Herbicide Strategies for Control of Glyphosate-Resistant and -Susceptible Kochia (Bassia

scoparia) in Chemical Fallow and Spring Wheat

by

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Abstract

Kochia [Bassia scoparia (L.) A.J. Scott], the first known glyphosate-resistant weed in western Canada, is an abundant and troublesome summer annual tumbleweed. Yet, knowledge gaps exist in kochia management, specifically as to what herbicide and herbicide mixes are effective to control glyphosate-resistant (GR) and glyphosate-susceptible (GS) kochia in chemical fallow and spring wheat (Triticum aestivum L.). Kochia's tolerance to saline soils, drought, and heat, as well as its ability to emerge early with multiple flushes, rapid growth, and late maturation, all contribute to its reproductive success and geographical expansion. This thesis research consisted of two field studies conducted in Alberta, Canada, from 2013 to 2015 and aimed to discover effective herbicidal control for GR and GS kochia in chemical fallow and spring wheat in western Canada. The most consistent control in chemical fallow ($\geq 80\%$ visual control in all environments with \geq 80% biomass reduction in 2014) was observed with glyphosate + dicamba, glyphosate + dicamba/diflufenzopyr, glyphosate + saflufenacil, and glyphosate + carfentrazone + sulfentrazone. Reduced efficacy was observed for several herbicide mixtures when they were applied to GR compared with GS kochia accessions. Effective modes of action mixed with glyphosate include synthetic auxins (group 4), a combination of a synthetic auxin and an auxin transport inhibitor (group 19), or protoporphyrinogen oxidase inhibitors (group 14). The most effective and consistent treatments for kochia management in spring wheat included sulfentrazone applied pre-emergence and fluroxypyr/bromoxynil/2,4-D or pyrasulfotole/bromoxynil applied post-emergence. All of these treatments resulted in \geq 90% visible control in all environments and \geq 90% kochia biomass reduction compared with the untreated control in Lethbridge 2014 and 2015.

MCPA/dichlorprop-p/mecoprop-p, dicamba/2,4-D/mecoprop-p, and dicamba/fluroxypyr resulted in acceptable control among environments (\geq 80% visible control in all environments and \geq 80% kochia biomass reduction in Lethbridge 2014 and 2015); however, the latter two options caused unacceptable (\geq 10%) wheat visible injury in Coalhurst 2014. Confirmations of auxinic herbicideresistant kochia in western Canada, partly due to their increase of use on GR kochia in spring grains, will limit these herbicide options. If designed appropriately, an integrated herbicide program for kochia including mixing, rotating, and layering alternative herbicide modes of action could help mitigate further selection for herbicide resistance.

Preface

This document is the original work of the candidate with editorial assistance by Dr. Charles M. Geddes. Dr. Linda M. Hall was responsible for the experimental design and financial management of the research program. Chapters 3 and 4 were conducted with Dr. Robert E. Blackshaw's technical staff in Lethbridge, AB, and with Dr. Bill Hamman's technical staff in Coalhurst, AB. The candidate assisted in trial establishment, data collection, and harvest. The candidate was responsible for compilation of data and manuscript preparation with editing and suggestions from Dr. Charles M. Geddes.

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Foreward

The thesis includes an introduction (Chapter 1), literature review (Chapter 2), two research chapters (Chapters 3 and 4), and a general discussion and conclusions (Chapter 5). Chapters 3 and 4 include work conducted at Lethbridge Agriculture and Agri-Food Canada (AAFC) and Hamman AG Research in Coalhurst in 2013, 2014, and 2015. The research chapters are written in the format of the Canadian Journal of Plant Science and follow the style defined by the Faculty of Agricultural, Life & Environmental Sciences, University of Alberta, Edmonton, AB.

Chapter One: Introduction

1.1. Background and Research Objectives

Kochia [Bassia scoparia (L.) A.J. Scott] is a troublesome, summer-annual tumbleweed that is found throughout the Great Plains of North America on agriculture lands and ruderal areas. Kochia is presently the 15th most abundant weed among annual crops in Alberta and Saskatchewan, and the most abundant weed in the mixed grassland ecoregion of Alberta (Leeson 2016; Leeson et al. 2019). It was introduced to North America from Eurasia as an ornamental garden forb (Friesen et al. 2009), but its invasive attributes have facilitated its northward expansion that is only limited by growing season length (Beckie et al. 2012). Kochia's weedy characteristics include early spring germination, prolonged season periodicity, rapid growth, high genetic variation, tolerance to abiotic stresses (drought, heat, and acid soil), protogynous flowering, prolific seed production, and long-distance seed dispersal by a wind-blown tumbleweed (Schwinghamer and Van Acker 2008; Beckie et al. 2016; Endo et al. 2014; Friesen et al. 2009; Bilski and Foy 1988; Mengistu and Messersmith 2002). Due to kochia's high level of genetic variation and the high amount of selection pressure from herbicides (e.g., glyphosate), kochia has adapted and can exhibit resistance to up to four herbicide modes of action (Heap 2021). Glyphosate is used frequently in westem Canada in no-till chemical fallow, pre-seed, post-emergence in glyphosate-tolerant crops, and preand post-harvest (Powles 2008; Benbrook 2016; Martin et al. 2017). Western Canada has an unprecedented rate of spread of glyphosate-resistant (GR) kochia. Glyphosate-resistant kochia increased from about 4% to 50% of kochia populations surveyed in Alberta between 2012 and 2017 (Beckie et al. 2013; Hall et al. 2014; Beckie et al. 2019). Herbicide-resistant kochia has become difficult to manage successfully using herbicides in southern Alberta.

Wheat is the most seeded crop by area in the Canadian prairies where it was grown on about 9.4 million hectares in 2020 (Statistics Canada 2020). Many farmers in the dry regions of southern Alberta rotate among spring wheat (Triticum aestivum L.) and canola (Brassica napus L.) A fallow year is favorable in semi-arid regions to retain or build up soil moisture for the subsequent cash crop. Using conventional tillage for weed control in a fallow season may require 3 to 5 passes with a field cultivator and can cause wind and water erosion, soil fertility loss, soil salinization, and decrease of soil organic matter (Haas et al. 1974; Action and Gregorich 1995; Campbell et al. 1990). Growers have rapidly adopted no-till systems in the prairie provinces of Alberta, Saskatchewan, and Manitoba (Geddes 2019) and are using effective, non-selective, and broad-spectrum herbicides (e.g., glyphosate) for weed control instead of tillage. Some benefits of chemical fallow are: an increase in soil moisture available to the subsequent cash crop, maintaining crop residue on the soil surface, and allowing for a period of mineralization making nutrients more available for plant uptake (Campbell et al. 1990; Tanaka et al. 1987; Wicks and Smika 1973; Fenster et al. 1965; Lindwall and Anderson 1981). However, the cost of multiple herbicide applications is the high selection pressure applied to weeds causing adaptations resulting in herbicide resistance. There ways in which herbicide use can be optimized to help mitigate the selection pressure for herbicide-resistant weeds, including: herbicide mixtures, rotation, layering, and site-specific applications (Beckie and Harker 2017). Herbicide-resistant weed management tools used in conjunction with non-herbicide integrated weed management (IWM) (cultural and mechanical) strategies can help decrease the rate of HR in kochia (Ball et al. 1992; Blackshaw 1990; Zorner et al. 1984).

