}), seven were confirmed as GR (Figure 5). The seven fields had been chem-fallowed or cropped to small-grain cereals in 2011. The frequency of GR plants in a population ranged from 18% (sample point [SP] 21) to 79% (SP22) (Table 3). The site (SP27) farthest from the chem-fallow fields (F1, F2, F3) was located about 13 km to the southeast. All of the sites were located east of the three chem-fallow fields. The same grower farmed fields F1 and SP23; another grower fields F2 and SP20, and another grower fields SP2 and SP22. Therefore, kochia seed may have been spread by farm equipment, in addition to wind. Molecular markers will be needed to determine the relative contribution of evolution through selection and gene flow, primarily seed dispersal.

In addition to the seven confirmed GR kochia populations found in this survey, an additional population more than

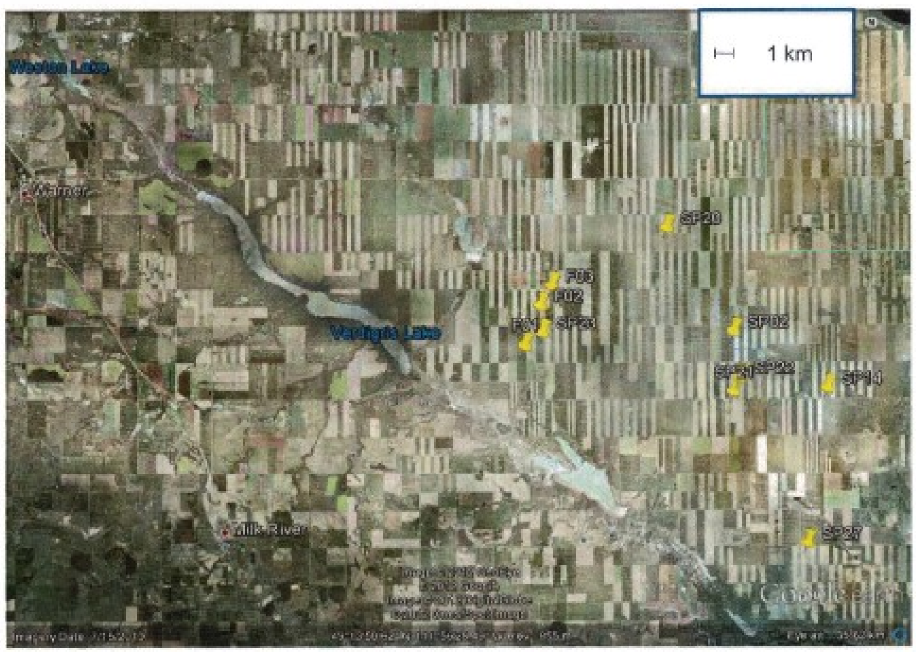

Figure 5. Seven sites (labeled SP) in the County of Warner in southern Alberta in fall, 2011 with confirmed glyphosate-resistant (GR) kochia; F01, F02, and F03 denote the chem-fallow fields where GR kochia was first confirmed.

Table 3. Frequency of glyphosate- and acetolactate synthase (ALS)-inhibitor–resistant plants in Alberta kochia populations.[a]

Population	Glyphosate resistance	ALS-inhibitor resistance
	%	
F1	100	13
F2	100	100
F3	100	90
SP2	71	52
SP14	53	100
SP20	66	85
SP21	18	100
SP22	79	100
SP23	61	90
SP27	65	89

[a] Glyphosate applied at 900 g ae ha^{-1} and thifensulfuron : tribenuron premixture applied at 15 g ai ha^{-1}. A minimum of 100 plants per population were screened with each herbicide.

100 km northwest of these survey populations was recently confirmed as GR. An expanded survey (300 sites) was completed in the fall of 2012 throughout southern Alberta to estimate the prevalence of GR kochia in the region. In the neighboring province of Saskatchewan in 2012, a number of GR kochia populations in chem-fallow fields covering a wide geographic area were confirmed (Beckie, unpublished data). Surveys will be conducted in central and southern Saskatchewan and southern Manitoba in 2013 to determine the incidence of GR kochia.

ALS-Inhibitor Resistance in GR Kochia Populations and Molecular Characterization. When the confirmed GR kochia populations were screened with thifensulfuron : tribenuron premixture at 15 g ha^{-1}, all of them were ALS-inhibitor HR (Table 3). These results were expected, as previous surveys had documented about 90% of Canadian prairie kochia populations exhibiting resistance to ALS-inhibiting herbicides (Beckie et al. 2011b). Most of the GR populations had a high frequency of ALS-inhibitor–HR plants, except sites F1 (13%) and SP2 (52%). However, all populations were susceptible to dicamba, an auxinic herbicide (data not shown). Dicamba-HR kochia has not yet been reported in Canada, although numerous populations are found in the northwestern United States (Heap 2012).

The following amino acid substitutions that previously were known to confer ALS-inhibitor resistance were found in this study: Pro_{197}, Asp_{376}, and Trp_{574} sites (Table 4). Pro_{197} mutations resulting in amino acid substitutions conferring resistance were the following: CCG Pro to CAG Gln, CCG Pro to TCG Ser, and CCG Pro to CGG Arg. At the Asp_{376} site, a T to G mutation resulted in an amino acid substitution of GAT Asp to GAG Glu. At the Trp_{574} amino acid site, a mutation of G to T resulted in an amino acid substitution of TGG Trp to TTG Leu.

In the Alberta F2 population, 11 of the 20 individuals tested had amino acid substitutions conferring ALS-inhibitor resistance (Table 4). One individual in the F2 population was homozygous for Leu_{574}, whereas eight individuals from that population revealed the target-site mutation T(T/G)G, resulting in a Trp/Leu_{574} amino acid substitution. The polymorphism GA(T/G) results in the substitution Asp/Glu_{376}, and was found in two individuals in the F2 population. Three individuals possessed the Pro/Gln_{197} substitution. Three individuals had two amino acid substitutions: two individuals with both Pro/Gln_{197} and Trp/Leu_{574} substitutions, and one individual with both Asp/Glu_{376} and Trp/Leu_{574} substitutions.

In the Alberta F3 population, five of the 20 individuals had the polymorphism C(C/A)G, resulting in the amino acid substitution Pro/Gln_{197}. One individual in the Kansas (Phillip) population had a polymorphism (T/C)CG, resulting in a Pro/Ser_{197} substitution, whereas a polymorphism C(C/G)G resulting in a Pro/Arg_{197} substitution was found in another individual. A third individual revealed the target-site mutation T(T/G)G, resulting in the amino acid substitution Trp/Leu_{574}. As expected, no target-site mutations were found in the 10 sequenced plants of the Hanley HS population (data not shown).

Unexpectedly, no target-site mutations were found in the 20 sequenced plants of the Alberta F1 population (data not shown). The low frequency of ALS-inhibitor–HR individuals in the Alberta F1 population (13%; Table 3) likely explains

Table 4. Acetolactate synthase (*ALS*) target-site mutations in glyphosate-resistant (GR) Alberta kochia populations F2 and F3, and a GR Kansas population, Phillip (total of 20 individuals per population sequenced).[a]

Population	Pro197	Asp376	Trp574
F2	CCG Pro197	GAT Asp	T(T/G)G Trp/**Leu**
F2	C(C/A)G Pro/**Gln**	GAT Asp	TGG Trp
F2	CCG Pro	GA(T/G) **Asp/Glu**	T(T/G)G Trp/**Leu**
F2	CCG Pro	GAT Asp	T(T/G)G Trp/**Leu**
F2	C(C/A)G Pro/**Gln**	GAT Asp	T(T/G)G Trp/**Leu**
F2	CCG Pro	GA(T/G) **Asp/Glu**	TGG Trp
F2	CCG Pro	GAT Asp	T(T/G)G Trp/**Leu**
F2	CCG Pro	GAT Asp	**TTG Leu**
F2	CCG Pro	GAT Asp	T(T/G)G Trp/**Leu**
F2	C(C/A)G Pro/**Gln**	GAT Asp	T(T/G)G Trp/**Leu**
F2	CCG Pro	GAT Asp	T(T/G)G Trp/**Leu**
F3	C(C/A)G Pro/**Gln**	GAT Asp	TGG Trp
F3	C(C/A)G Pro/**Gln**	GAT Asp	TGG Trp
F3	C(C/A)G Pro/**Gln**	GAT Asp	TGG Trp
F3	C(C/A)G Pro/**Gln**	GAT Asp	TGG Trp
F3	C(C/A)G Pro/**Gln**	GAT Asp	TGG Trp
Phillip	(T/C)CG Pro/**Ser**	GAT Asp	TGG Trp
Phillip	CCG Pro	GAT Asp	T(T/G)G Trp/**Leu**
Phillip	C(C/G)G Pro/**Arg**	GAT Asp	TGG Trp

[a] A dash (/) between nucleotides indicates that both nucleotides are present at that position, i.e., heterozygosity.

Table 5. Weed risk assessment of selection for glyphosate resistance in the semiarid Grassland and subhumid Parkland regions of the Canadian prairies (top three weeds in each region in bold font).