There is research on kochia abundance and distribution on the Canadian prairies, but few reports compare effective herbicides and herbicide mixtures for GR and glyphosate-susceptible (GS) kochia control in chemical fallow and spring wheat. The overall objectives of this thesis were to (a) summarize the existing literature, (b) identify current knowledge gaps, and (c) fill some of the most pertinent knowledge gaps for effective control of GR and GS kochia in chemical fallow and spring wheat in western Canada. The research gaps were addressed through the design and implementation of two specific field research experiments conducted in Lethbridge and Coalhurst, Alberta, Canada from 2013 to 2015. The main objectives of this research were:

- Determine which herbicides and herbicide mixtures remain effective for control of GR and GS kochia in chemical fallow fields in western Canada (Chapter 3)
- Determine which herbicide options remain effective for control of GR and GS kochia in spring wheat in western Canada (Chapter 4)
- Determine whether herbicide efficacy differs among GS and GR kochia (Chapters 3 and 4)

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Chapter Two: Literature Review

2.1. Kochia Biology

2.1.1. Distribution and Abundance

Kochia [Bassia scoparia (L.) A.J. Scott] is a problematic weed that is disseminated throughout the U.S. Great Plains and Canadian prairies. Brought over from Eurasia as an ornamental garden plant and introduced into North America in the 1800s, its northern expansion continues to grow but is limited by growing season length (Friesen et al. 2009; Beckie et al. 2012). In the United States, kochia is found in the Central Valley, San Francisco Bay region, Central and South Coast, Mojave and Sonoran deserts, the Great Basin (up to 1500m in elevation), California (spreading into), most contiguous states (except Maryland), and a few southern states (DiTomaso and Healy 2007). In Canada, kochia is found in all provinces except for Newfoundland and Labrador, but most populations are concentrated in the prairie provinces of Alberta, Saskatchewan, and Manitoba (Friesen et al. 2009; Meades et al. 2000). Kochia has many invasive weedy characteristics that aid its rapid geographic spread including early spring germination, prolonged emergence periodicity, rapid growth, prolific seed production, and efficient long-distance seed dispersal (Schwinghamer and Van Acker 2008; Beckie et al. 2016). Kochia had the highest rate of spread compared with 40 other invasive weeds in the northwestern United States (Forcella 1985). Kochia is found in agricultural and non-agricultural areas namely annual crops, perennial forages, hay fields, rangelands, roadsides, railways, and oil well sites (Friesen et al. 2009).

Kochia is an abundant weed in western Canada. In the Mixed Grassland ecoregion of Alberta, kochia was the most abundant weed among annual field crops after post-emergence herbicide application, and the 2nd most abundant weed in spring wheat (Leeson et al. 2019). Kochia was the 7th most abundant midseason weed among annual crops in the Mixed Grassland ecoregion of Saskatchewan (Leeson 2016a). In Manitoba, kochia was the 20th most abundant midseason weed in the Aspen Parkland ecoregion and Southwest Crop Reporting District on average, but was more prevalent in certain districts (e.g., 5th most abundant weed in annual crops in Brenda-Waskada, Boissevain-Morton & Deloraine-Winchester, 6th most abundant in annual crops in Elton & Cornwallis, and 7th most abundant in annual crops in Grassland in the Southwest Crop Reporting District) (Leeson et al. 2016b).

2.1.2. Morphology

Kochia seeds, cotyledons, and basal rosettes vary widely among kochia accessions. Kochia seeds are small (2-3mm long), rough, flat, and are ovate shaped that are enclosed within a fragile hull (Mulugeta 1991). Kochia cotyledons are short, narrow, and commonly have a distinguishing bright pink underside (Friesen et al. 2009). A basal rosette forms with many linear leaves that are covered in soft hairs (Bubar et al. 2000).

The juvenile to mature kochia plant is phenotypically plastic, and plant morphology at these growth stages is dependent on the environment (light, stand density, and competition) (Barnes et al. 1990; Becker 1978; Mulugeta 1991). Multiple biotypes within the same field can differ in phenotype (Stallings et al. 1995). As a juvenile, kochia has a branched stem with sessile, alternate leaves and a grey/green stem that can have distinguishing red stripes when mature (Bubar et al. 2000). It forms into a bushy to pyramid-shaped, 0.15 m to 2 m tall plant with a ~5 m long taproot and ~7m long lateral roots (Davis et al. 1967; Friesen et al. 2009). Small, green, inconspicuous flowers develop that are sessile and clustered in groups of two to six (Friesen et al. 2009). Kochia has two different recognized morphological types, weedy and ornamental. The

ornamental type is shorter (0.5 to 0.8m tall compared to the 0.15 to >2.0m tall weedy type) and its appearance resembles a small, sheared evergreen tree with dense, pyramidal foliage that turns a bright purple/red in the fall (Friesen et al. 2009). The weedy type is generally taller and less dense with fewer branches.

2.1.3. Seed Persistence, Viability and Germination

Kochia does not have a persistent seed bank in the soil because the seed exhibits little-tono dormancy. Kochia seed generally does not persist in soil for more than a year or two (Beckie et al. 2018; Burnside et al. 1981; Schwinghamer and Van Acker 2008). In the Central Great Plains, the majority of kochia seed (>95%) did not persist for more than 2 years (Dille et al. 2017). Kochia seed burial depths (0, 2.5, and 10 cm) did not have an effect on seed viability in the Central Great Plains (>80% of kochia seeds were viable when exhumed after 6 months of burial, but this declined to 5% seed viability when exhumed after 1 year) (Dille et al. 2017), or in the Northern Great Plains (seeds buried at 2.5 cm and 10 cm depths did not lose viability compared with unburied seeds) (Beckie et al. 2018). Kochia seeds can germinate rapidly (within hours) under favorable conditions (Zorner et al. 1984; Lodhi 1979). Kochia germinates best on the soil surface and germination declines as seed depth increases (Everitt et al. 1983).