			Proportional emergence							
Weed species	RA[a]	Efficacy[b]	Preseed	In-crop1	In-crop-2	Postharvest	Total SP[c]	Seedbank (yr)[d]	SP: In-crop/preseed	Risk rating: SP/seedbank
Grassland region										
Green foxtail[e]	68.8	0.95	0.00	0.85	0.15	0.0	65.4	10	> 60.0	**6.54**
Wild oat	47.3	0.95	0.10	0.80	0.10	0.0	44.9	4.5	9.0	**9.99**
Wild buckwheat	28.9	0.75	0.04	0.94	0.02	0.0	21.7	8	27.6	2.71
Common lambsquarters	10.7	0.90	0.0	1.00	0.0	0.0	9.6	14	> 10.0	0.69
Chickweed	0.4	0.95	0.05	0.90	0.05	0.0	0.4	7.5	19.0	0.05
Field pennycress	16.7	0.95	0.06	0.81	0.12	0.0	15.9	15	15.0	1.06
Redroot pigweed	14.0	0.95	0.0	0.35	0.50	0.15	13.3	15	> 10.0	0.89
Cleavers	1.5	0.90	0.05	0.90	0.05	0.0	1.4	2	19.0	0.69
Kochia	20.9	0.90	0.60	0.40	0.0	0.0	18.8	1	0.7	**18.8**
Wild mustard	2.9	0.95	0.05	0.90	0.05	0.0	2.8	25	19.0	0.11
Parkland region										
Green foxtail	43.0	0.95	0.00	0.85	0.15	0.0	40.9	10	> 40.0	**4.09**
Wild oat	37.1	0.95	0.10	0.80	0.10	0.0	35.2	4.5	9.0	**7.83**
Wild buckwheat	32.1	0.75	0.04	0.94	0.02	0.0	24.1	8	27.6	3.01
Common lambsquarters	13.8	0.90	0.0	1.00	0.0	0.0	12.4	14	> 13.0	0.89
Common chickweed	16.0	0.95	0.05	0.90	0.05	0.0	15.2	7.5	19.0	2.03
Field pennycress	9.2	0.95	0.06	0.81	0.12	0.0	8.7	15	15.0	0.58
Redroot pigweed	7.4	0.95	0.0	0.35	0.50	0.15	7.0	15	> 6.0	0.47
Cleavers	9.0	0.90	0.05	0.90	0.05	0.0	8.1	2	19.0	**4.05**
Kochia	1.9	0.90	0.60	0.40	0.0	0.0	1.7	1	0.7	1.71
Wild mustard	3.7	0.95	0.05	0.90	0.05	0.0	3.5	25	19.0	0.14

[a] RA, relative abundance (derived from Leeson et al. 2005); see text for description.
[b] Based on expert opinion (see Acknowledgments)" preseeding (early May), in-crop1 (early June), in-crop2 (late June), postharvest (September).
[c] SP, selection pressure, calculated as RA × efficacy.
[d] Derived from Van Acker (2009).
[e] Green foxtail, *Setaria viridis* (L.) Beauv.; wild oat, *Avena fatua* L.; wild buckwheat, *Polygonum convolvulus* L.; common lambsquarters, *Chenopodium album* L.; common chickweed, *Stellaria media* (L.) Vill.; field pennycress, *Thlaspi arvense* L.; redroot pigweed, *Amaranthus retroflexus* L.; cleavers, *Galium spurium* L. (false cleavers), or *G. aparine* L. (catchweed bedstraw); wild mustard, *Sinapis arvensis* L.

the lack of detection of target-site mutations in tissue-sampled GR plants.

Risk Assessment of Glyphosate Resistance in Prairie Weeds. Kochia, which emerges early in the growing season, was the only weed examined in which preseed glyphosate selection pressure was greater than in-crop selection pressure (Table 5: column SP: In-crop/preseed = 0.7). In the Grassland region of the prairies, the top three weeds predicted at greatest risk of glyphosate resistance were kochia, wild oat, and then green foxtail. In the Parkland region, wild oat and green foxtail, followed by cleavers species were the top three species. Based on numerous surveys of HR weeds in the prairies since 1988, weed population abundance is a key HR risk factor. The risk rating for kochia was twice that of any other species. Those predictions were originally presented in a poster presented at the 2010 Canadian Weed Science Society annual meeting (Beckie 2010) in response to repeated questions of prairie weeds at greatest risk of glyphosate resistance.

For predicting invasive weed species, a history of invasion elsewhere is probably the best indicator. Thus, the risk of GR kochia in the prairies was elevated following the report of GR kochia in Kansas in 2007 (Heap 2012). Regardless of whether the predictions prove accurate or not (other than kochia), the simple empirical modeling exercise was successful in raising awareness among all regional stakeholders, via the media, of the risk of selection of GR weeds and the urgency of proactive management—which was the original intent of the project.

Herbicides to control ALS-inhibitor (group 2)–HR kochia or group 2 plus glyphosate (group 9)–HR kochia in field crops in Canada are listed in Appendix Table 1—at preseeding (burndown), in-crop, or in chem-fallow situations. Based on previous survey results, growers must assume kochia populations are group 2–HR. There are sufficient alternative herbicides to control group 2 + 9–HR kochia in most cereal crops. However, there currently are no registered in-crop herbicides to control the multiple-HR biotype in mustard, sunflower (*Helianthus annuus* L.), lentil (*Lens culinaris* Medik), chickpea (*Cicer arietinum* L.), dry bean (*Phaseolus vulgaris* L.), soybean, or potato (*Solanum tuberosum* L.). Broadleaf crops with very few alternative herbicides include sugar beet (*Beta vulgaris* L.; phenmedipham + desmedipham, a group 5 herbicide); field pea (*Pisum sativum* L.; MCPA, group 4); and canola (glufosinate, group 10). We are presently exploring various herbicide treatments to control GR kochia under field conditions.