2.1.4. Emergence and Growth

Kochia emergence timing is dependent on soil moisture, soil temperature, and thermal time (growing degree days, GDD). The majority of kochia in the Great Plains region emerges in late March/early April within a few distinct emergence flushes (taking advantage of the spring moisture or are concomitant with precipitation), but kochia continues to emerge in lesser amounts throughout the growing season (Anderson and Nielsen 1996; Geddes and Davis 2021; Kumar et al. 2018). Kochia has shown an average date of emergence when the daily soil temperature is

between 3°C and 8°C (Nussbaum et al. 1985). Kochia biotypes along the north south transect in the Great Plains differ in thermal time required to emerge (cumulative GDD required for 10% emergence) and emergence duration (Kumar et al. 2018). Biotypes from Kansas required 168 cumulative GDDs (early March) for 10% emergence, while biotypes from Wyoming and Nebraska needed 90 (late March) (Dille et al. 2017). Biotypes from Manitoba had an initial first flush of emerged seedlings after 50 GDDs (at $T_{base} 0^{\circ}$ C) based on a 30-year average (Schwinghamer and Van Acker 2008). In southern Alberta, kochia emergence often takes place between early April to late June, but seedlings have been identified until late August (A.T. Torbiak, personal observation).

Kochia grows rapidly and aggressively (Christofoleti et al. 1997) and is tolerant to many abiotic stresses which make it a favorable forage crop (otherwise known as 'poor man's alfalfa') that grows well in unproductive areas of fields. Kochia is similar to alfalfa in regard to being highly palatable, having a high protein content, and production amount (Bell et al. 1972; Nair et al. 2021; Sherrod 1971). In semi-arid environments with saline soils, kochia is a productive dryland forage crop due to its high water-use efficiency as a C₄ plant and its salt tolerance (facultative alkali halophyte) (Endo et al. 2014; Friesen et al. 2009). Kochia is also tolerant to acid soil factors (Al and Mn) that can be toxic to other plants (Bilski and Foy 1988).

2.1.5. Flowering, Pollination and Genetic Diversity

Flowering time and duration vary among kochia biotypes. Kochia is a short-day plant and initiates flowering when the photoperiod is shorter than 13 to 15 hours, generally 8 to 14 weeks after emergence (Bell et al. 1972). Kochia flowers indeterminately for about 53 to 54 days but varies within species (different ecotypes are adapted to certain ecosystems) and to environmental factors, such as light, temperature, soil fertility, and moisture. (Bell et al. 1972; Mulugeta 1991; Stallings et al. 1995).

Kochia is primarily self-pollinated but can have substantial outcrossing (Guttieri et al. 1998). Protogynous flower development increases the chance of genetic exchange, with the stigmas emerging about one week before the anthers (female reproductive organs mature before male reproductive organs) (Stallings et al. 1995). This provides the opportunity for cross pollination with other kochia plants. An abundance of pollen is produced for 5 to 10 days and is dispersed by wind and bees (Beckie et al. 2016; Mulugeta 1991). Kochia pollen is viable for less than one day to 12 days depending on environmental conditions and is usually deposited less than 154 m from the pollen source (Mulugeta et al. 1994).

Kochia has a high level of genetic diversity and maintains this diversity despite herbicide selection pressures (Martin et al. 2020). Some accessions of kochia have shown the degree of genetic variability within populations to be the same as among populations, and other accessions have shown within population genetic variability to account for the majority of variation (Dyer et al. 1993; Mengistu and Messersmith 2002). Since kochia has high levels of gene flow through seed and pollen transfer, it is able to maintain its diverse genome even with abundant and frequent selection pressure from herbicides in cropping systems.

2.1.6. Maturity and Seed Production

Fully mature kochia plants abscise at the base of the stem creating a tumbleweed that is blown in the wind and disperses seeds over long distances. Kochia matures late and has a long growing season compared to other summer-annual weeds found on the prairies (Nussbaum et al. 1985). About 3% of kochia seeds fall off the mother plant onto the soil beneath (over a two-year average of 12 plants per year in Lethbridge AB and Scott SK, 2.9% of total seed produced by kochia plant fell off the mother plant onto the soil surface) (Beckie et al. 2016), and the remaining seed often gets dispersed by the tumbleweed over long distances. In Montana, kochia start to tumble in early October (Mulugeta 1991). Moderate wind speed can abscise the senesced mature kochia plant (40 to 48 km hr⁻¹ wind with a kochia plant at 5-10% moisture content level has a mean force of 223.4 kg cm⁻²) (Becker 1978). Under greater soil moisture conditions, greater force is required to abscise the kochia stem (Baker et al. 2008). Wind dispersal distance of tumbleweeds is affected by soil surface condition and the tumbleweed can travel at speeds of up to 300 cm s⁻¹ dispersing most seeds over the first km traveled (Beckie et al. 2016; Mulugeta 1991).

Since kochia matures late in the growing season (Bell et al. 1952; Nussbaum et al. 1985), it is often green and immature when summer annual crops are harvested leaving behind low, green lateral branches that can regrow following early harvest dates. Following decapitation due to harvest in late July to September, kochia can regrow reducing harvest efficiency and adding seed to the soil seedbank (Mickelson et al. 2004).

A mature kochia plant can produce a prolific quantity of seeds. The number of seeds produced per plant is dependent on weather (warmer, drier weather can increase seed production) (Stallings et al. 1995), inter- and intra-specific competition, and stand density (A.T. Torbiak, personal observation). Non-competitive kochia plants in the field or greenhouse can produce up to 100,000 seeds plant⁻¹ with a normal range between 12,000 and 25,000 seeds plant⁻¹ (Stallings et al. 1995; Thill and Mallory-Smith 1996; Thompson et al. 1994; Watson et al. 2001) and over 2.5 million seeds m⁻² (Nussbaum et al. 1985).

2.1.7. Herbicide Resistance

High selection pressure through repeated use of the same or similar herbicide (Regehr and Morishita 1989) in combination with high adaptive potential of kochia have contributed to the rapid evolution of herbicide resistance in this species. The high degree of genetic diversity in kochia is maintained via seed- and pollen-mediated gene flow (Beckie et al. 2016). Rapid evolution in response to recurrent selection pressure is facilitated by quick population turnover of kochia in the soil seedbank due to short seed longevity (Beckie et al. 2018), prolific seed production in combination with tumbleweed seed dispersal, and outcrossing caused by protogynous flowering (Becker 1978; Stallings et al. 1995; Beckie et al. 2016; Mulugeta 1991). Resistance traits can be spread by seeds or pollen (Thompson et al. 1994).