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Literature Cited

Agriculture and Agri-Food Canada. 2003. A National Ecological Framework for Canada: GIS Data. http://sis.agr.gc.ca/cansis/nsdb/ecostrat/gis_data.html. Accessed: May 2012.

Beckie, H. J. 2010. Predicting Prairie Weeds at Risk for Glyphosate Resistance. Page 9 *in* Canadian Weed Science Society Annual Meeting Archives. http://www.weedscience.ca/annual-meeting/archives. Accessed: May 2012. [Abstract]

Beckie, H. J. 2012. Herbicide-resistant weed management: focus on glyphosate. Pest. Manag. Sci. 67:1037–1048.

Beckie, H. J., K. N. Harker, L. M. Hall, F. A. Holm, and R. H. Gulden. 2011a. Risk assessment of glyphosate resistance in western Canada. Weed Technol. 25:159–164.

Beckie, H. J., C. Lozinski, and S. Shirriff. 2009. Alberta Weed Survey of Herbicide-Resistant Weeds in 2007. Saskatoon, SK: Agriculture and Agri-Food Canada Weed Survey Series Publ. 09-1. 36 p.

Beckie, H. J. and F. J. Tardif. 2012. Herbicide cross resistance in weeds. Crop Prot. 35:15–28.

Beckie, H. J., S. I. Warwick, C. A. Sauder, C. Lozinski, and S. Shirriff. 2011b. Occurrence and molecular characterization of acetolactate synthase (ALS) inhibitor-resistant kochia (*Kochia scoparia*) in western Canada. Weed Technol. 25:170–175.

Beckie, H. J., R. M. Weiss, J. Y. Leeson, and O. O. Olfert. 2012. Range expansion of kochia (*Kochia scoparia*) in North America under a changing climate. Pages 33–46 *in* J. A. Ivany and R. E. Blackshaw, eds. Climate Change and the Canadian Agricultural Environment. Topics in Canadian Weed Science. Volume 8. Pinawa, MB: Canadian Weed Science Society.

Bullied, W. J., A. M. Marginet, and R. C. Van Acker. 2003. Conventional- and conservation-tillage systems influence emergence periodicity of annual weed species in canola. Weed Sci. 51:886–897.

Foes, M. J., L. Liu, G. Vigue, E. W. Stoller, L. M. Wax, and P. J. Tranel. 1999. A kochia (*Kochia scoparia*) biotype resistant to triazine and ALS-inhibiting herbicides. Weed Sci. 47:20–27.

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.

Gomez, K. A. and A. A. Gomez. 1984. Statistical Procedures for Agricultural Research. 2nd ed. New York: J. Wiley. 680 p.

Gressel, J. and L. A. Segel. 1982. Interrelating factors controlling the rate of appearance of resistance: the outlook for the future. Pages 325–347 *in* H. M. LeBaron and J. Gressel, eds. Herbicide Resistance in Plants—Biology and Biochemistry. New York: J. Wiley.

Guttieri, M. J., C. V. Eberlein, C. A. Mallory-Smith, D. C. Thill, and D. L. Hoffman. 1992. DNA sequence variation in domain A of the acetolactate synthase genes of herbicide-resistant and -susceptible weed biotypes. Weed Sci. 40:670–676.

Guttieri, M. J., C. V. Eberlein, and D. C. Thill. 1995. Diverse mutations in the acetolactate synthase gene confer chlorsulfuron resistance in kochia (*Kochia scoparia*) biotypes. Weed Sci. 43:175–178.

Heap, I. M. 2012. International Survey of Herbicide Resistant Weeds. http://www.weedscience.org. Accessed: April 2012.

Koutsoyiannis, A. 1977. Theory of Econometrics. 2nd ed. London: MacMillan Education. Pp. 81–91.

Kvalseth, T. O. 1985. Cautionary note about R^2. Am. Statistician 39:279–285.

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, SK: Agriculture and Agri-Food Canada Weed Survey Series Publ. 05-1. 395 p.

Morrison, I. N. and M. D. Devine. 1994. Herbicide resistance in the Canadian prairie provinces: five years after the fact. Phytoprotection 75(Suppl.):5–16.

Preston, C., A. M. Wakelin, F. C. Dolman, Y. Bostamam, and P. Boutsalis. 2009. A decade of glyphosate-resistant *Lolium* around the world: mechanisms, genes, fitness and agronomic management. Weed Sci. 57:435–441.

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

Saari, L. L., J. C. Cotterman, and M. M. Primiani. 1990. Mechanism of sulfonylurea herbicide resistance in the broadleaf weed, *Kochia scoparia*. Plant Physiol. 93:55–61.

Salava, J., D. Chodová, and J. Mikulka. 2004. Molecular basis of acetolactate synthase-inhibitor resistance in Czech biotypes of kochia. J. Plant Dis. Prot. 19:915–919.

SAS. 1999. SAS/STAT User's Guide. Version 8. Cary, NC: SAS Institute. 1243 p.

Saskatchewan Ministry of Agriculture. 2012. Guide to Crop Protection: Weeds, Plant Diseases, Insects. Regina, SK: Saskatchewan Ministry of Agriculture. 482 p. http://www.agriculture.gov.sk.ca/Guide_to_Crop_Protection. Accessed: April 2012.

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

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. 9:218–227.

Sikkema, P. H., D. E. Robinson, F. J. Tardif, M. B. Lawton, and N. Soltani. 2013. Discovery of glyphosate-resistant weeds in Ontario, Canada—distribution plus control. Global Herbicide Resistance Challenge Conference. Perth, Australia. In press [Abstract]

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.

Statistics Canada. 2012. July estimates of production of principal field crops. Field Crop Reporting Series. Catalogue no. 22-002-X. http://www.statcan.gc.ca. Accessed: November 2012.

Steel, G. D. and J. H. Torrie. 1980. Principles and Procedures of Statistics: A Biometrical Approach. 2nd ed. New York: McGraw-Hill. 633 p.

Thomas, A. G. and J. Y. Leeson. 2007. Tracking long-term changes in the arable weed flora of Canada. Pages 43–69 *in* D. R. Clements and S. J. Darbyshire, eds. Invasive Plants: Inventories, Strategies and Action. Topics in Canadian Weed Sci. Volume 5. Sainte Anne de Bellevue, QC: Canadian Weed Science Society.

Thompson, C. R., D. C. Thill, C. A. Mallory-Smith, and B. Shafii. 1994. Characterization of chlorsulfuron resistant and susceptible kochia (*Kochia scoparia*). Weed Technol. 8:470–476.

Van Acker, R. C. 2009. Weed biology serves practical weed management. Weed Res. 49:1–5.

Vink, J. P., N. Soltani, D. E. Robinson, F. J. Tardif, M. B. Lawton, and P. H. Sikkema. 2012. Occurrence and distribution of glyphosate-resistant giant ragweed (*Ambrosia trifida* L.) in southwestern Ontario. Can. J. Plant Sci. 92:533–539.

Warwick, S. I., C. A. Sauder, and H. J. Beckie. 2010. Acetolactate synthase (*ALS*) target-site mutations in *ALS* inhibitor-resistant Russian thistle (*Salsola tragus*). Weed Sci. 58:244–251.

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

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Appendix 2: Survey of glyphosate-resistant kochia (*Kochia scoparia* L. Schrad.) in Alberta

SHORT COMMUNICATION

Survey of glyphosate-resistant kochia (*Kochia scoparia* L. Schrad.) in Alberta

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[1]Agricultural, Food and Nutritional Science, 410 Agriculture/Forestry, University of Alberta, Edmonton, Alberta, Canada T6G 2P5 (e-mail: linda.hall@ualberta.ca); [2]Agriculture and Agri-Food Canada (AAFC), 107 Science Place, Saskatoon, Saskatchewan, Canada S7N 0X2; [3]Agriculture and Agri-Food Canada, Lethbridge Research Centre, P.O. Box 3000, 5403 1st Avenue S., Lethbridge, Alberta, Canada T1J 4B1; [4]Alberta Agriculture and Rural Development, 17507 Fort Road N.W., Edmonton, Alberta, Canada T5Y 6H3; and [5]Alberta Agriculture and Rural Development, 301 Horticultural Station Road E., Brooks, Alberta, Canada T1R 1E6.

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Hall, L. M., Beckie, H. J., Low, R., Shirriff, S. W., Blackshaw, R. E., Kimmel, N. and Neeser, C. 2014. **Survey of glyphosate-resistant kochia (*Kochia scoparia* L. Schrad.) in Alberta**. Can. J. Plant Sci. **94**: 127–130. Glyphosate-resistant (GR) kochia was identified in Warner county in southern Alberta in 2011. To determine the scale of the distribution and frequency of GR kochia, a randomized stratified survey of more than 300 locations (one population per location) in southern Alberta was conducted in the fall of 2012. Mature plants were collected, seed separated, and F_1 seedlings screened by spraying with glyphosate at 900 g a.e. ha^{-1} under greenhouse conditions. Screening confirmed 13 GR kochia sites: seven in Warner county, five in Vulcan county, and one in Taber county. The frequency of GR individuals in a population ranged from 0.3 to 98%. GR kochia were found in arid areas where chemical fallow is a significant component of the rotation. Economic and agronomic impact of this GR weed biotype is compounded because of multiple resistance to acetolactate synthase-inhibiting herbicides.