Kochia can exhibit resistance to up to four herbicide modes of action in North America (Heap 2021). The first herbicide-resistant (HR) kochia documented in North America was found along railways in Kansas in 1976, where atrazine (group 5, photosystem II (PSII) inhibitor) was applied at high rates for broad-spectrum weed control (Bandeen et al. 1982; Heap 2021). Photosystem II inhibitor resistance in kochia is not known to occur in Canada. Kochia that was resistant to chlorsulfuron and metsulfuron-methyl (acetolactate synthase (ALS)-inhibiting herbicides; group 2) was confirmed first in wheat in Kansas and North Dakota in 1987 (Primiani et al. 1989). In 1988, the first ALS inhibitor-resistant kochia in Canada was found in Manitoba and Saskatchewan, and then the following year in Alberta (Morrison and Devine 1994). A 2007 kochia survey of the Canadian prairies found that 85% of kochia sampled in fields was ALS inhibitor resistant (Beckie et al. 2011b). The first kochia resistant to glyphosate [the only 5enolpyruvylshikimate-3-phosphate synthase (EPSPS)-inhibiting herbicide; group 9] was confirmed in Kansas in 2007 (Waite 2008). In 2011, the first multiple HR kochia - resistant to both ALS inhibitors and EPSPS inhibitors - was documented in Canada, and a survey the following year determined that 4% of the kochia populations sampled in Alberta were glyphosate-resistant (GR) (Beckie et al. 2013, Hall et al. 2014). Five years later, a kochia survey found that 50% of kochia populations sampled in Alberta were GR, which represents an unprecedented rate of spread

of HR traits over a short timeframe (Beckie et al. 2019). A similar increase in GR kochia (from 1% to 58% of sampled populations) was observed in Manitoba between 2013 and 2018 (Beckie et al. 2015; Geddes et al. 2022a). Synthetic auxin (group 4)-resistant kochia (resistant to dicamba and fluroxypyr) was first reported in Montana in 1994 (Cranston et al. 2001; Heap 2021). Synthetic auxin-resistant kochia in Canada was first identified (with multiple resistance with ALS inhibitors) in Saskatchewan (Heap 2021), and then in AB in 2017 (resistant to both dicamba and/or fluroxypyr) which also ended up being the first triple-resistant kochia in Canada (resistant to ALS inhibitors, synthetic auxins, and the EPSPS-inhibiting herbicide glyphosate) (Beckie et al. 2019; Geddes et al. 2022b, 2022c). In 2013, the first four-way resistant kochia [resistant to atrazine (PSII inhibitor), chlorsulfuron (ALS inhibitor), dicamba (synthetic auxin), and glyphosate (EPSPS inhibitor)] was identified in Kansas corn fields (Heap 2021; Varanasi et al. 2015).

2.1.8. Mechanism of Glyphosate Resistance

Glyphosate (first introduced in 1974) is the most widely used herbicide in the world, often called a 'once-in-a-century' herbicide due to its broad-spectrum weed efficacy, low mammalian toxicity, environmentally benign nature, and low economic cost (Duke and Powles 2008). However, glyphosate-resistant weeds have evolved (including GR kochia) due to widespread, frequent, and abundant use of glyphosate for non-selective pre-seed weed control and repeated usage (multiple times a season) in glyphosate-tolerant crops (Powles 2008; Benbrook 2016; Martin et al. 2017). Based on grower management surveys, glyphosate use between about 1997 and 2007 increased by 5X, 16X, and 4X, in Alberta, Saskatchewan and Manitoba, respectively (Geddes 2019).

Glyphosate's (N-(phosphonomethyl) glycine) physiochemical properties allow it to be taken up rapidly through plant surfaces through diffusion (or possibly a phosphate transport protein). It is a water soluble, weak acid that gets trapped in the cell and moves passively with sugars in the phloem in a source to sink direction (it must translocate from leaf to the meristematic tissues to be effective) (Hall et al. 1999; Siehl and Roe 1997). Glyphosate enters the cytoplasm of the plant cell and is transported into the chloroplast to the target site where the EPSPS protein is located.

Glyphosate inhibits the EPSPS enzyme of the shikimate pathway. In this pathway, EPSPS catalyzes the reaction where the enolpyruvoyl group from PEP is transferred to the 5-hydroxyl of shikimate-3-phosphate (S3P) to form EPSP and inorganic phosphate (Pi) (Alibhai and Stallings 2001). Glyphosate is a transition state analog of phosphoenolpyruvate (PEP) and competes with PEP to bind to EPSPS. When glyphosate binds to EPSPS, it inhibits the enzyme from synthesising the aromatic amino acids phenylalanine, tyrosine, and tryptophan (Steinrucken and Amrhein 1980; Alibhai and Stallings 2001). This causes two toxic effects within the cell, 1) depletion of aromatic amino acids for protein synthesis, and 2) the increase of carbon to the shikimate pathway leaves a shortage of essential carbon for other plant pathways (Herman and Weaver 1999) and results in chlorosis and necrosis of the treated weed.

Glyphosate has 4 resistance mechanisms identified to date which include altered target site mutation (mutations changing one or two amino acids in EPSPS), reduced glyphosate translocation, compartmentalization/sequestration (in a vacuole), and overproduction of EPSPS (extra gene copies are produced requiring more glyphosate to cause complete inhibition); the latter of which is found in kochia (Gaines et al. 2019; Hall et al. 1999). Kochia populations have been found with 3 to 16 extra EPSPS gene copies that occur from a tandem duplication of 45-70 kbp at a single locus and may have been triggered by insertion of a mobile genetic element (Gains et al. 2019; Godar et al. 2015; Wiersma et al. 2015).

2.1.9. Integrated Chemical Management

Herbicide-resistant weed management (HRWM) strategies are needed to decrease herbicide selection pressure due to the rapid evolution of kochia populations in North America, now resistant to four herbicide modes-of-action (PSII inhibitors, ALS inhibitors, EPSPS inhibitors, and synthetic auxins) (Heap 2021). Herbicides in these groups are routinely used for weed control in chemical fallow and annual production systems. Integrated chemical management through mixing (single application with different modes of action), rotating (multiple applications among years with different modes of action), or layering (multiple applications in a single year with different modes of action) effective herbicide modes of action can help extend the longevity of these herbicides to aid in future weed control efforts (Beckie and Harker 2017). Customizing these strategies to the agro ecoregion is important due to different soil types, climate, crop rotations, and production systems.

2.1.10. Integrated Weed Management

The abundance of HR kochia in the Great Plains region necessitates the use of true integrated weed management (IWM) where non-chemical tools are used in tandem with remaining herbicide options. Mechanical weed management through primary tillage (e.g., moldboard plowing and chisel plowing) and secondary tillage (row cultivation) can help control kochia and mitigate kochia seeds from entering the seedbank (Ball and Miller 1990; Ball 1992). Cultural strategies such as rotating cropping sequence, increasing crop competition, choosing a competitive species (lentils versus barley), cultivar selection, early and uniform crop emergence, crop density and row spacing, and using silage crops/green manure/cover crops are all useful tools for long-term weed management systems (Blackshaw et al. 2007; Geddes and Kimmins 2021). Ecological

tactics such as exploiting emergence patterns and weed seed persistence in soil seedbanks can also aid in kochia management programs (Geddes and Davis 2021; Kumar et al. 2018).

Kochia's short seed longevity in the soil seedbank could be an effective target for integrated kochia management programs. Kochia emerges best when the seeds are on the soil surface and emergence decreases as burial depth increases (Everitt et al. 1983); however, seed persistence can also increase with burial depth (Zorner et al. 1984) [although more-recent research has shown this not to hold true under all conditions (Beckie et al. 2018; Dille et al. 2017)]. Altering tillage depth can change where the kochia seeds are present in the soil seedbank. This suggests that shallow tillage will result in more kochia seeds from the seed bank emerging successfully and that deep tillage will reduce seedling emergence but could increase soil seedbank persistence (Zorner et al. 1984). Shallow tillage with early (chemical or other) control of emerged seedlings could be a good strategy to deplete the kochia seedbank, but deep tillage of 30 cm is predicted to limit kochia seedling emergence to 12% but result in twice the number of persistent kochia seeds after 24 months (Zorner et al. 1984).

More recent studies in Lethbridge, AB (and Scott, SK) using GS and GR kochia showed no difference in seed viability loss over time in relation to seed burial depth of surface, 2.5cm, and 10cm (Beckie et al. 2018), and similar results were found in the Central Great Plains of the USA (Wyoming, Nebraska, Colorado, and Kansas) with GS kochia (seed viability was >80% when seeds were exhumed within 6 months and dropped to <5% after the first year) (Dille et al. 2017). In contrast with Zorner et al. (1984), these results suggest that tillage depth may play a less important role compared with the presence versus absence of tillage for weed control. Combining inter-row tillage with chemical (atrazine) weed control in corn was effective for kochia management (Blackshaw et al. 1990). Implementing cultural practices that complement kochia phenology (emergence and seed set) can be an effective kochia control strategy. Combining different herbicide treatments with different crop canopies can be influential on kochia seed production per unit area which is a key indicator of long-term management success (Mosqueda et al. 2020). Herbicides were found to influence weed density/survival to the greatest extent, while crop choice (which indicates the amount of competitiveness and seeding/harvest time) was found to be the most important factor in influencing kochia seed production among the four crops: wheat (*Triticum aestivum* L.), sugar beet (*Beta vulgaris* L.), dry bean (*Phaseolus vulgaris* L.), and corn (*Zea mays* L.). Spring wheat had the lowest kochia density and seed production (nil), suggesting that wheat had the greatest ability to suppress this weed. This could partially be due to the early spring wheat seeding time, dense crop canopy, and early harvest (before kochia matured to seed set). This suggests that early-harvested crops in tandem with effective herbicides can be a successful combination to reducing the kochia seed bank (Mosqueda et al. 2020).

Cultural strategies such as crop diversity and practices that promote crop competitiveness (for example, competitive crop cultivars and higher seeding rates) are important IWM tactics for HRWM (Beckie and Harker 2017). Other important strategies include alternative crop life cycles (e.g., exchanging summer-annuals for winter annuals or perennials), weed scouting and surveys, weed sanitation and field border control, keeping a field logbook of weed control, attending meetings/reading and keep up to date with current literature, visiting field demonstrations, and utilizing web-based field HR weed assessment services (Beckie and Harker 2017; Beckie et al. 2011*a*; Sutherland 2001; Beckie et al. 2017; Kumar et al. 2019). A proactive multifaceted approach to IWM is required to mitigate future weed resistance and to sustain the effectiveness of current herbicide options.

2.2. Chemical Fallow and Wheat in Western Canada

2.2.1. Chemical Fallow in Western Canada

Summer-fallow is a no-crop year that is typically used in a dryland field crop rotation (e.g., spring wheat-fallow) in semi-arid regions of the Great Plains. In Canada, about 893,000 ha of land remains unseeded per annum, and this land is managed as summer-fallow (Statistics Canada 2016a). Traditionally, three to five tillage passes were used each year for weed control in summer-fallow (Fenster et al. 1965). The benefits of summer-fallow with conventional tillage can include moisture conservation for the subsequent crop, disease and weed control, and increased crop yield for the following year; however, detriments can include wind and water soil erosion, soil fertility loss, decrease of soil organic matter, and soil salinization (Haas et al. 1974; Action and Gregorich 1995; Campbell et al. 1990).

Rapid adoption of reduced and zero-tillage in Canada in the last two decades was facilitated using non-residual, non-selective herbicides (i.e., glyphosate) for weed control in place of tillage (Geddes 2019). Over this timeframe, chemical fallow replaced summer-fallow by conventional tillage. Chemical fallow is when growers use multiple applications of herbicides instead of repeated tillage passes for weed management in a no-crop growing season (i.e., zero-till summer-fallow using herbicides). In western Canada about 59% of growers practice zero-tillage, 24% practice reduced tillage (retaining most crop residue on the soil surface), and 17% use conventional tillage systems (incorporating most crop residue into the soil) (Statistics Canada 2016*a*). For weed control in summer-fallow, 42% of unseeded hectares use chemical fallow only, 34% use tillage only, and 24% use chemical control and tillage in combination (Statistics Canada 2016*b*).

Including chemical fallow in a crop rotation can have benefits in a semi-arid environment like southern Alberta. One of the most important outcomes of chemical fallow is to retain or build soil moisture, and to increase soil water storage and availability for subsequent cash crops (Campbell et al. 1990; Tanaka et al. 1987; Wicks and Smika 1973). Chemical fallow can maintain crop residue on the soil surface and allow for a period of mineralization making soil nutrients more available for plant uptake (Fenster et al. 1965; Lindwall and Anderson 1981; Wicks and Smike 1973). Drawbacks to chemical fallow include field susceptibility to wind and water erosion, organic matter degradation, and the economic loss of a cash crop year. In addition, several applications of herbicide used to control weeds in chemical fallow results in greater selection pressure for herbicide resistance when weed escapes remain unhindered by crop competition.

2.2.2. Wheat in Western Canada

Wheat is a prominent crop in western Canada. Wheat (including summer- and winterannual spring and durum wheat varieties) was the second highest grown crop based on seeded area in Alberta, Saskatchewan, and Manitoba which equated to a combined 8.5 million hectares in 2017 (canola was the most common at 9.2 million hectares) (Statistics Canada 2020). A conventional tillage, two-year wheat-fallow rotation was one of the main cropping systems for many decades in the semi-arid Great Plains of North America where water availability was the main factor limiting crop yield (Campbell et al. 1986; McConkey et al. 2012). However, in recent decades the majority of prairie farmers have transitioned to continuous cropping (wheat rotated with canola is common) and reduced or zero-tillage systems (Geddes 2019).