Key words: Chemical fallow, *Kochia scoparia* (L.) Schrad., glyphosate resistance, multiple herbicide resistance

Hall, L. M., Beckie, H. J., Low, R., Shirriff, S. W., Blackshaw, R. E., Kimmel, N. et Neeser, C. 2014. **Étude de la kochie (*Kochia scoparia* L. Schrad.) résistante au glyphosate en Alberta.** Can. J. Plant Sci. **94**: 127–130. En 2011, des plants de kochie résistants au glyphosate (RG) étaient identifiés dans le comté de Warner, dans le sud de l'Alberta. Pour avoir une meilleure idée de l'importance de la distribution et de la fréquence de la kochie RG, les auteurs ont procédé à une étude stratifiée randomisée à plus de 300 emplacements (une population par emplacement) du sud de l'Alberta, à l'automne 2012. Ils ont recueilli des plants adultes, ont séparé les graines puis sélectionné les plantules en les aspergeant avec 900 g de matière active de glyphosate par hectare, en serre. La sélection a confirmé l'existence de 13 sites où pousse la kochie RG, soit sept dans le comté de Warner, cinq dans le comté de Vulcan et un dans le comté de Taber. La fréquence des plants RG au sein de la population varie de 0,3 à 98 %. La kochie RG a été découverte dans les lieux arides où on recourt abondamment à la jachère chimique dans les assolements. L'impact économique et agronomique de cette adventice RG est d'autant plus important que celle-ci résiste aussi aux herbicides qui inhibent l'acétolactate synthase.

Mots clés: Jachère chimique, *Kochia scoparia* (L.) Schrad., résistance au glyphosate, résistance multiple aux herbicides

Glyphosate is a key herbicide for weed control in chemical fallow in arid to semiarid regions of the prairies, pre-seeding in direct-seeding systems, pre- and post-harvest control, and in glyphosate-resistant (GR) canola (*Brassica napus* L.), soybean [*Glycine max* (L.) Merr.], corn (*Zea mays* L.), and sugar beet (*Beta vulgaris* L.). Glyphosate was first introduced in 1974, and is the most widely used herbicide in the world. Frequent glyphosate use has selected for GR weeds – over 20 weed species in several countries, including eastern Canada (Vink et al. 2012; Heap 2013). Until 2011, GR weeds had not been identified in western Canada.

Kochia is a competitive tumbleweed with early emergence (Schwinghamer and Van Acker 2008), abundant seed production, and tolerant of stress (Friesen et al. 2009). It is one of the most common weeds of southern Alberta, being the fourth most abundant weed in the Mixed and Moist Mixed Grassland ecoregions (Leeson et al. 2005). A C_4 plant, it continues to grow under hot, dry conditions. Kochia is morphologically plastic, and occurs in agricultural areas, waste lands,

Abbreviations: ALS, acetolactate synthase; GR, glyphosate-resistant

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and rangelands. Kochia also matures later than many other weeds, usually after annual crop harvest. Kochia resistant to acetolactate synthase (ALS) inhibitors in the prairies was reported in 1989. Beckie et al. (2011b, 2013b) reported that about 90% of the prairie populations tested were resistant. Resistant genes may be transmitted through pollen movement (Stallings et al. 1995). However, long-distance transport of resistant genes occurs via seed dispersal from mature plants tumbling across the landscape.

GR kochia was first identified in Kansas in 2007 (Waite 2008; Waite et al. 2013), followed by South Dakota in 2009, and Nebraska in 2011; it was selected primarily in GR corn and soybean fields (Heap 2013). In 2011, kochia resistant to glyphosate and ALS inhibitors (multiple herbicide-resistant) was discovered in southern Alberta (Beckie et al. 2013a). Initially, three populations were identified in chemical-fallow fields. A 20-km survey around these sites confirmed an additional seven populations. Resistance level was considered low to moderate, with a resistance factor (ratio of the rates required for 50% control of the resistant and susceptible populations) of 4 to 7. However, resistant plants could not have been controlled in the field by a reasonable rate of glyphosate. To determine the frequency and distribution of GR kochia in southern Alberta, a random survey, stratified by cropped area, was conducted in the fall of 2012.

MATERIALS AND METHODS

Survey Methodology

A random survey of GR kochia was conducted in fall, 2012. The number of populations collected was stratified, proportional to cultivated land area per ecodistrict within the Southern Alberta agricultural extension region, covering four agricultural ecoregions (Leeson et al. 2005). Therefore, the proportional allocation of collection sites in each county was the same as that of the general weed survey. Surveyors drove to 309 predetermined sites during a 3-wk post-harvest survey period in September and October, 2012. Approximately 20 mature kochia plants were randomly collected at each site, and placed in a cotton bag to form a composite sample. A survey form was completed on-site for each population, and a photograph taken with GPS reference. Populations were sampled in field border areas and ruderal areas such as roadsides/ditches, railway rights-of-way, and oil well sites.

Sample Processing and Resistance Screening

Samples were threshed under contained conditions at the University of Alberta in Edmonton, and seed samples sent for screening to Agriculture and Agri-Food Canada, Saskatoon, SK. All remaining material was autoclaved to prevent distribution of kochia on the University of Alberta research station. Samples were also received from growers in Alberta and Saskatchewan who had experienced poor kochia control and were included in screening.