Managing kochia in wheat production can be challenging. Growers of zero-till wheat in western Canada typically rely on a pre-seed glyphosate application for weed control in place of tillage. Since kochia has prolonged emergence periodicity, it can emerge up to and after the preplant burndown and early post-emergence application windows (Anderson and Nielsen 1996; Christoffoleti et al. 1997; Schwinghamer and Van Acker 2008; Mickelson et al. 2004), making it difficult to manage in summer-annual crops. Low densities (14 and 21 plants m⁻²) and high densities (195 to 520 plants m⁻²) of kochia reduced wheat yield in Manitoba by 10-33% and 40-73%, respectively (Friesen et al. 2009). Kochia can hinder wheat harvest since it often remains immature and green when wheat is ready to harvest. In addition to harvest difficulties, kochia can regrow and produce seeds (Mickelson et al. 2004) following harvest warranting further weed control efforts.

2.3. Herbicide Strategies for Glyphosate-Susceptible and -Resistant Kochia in Chemical Fallow and Spring Wheat

2.3.1. Herbicide Strategies to Control Kochia in Chemical Fallow

Herbicide efficacy is dependent on weed growth stage, environmental conditions, and herbicide dose (Tonks and Westra 1997; Ou et al. 2018; Gains et al. 2017). Herbicide efficacy can vary widely among kochia biotypes due to morphological differences, particularly the leaf surface where herbicides are absorbed through the waxy cuticle. Kochia has difficult-to-wet, pubescent leaves with a crystalline epicuticular wax which can lead to reduced herbicide absorption (Friesen et al. 2009; Harbour et al. 2003). Kochia plants in the western USA often have shorter leaves and are more pubescent than plants from the mid-western states (Eberlein and Fore 1984), which can affect herbicide absorption. Different kochia accessions have found to greatly differ in control by 2,4-D and dicamba (Bell et al. 1972).

There are many registered herbicide options to control kochia in chemical fallow, spanning a range of different modes of action. Glyphosate is the predominant herbicide for pre-seed and chemical fallow burndown since it is non-selective, has a high efficacy, exhibits almost no residual activity, and is affordable (Benbrook 2016; Shaner 2014; Kniss 2018). Glyphosate has excellent visual control of GS kochia [>93% at 3-4 weeks after application (WAA)] (Kumar et al. 2015; Low 2016). Field studies in Alberta have shown high efficacy of glyphosate on kochia (97% visual control at 4 WAA with 80% biomass reduction compared to the untreated check) (Low 2016). With the spread of glyphosate resistance in the Great Plains region, mixing glyphosate with additional effective herbicide modes of action or rotating effective modes of action is required to mitigate and manage these biotypes effectively.

Herbicides with different modes of action can be combined with glyphosate to manage herbicide-susceptible kochia effectively in chemical fallow. Carfentrazone, sulfentrazone, and saflufenacil are all protoporphyrinogen oxidase (PPO) inhibitors (group 14) that are registered for use on kochia in chemical fallow (Anonymous 2020). Saflufenacil is absorbed rapidly by leaves and roots and is used for residual pre-emergence (or pre-seed) control of major broadleaf weeds (Grossman et al. 2011). Saflufenacil had 90% and 82% visual control of kochia at 1 and 3 WAA, respectively, in a field trial in Montana (Kumar and Jha 2015). Carfentrazone is a contact herbicide with little-to-no residual activity in soil, while sulfentrazone is systemic with a residual half-life of 121 to 302 days (Shaner 2014). Saflufenacil and carfentrazone controlled kochia (biomass reduction) pre-seed in spring wheat as effectively as glyphosate in three out of four environments in Alberta (Low 2016). Pyraflufen-ethyl/2,4-D (groups 14/4) and pyraflufen-ethyl/bromoxynil (groups 14/6) are registered for kochia control in western Canada and can be mixed with glyphosate for chemical fallow or pre-seed control.

Dicamba is a synthetic auxin (group 4) in the benzoic acid chemical family that is translocated within the plant in both the xylem and phloem (Hall et al. 1999), and often mixed with

glyphosate. Dicamba applied at 140 g ai ha⁻¹ provided <80% control of kochia (Burton et al. 2014; Low 2016), but 300 g ai ha⁻¹ resulted in excellent visual control and biomass reduction compared to the untreated check (>90%) (Wicks et al. 1994). Air temperature at spraying for glyphosate and dicamba should be considered due to a greater amount of active chemical needed for kochia control for hot summer day/nights (32.5° C/22.5° C) compared with lower application temperatures (25°C/15°C). This is due to less glyphosate being absorbed and less dicamba translocating to the active meristem in kochia (Ou et al. 2018). Some antagonism has been documented with dicamba + glyphosate mixtures (Flint and Barrett 1989*a*; Flint and Barrett 1989*b*).

A mixture of dicamba/diflufenzopyr has the dual activity of a synthetic auxin and an auxin transport inhibitor (group 19) which concentrates dicamba to the meristematic sink, resulting in greater kochia control with lesser amounts of the dicamba active ingredient (Shaner 2014). It is often mixed with glyphosate. Dicamba/diflufenzopyr has been effective at controlling GR and ALS inhibitor-resistant kochia in greenhouse studies, resulting in 82% biomass reduction (compared to untreated check) (Burton et al 2014); however, kochia control (biomass reduction) in spring wheat with dicamba/diflufenzopyr was only as effective as glyphosate in two out of four environments (Low 2016). It remains unclear whether these herbicides are as effective on GR kochia.

2.3.2. Herbicide Strategies to Control Kochia in Spring Wheat

Synthetic auxins are safe to use in wheat, and weed symptomology consists of twisting, bending, and leaf cupping 10-14 days after application (DAA). Synthetic auxin herbicide mixtures including dicamba/2,4-D, fluroxypyr + dicamba, fluroxypyr + 2,4-D, fluroxypyr + clopyralid/MCPA, MCPA/dichlorprop-p/mecoprop-p, MCPA/mecoprop-p/dicamba, dicamba/2,4-D/mecoprop-p, dichlorprop-p/2,4-D, fluroxypyr/halauxifen + MCPA, and florasulam (ALS inhibitor, group 2)/fluroxypyr/MCPA, are all registered for kochia control in spring wheat (Anonymous 2020). Poor kochia control has been documented with 2,4-D by itself in greenhouse and field studies (Tonks and Westra 1997; Wolf et al. 2000). Dicamba controlled (triazine-resistant) kochia in sorghum, resulting in 96% visual control and biomass reduction (compared to untreated check in three out of three environments) (Wicks et al. 1994). 2,4-D + dicamba resulted in 85% visual control of ALS inhibitor-resistant kochia in wheat 38 DAA (Wolf et al. 2000). Greenhouse studies showed that dicamba/fluroxypyr was effective for kochia control (>80% biomass reduction compared to untreated check) (Burton et al. 2014). Ninety percent or more of (chlorsulfuron-resistant) kochia seedlings in spring wheat did not survive when treated with fluroxypyr and tank mixes of fluroxypyr + 2,4-D ester, bromoxynil/MCPA, or dichlorprop/2,4-D ester (Friesen et al. 1993).

Bromoxynil is a quick acting PSII inhibitor resulting in chlorosis in 1-2 DAA and necrosis 3-6 DAA (Shaner 2014). Bromoxynil-containing herbicide mixtures for kochia control include bromoxynil/2,4-D, fluroxypyr/bromoxynil/2,4-D, and bromoxynil/pyrasulfotole (a 4hydroxyphenylpyruvate dioxygenase inhibitor, HPPD, group 27). Bromoxynil at 200 g ai ha⁻¹ controlled triazine-resistant kochia in sorghum at >87% visual control in two environments, but only >80% biomass reduction compared to the untreated in one out of the two environments (Wicks et al. 1994). Kochia control with bromoxynil can decrease over time due to its non-residual contact activity and the plant partially recovering after herbicide application (Kumar and Jha 2015). Thus, mixing bromoxynil with another active ingredient can provide more sustained control. Pyrasulfotole/bromoxynil and bromoxynil/2,4-D have managed kochia effectively in spring wheat (as effective as glyphosate in biomass reduction in 3 out of 4 environments) (Low 2016). Bromoxynil/MCPA has been effective on (GR and ALS inhibitor-resistant) kochia in greenhouse studies (Burton et al. 2014), and best when applied to kochia that is <15 cm in height (Tonks and Westra 1997).

Sulfentrazone (registered as a pre-seed herbicide for kochia control) is a PPO inhibitor (group 14) that provides residual activity and helps prevent kochia from emerging. Prolonged residual activity from a pre-seed herbicide can reduce the need for multiple post-emergence herbicide applications. Sulfentrazone applied pre-emerge resulted in \geq 91% kochia control 12 WAA in Montana (Kumar and Jha 2015).

These aforementioned herbicides with the exception of dicamba, are registered for kochia management; with many of them exhibiting control of ALS inhibitor-resistant kochia biotypes. Glyphosate-resistant kochia is relatively new to the Canadian prairies (2011) but has spread rapidly (Alberta surveyed kochia went from 4% GR to 50% in five years) (Hall et al. 2014; Beckie et al. 2019). The efficacy of these herbicides for control of GR kochia in western Canada remains unknown, and this represents a significant knowledge gap for Canadian farmers.

2.4. Remaining Questions

In summary, several knowledge gaps regarding chemical management of herbicideresistant kochia remain. These include: 1) few publicly available research studies assessing herbicide options for control of GR kochia on the Canadian prairies, and 2) whether herbicides and herbicide mixtures registered for control of GS kochia remain effective for control of GR kochia. In response to these knowledge gaps, this thesis research was designed to determine which chemical options remain effective for control of GR and GS kochia in chemical fallow and spring wheat in western Canada. The main questions addressed by this thesis research include:

- Which herbicides and herbicide mixtures remain effective for control GS and GR kochia in chemical fallow? (Chapter 3)
- Which herbicide options remain effective for control GS and GR kochia in spring wheat? (Chapter 4)
- Are there different levels of herbicide activity on GS and GR kochia? (Chapters 3 and 4)

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Chapter Three: Herbicide Mixtures Control Glyphosate-resistant Kochia (*Bassia scoparia*) in Chemical Fallow, but Their Longevity Warrants Careful Stewardship

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3.1. Introduction

Kochia [*Bassia scoparia* (L.) A.J. Scott] is an abundant and troublesome weed throughout the Great Plains region. It is the most abundant weed in annual crops of the mixed grassland ecoregion of Alberta, and the 15th most abundant weed among annual crops in Alberta and Saskatchewan (Leeson 2016; Leeson et al. 2019). Kochia is an invasive, summer annual weed that was introduced to the Americas in the late 1800s as an ornamental garden forb from central Europe and western Asia (Friesen et al. 2009). Its unique weedy characteristics, including early spring germination, prolonged emergence periodicity, rapid growth, prolific seed production, efficient pollen-mediated gene flow, and long-distance seed dispersal (Schwinghamer and Van Acker 2008; Beckie et al. 2016), contribute to its geographic spread. Forcella (1985) found that kochia had the highest rate of spread compared with 40 other invasive weed species in the northwestern United States. Kochia is a competitive C4 plant that favors arid and semi-arid conditions, and is tolerant to drought, heat, and saline soils (Friesen et al. 2009). These traits enable kochia to be problematic in annual cropping systems, forage crops and hay fields, rangeland, roadsides, oil well sites, and waste areas.

Kochia has a high level of genetic diversity within and among populations (Mengistu and Messersmith 2002), and this diversity is maintained via seed- and pollen-mediated gene flow (Beckie et al. 2016). Protogynous flowering (where the stigmas emerge and are receptive to pollen before the anthers fully mature on the same plant) promotes initial outcrossing prior to self-pollination and increases the chance of pollen transfer to other kochia plants (Mulugeta et al. 1994; Stallings et al. 1995). Resistance alleles are spread among kochia plants and populations through pollen-mediated gene flow and seed dispersal resulting from abscised mature kochia plants tumbling in the wind (Beckie et al. 2016). Kochia seed longevity in soil lasts about 1 to 2 years (Beckie et al. 2018), which can lead to the rapid evolution of herbicide resistance (Beckie et al. 2013).

Outcrossing of kochia increases the chance of spreading herbicide resistance, and resistance to four herbicide modes of action: photosystem II inhibitors (group 5) (not known to be present in Canada), acetolactate synthase (ALS) inhibitors (group 2), the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) inhibitor glyphosate (group 9), and synthetic auxins (group 4) have been found; in some cases within the same kochia population (Heap 2020). In 1988 the first herbicide-resistant kochia population, resistant to the ALS inhibitor chlorsulfuron, was found in Manitoba and Saskatchewan, then in Alberta the following year (Morrisson and Devine 1994). This type of resistance was found in 85% of the kochia populations surveyed across the three Canadian prairie provinces in 2007 (Beckie et al. 2011), and 100% of kochia populations in Alberta in 2017 (Beckie et al. 2019). The first synthetic auxin-resistant kochia in Canada was confirmed in Saskatchewan in 2015, and subsequently in 2017 the first triple-resistant kochia populations (to synthetic auxins, ALS inhibitors, and an EPSPS inhibitor) were found in Alberta (Beckie et al. 2019).

Glyphosate-resistant (GR) kochia was first reported in wheat fields in Kansas in 2007, and since then it has been identified in ten of the U.S. states of the American Great Plains (Heap 2020; Kumar et al. 2019). In 2011, the first cases of GR kochia in Canada were confirmed in chemical fallow fields located in Warner County, Alberta (Beckie et al. 2013). This was the first GR weed confirmed in western Canada. Rapid spread of GR kochia was observed in Alberta, increasing from an estimated 5% of kochia populations in 2012 to 50% of kochia populations in 2017 (Beckie et al. 2019). This rapid spread of glyphosate resistance represents an unprecedented rate of herbicide resistance gene flow present among kochia populations.