From each population, a minimum of 100 seeds were planted in flats filled with potting soil in a greenhouse, and glyphosate, tribenuron/thifensulfuron, or dicamba was applied at 900, 15 (5+10), and 480 g a.e./ a.i. ha^{-1}, respectively, when seedlings were 3 to 5 cm tall, using a moving-nozzle cabinet sprayer equipped with a flat-fan nozzle tip (TeeJet 8002VS, Spraying Systems Co., Wheaton, IL) calibrated to deliver 200 L ha^{-1} of spray solution at 275 kPa (Beckie et al. 2013a). Three weeks after treatment, plant response to herbicide application was visually scored as susceptible: dead or nearly dead, or resistant: some injury but new growth, or no injury (Beckie et al. 2013a). Assessments were made relative to known herbicide-treated and -untreated susceptible and resistant populations.

RESULTS AND DISCUSSION

Kochia resistant to glyphosate was identified at 13 of 309 sites surveyed (4.2% of fields) (Fig. 1). Seven sites were located in Warner county, where GR kochia was previously confirmed at 10 other sites in a fall, 2011 survey (Beckie et al. 2013a). Five sites were located in Vulcan county to the north, and one site to the east in Taber county. Besides these 13 confirmed sites, 9 sites were also confirmed in Alberta in 2012 from samples submitted by growers: three sites in Warner county, one site in Lethbridge county, four sites in Forty Mile county, and one site in Cypress county (Fig. 2). Moreover, 10 kochia samples submitted by growers in west-central and southwestern Saskatchewan that year were confirmed as GR (Fig. 2). In this survey, two of the locations where GR kochia was found were non-agricultural areas (ditch and railway rights-of-way) adjacent to agricultural areas (Table 1). Kochia can be an abundant weed in ruderal areas of southern Alberta where glyphosate may be used for non-selective weed control.

The frequency of glyphosate resistance in confirmed populations varied from 0.3 to 98% (Table 1). Differences may be due to the time since glyphosate resistance was selected or introduced (either via seed or pollen), the amount of glyphosate selection that occurred in that population over time, or the amount of selection that had occurred in 2012 to reduce the frequency of susceptible individuals in the population. It should be noted that even though glyphosate resistance was at low levels in some samples, that frequency would be expected to increase with the use of glyphosate applied alone.

Only three populations tested were < 50% resistant to the ALS-inhibiting herbicide tribenuron/thifensulfuron (Table 1). High frequency of resistance to ALS inhibitors in kochia populations had been previously reported in Alberta (Beckie et al. 2011b). None of the GR populations were resistant to dicamba, suggesting that dicamba can control GR kochia prior to seeding of most cereal crops and in chemical fallow. Dicamba-resistant kochia has not been identified previously in

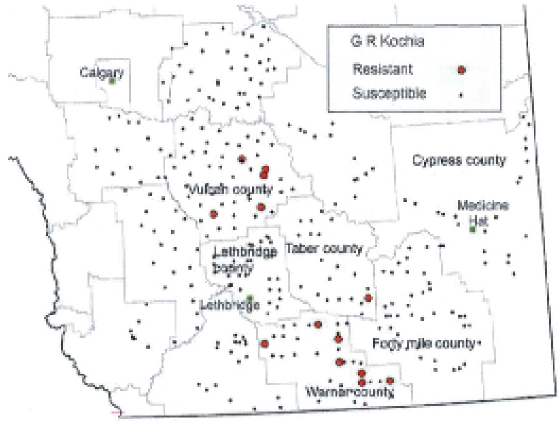

Fig. 1. Location of glyphosate-resistant (GR) (large red circle) and glyphosate-susceptible (small black circle) kochia, surveyed in fall, 2012: seven populations in Warner county, five populations in Vulcan county, and one population in Taber county.

Alberta, but has been reported in the midwestern USA (Cranston et al. 2001; Preston et al. 2009).

In Vulcan, Taber, and Warner counties, where resistant populations were located, frequent chemical fallow (strip cultivation) is practiced. In chemical fallow, glyphosate is typically applied alone, and at multiple times during the fallow year to control vegetation. Kochia is a very abundant weed in these arid locations. The combination of frequent applications of a single herbicide on an abundant weed population has led to selection of herbicide resistance in other parts of the world. Unfortunately, kochia will not be confined to

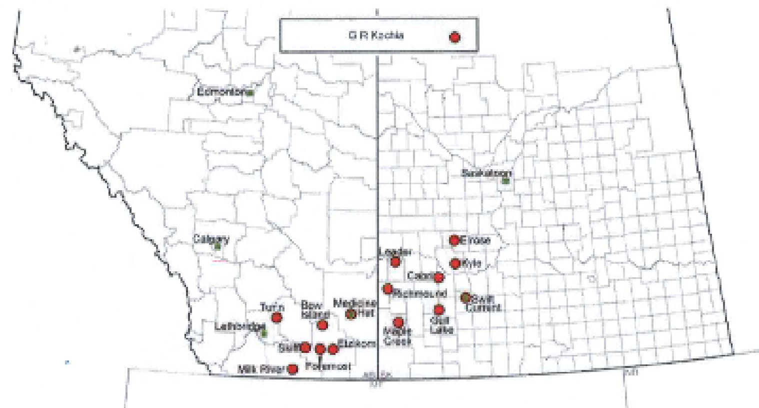

Fig. 2. Glyphosate-resistant (GR) kochia confirmed in 2012 from samples submitted by growers. Note: the site at Milk River, Alberta, represents three confirmed fields; the site at Cabri and Kyle, Saskatchewan represent two confirmed fields each.

Table 1. Percentage of plants in a population resistant (R) to glyphosate, dicamba, or tribenuron/thifensulfuron, and the habitat and Alberta county where populations were located

Site	County	Habitat	Glyphosate-R	Dicamba-R	Tribenuron/ thifensulfuron-R
1	Warner	Field	98	0	20
2	Vulcan	Field	95	0	1
3	Vulcan	Ditch	85	0	98
4	Vulcan	Field	85	0	75
5	Warner	Field	80	0	70
6	Vulcan	Field	80	0	80
7	Warner	Field	70	0	15
8	Taber	Field	50	NA[z]	NA
9	Warner	Field	50	0	98
10	Warner	Field	50	0	98
11	Vulcan	RR[y]	10	0	55
12	Warner	Field	0.7	0	98
13	Warner	Field	0.3	0	50

[z]NA, data not available due to limited sample size.
[y]Railway right-of-way.

areas where it was selected, as it is capable of moving long distances and infesting other areas – agricultural, industrial, and waste areas.

This survey shows that GR kochia has established in discontinuous areas in southern Alberta where chemical fallow (strip cultivation) is practiced. Because of wind dispersal, GR kochia is an imminent threat for growers in southern Alberta who practice chemical fallow, direct-seeding, or grow GR sugar beet, corn, or canola. All GR populations tested for ALS-inhibitor resistance were resistant to the sulfonylurea herbicide tribenuron/thifensulfuron; cross-resistance would probably occur to triflusulfuron, marketed as Upbeet®, the only other herbicide that effectively controls kochia in sugar beet. In areas where GR kochia may already be present, glyphosate should not be used alone for pre-seeding or chemical fallow weed control.

Kochia was the first of several species predicted to be at risk for glyphosate resistance (Beckie et al. 2011a, 2013a). Other abundant species, selected during pre-seeding applications or present in large numbers in chemical-fallow fields are also at risk, including wild oat (*Avena fatua* L.), green foxtail (*Setaria viridis* L. Beauv.), and wild buckwheat (*Polygonum convolvulus* L.) (Beckie et al. 2013a). Like kochia, these weeds have already been selected for resistance to herbicides with different modes of action used in-crop. Worldwide, the incidence of multiple-resistant weed biotypes is increasing at an alarming rate. Across the prairies, multiple-resistant weeds will continue to challenge growers and agronomists, especially when one of those modes of action is glyphosate.

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

Beckie, H. J., Harker, K. N., Hall, L. M., Holm, F. A. and Gulden, R. H. 2011a. Risk assessment of glyphosate resistance in western Canada. Weed Technol. **25**: 159–164.

Beckie, H. J., Lozinski, C., Shirriff, S. and Brenzil, C. A. 2013b. Herbicide-resistant weeds in the Canadian Prairies: 2007 to 2011. Weed Technol. **27**: 171–183.

Beckie, H. J., Warwick, S. I., Sauder, C. A., Lozinski, C. and Shirriff, S. 2011b. Occurrence and molecular characterization of acetolactate synthase (ALS) inhibitor–resistant kochia (*Kochia scoparia*) in western Canada. Weed Technol. **25**: 170–175.

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

Friesen, L. F., Beckie, H. J., Warwick, S. I. and 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. 2013. International survey of herbicide resistant weeds. [Online] Available: http://www.weedscience.org [2013 Jul. 15].

Leeson, J. Y., Thomas, A. G., Hall, L. M., Brenzil, C. A., Andrews, T., Brown, K. R. and Van Acker, R. C. 2005. Prairie weed surveys of cereal, oilseed and pulse crops from the 1970s to the 2000s. Weed Survey Series Publ. 05-1. Agriculture and Agri-Food Canada, Saskatoon, SK. 395 pp.

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

Schwinghamer, T. D. and 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. and Shafii, B. 1995. Pollen-mediated gene flow of sulfonylurea-resistant kochia (*Kochia scoparia*). Weed Sci. **43**: 95–102.

Vink, J. P., Soltani, N., Robinson, D. E., Tardif, F. J., Lawton, M. B. and Sikkema, P. H. 2012. Glyphosate-resistant giant ragweed (*Ambrosia trifida*) control in dicamba-tolerant soybean. Weed Technol. **26**: 422–428.

Waite, J. C. 2008. Glyphosate resistance in kochia. M.Sc. dissertation. Kansas State University, Manhattan, KS. 31 pp.

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