Growers located in the semi-arid environment of the Canadian Prairies, east of the Rocky Mountains, include fallow in rotation with annual crops to improve soil water storage and water availability for subsequent cash crops (Campbell et al. 1990). There are about 1 415 600 ha of summer fallow left unseeded in western Canada per annum (10-year average between 2011 and 2020) (Statistics Canada 2020*a*). About 59% of growers in western Canada practice zero tillage, while 24% practice reduced tillage (retaining most crop residue on the soil surface), and 17% use conventional tillage systems (incorporating most crop residue into the soil) (Statistics Canada 2020*b*). In reduced or zero tillage systems, growers use herbicides for weed control in place of tillage to maintain a weed-free environment while the field remains absent of a crop throughout the growing season (known as chemical fallow). Chemical fallow can help retain or build soil moisture, maintain crop residue on the soil surface, and allow for a period of mineralization making soil nutrients more available for plant uptake (Fenster et al. 1965; Lindwall and Anderson 1981). Summer fallow can also increase soil susceptibility to wind and water erosion, salinization, moisture storage inefficiencies, and result in the economic loss of a cash crop for one growing season. In winter wheat-fallow rotations, zero tillage chemical fallow can retain more soil moisture, maintain greater surface residue, and result in reduced weed growth compared with tilled fallow (Wicks and Smika 1973).

Kochia is difficult to control in chemical fallow because it continues to emerge after herbicide applications in early spring (Schwinghamer and Van Acker 2008), then grows aggressively in the absence of crop competition. The risk of selecting for herbicide resistance is greater in chemical fallow because uncontrolled weeds may grow and produce copious amounts of seed when they are uninhibited by plant competition. Many farmers rely on glyphosate for costeffective non-selective weed control in chemical fallow systems, which can result in large selection pressure for glyphosate resistance if this herbicide is used as the sole source of weed management. Including multiple effective modes of action in chemical fallow is essential to mitigate the selection for herbicide-resistant weeds. In western Canada, there are no research reports on alternative herbicide options for control of GR kochia in chemical fallow. Due to the reliance of glyphosate in chemical fallow systems, and the increasing abundance and distribution of GR kochia, alternative control options are warranted to manage kochia effectively. The objective of this study was to determine herbicide mixtures including multiple modes of action to manage GR and glyphosate-susceptible (GS) kochia in chemical fallow fields.

3.2. Materials and Methods

3.2.1. Site Description

Field experiments were conducted in 2014 and 2015 at the Agriculture and Agri-Food Canada Lethbridge Research and Development Centre located near Lethbridge, AB (49.69° N, 112.77° W; clay loam textured soil; 3.6% OM; 7.8 pH), and Hamman Ag Research Inc. located near Coalhurst, AB (49.79° N, 112.99° W; loam textured soil; 2.5% OM; 8.3 pH). Soils at these locations were classified as dark brown chernozems. The previous crop in both years at Lethbridge was silage barley, and at Coalhurst was chemical fallow.

3.2.2. Experimental Design and Treatment Structure

The experiment used a randomized complete block design with four replications (blocks). The main plot size at Coalhurst was 2.5 x 6.0 m, and at Lethbridge was 2.5 x 5.5 m. Blocks were split randomly with GR and GS kochia accessions. One seeder pass (2.1 m width) including nine seed rows of each kochia accession was seeded across each experimental replication (perpendicular to herbicide treatment) in early spring. One meter spacing was left between each kochia accession, for a sub-plot size of 2.5 x 2.1 m. Kochia was seeded at a rate of 300 viable seeds m⁻² in all environments, with the exception of Lethbridge in 2015 where it was seeded at 400 viable seeds m⁻². Seeds were placed on the soil surface using a Fabro cone seeder (Fabro Enterprises Ltd., Swift Current, SK, Canada) with double disc seed-row openers spaced 23 cm apart. The seeder packer tires were left on the ground and packed the seed firmly into the soil.

Weeds were controlled at each experimental location prior to kochia seeding. Coalhurst used glyphosate at 900 g ae ha⁻¹ as a pre-seed burndown, while Lethbridge used glyphosate at 1334 g ae ha⁻¹ and glyphosate + bromoxynil at 1334 + 348 g ae/ai ha⁻¹ in 2014 and 2015, respectively.

Both kochia seed accessions were sourced from the Agriculture and Agri-Food Canada Lethbridge Research and Development Centre. The GR kochia accession was selected over multiple generations of in-field glyphosate use at 900 g ae ha⁻¹. The GS kochia accession was ALS inhibitor-resistant and was selected in the field using recurrent applications of tribenuron-methyl + thifensulfuron-methyl (Refine® SG; FMC of Canada, Mississaga, ON, Canada) at 10 + 5 g ai ha⁻¹ over several years.

The herbicide treatments tested included an untreated control and glyphosate applied alone or in mixture with 13 other herbicide combinations, which were either registered for kochia management in chemical fallow or to determine whether they would be effective for this usage (Table 3-1). Herbicide treatments were applied post-emergence when kochia plants reached 10 cm in height. Coalhurst used a 2.0 m hand-held, propane-propelled sprayer equipped with John Deere LDX01 nozzles (John Deere, Moline, IL, USA). The sprayer applied the herbicide mixtures with 100 L ha⁻¹ water carrier at 242 kPa and a speed of 4 km hr⁻¹. Lethbridge used a 2.0 m bicycle CO₂ sprayer equipped with Greenleaf Air Mix 110-01 nozzles (Greenleaf Technologies, Covington, LA, USA). This sprayer applied herbicide mixtures with 100 L ha⁻¹ water carrier at 290 kPa and a speed of 5 km hr⁻¹.

3.2.3. Data Collection

Kochia seedling emergence was determined for each kochia accession two weeks after emergence by counting all kochia seedlings present within one 0.25 m^2 quadrat placed randomly within each sub-plot. Kochia control was visually assessed for herbicide efficacy as a percentage from 0 (visually similar to untreated control) to 100% (complete necrosis) 3 weeks after herbicide application (WAA). Kochia aboveground biomass was sampled 6 WAA. Kochia fresh weight was determined for each accession from a 0.34 m^2 area (3 rows by 0.5 m) in each sub-plot in all

Herbicide common names	Herbicide trade name	MOA	Concentration/ formulation	Rate (g ai/ae ha ⁻¹)	Merge adjuvant	Company
Glyphosate	Roundup WeatherMAX®	9	540 g L ⁻¹ SN	450	-	Monsanto Canada Inc.
Glyphosate +	Roundup WeatherMAX® +	9	540 g L^{-1} SN	450 +		Monsanto Canada Inc.
dicamba	Banvel® II	4	480 g L^{-1} SN	290		BASF Canada
Glyphosate +	Roundup WeatherMAX® +	9	540 g L ⁻¹ SN	450 +		Monsanto Canada Inc.
dicamba	Banvel® II	4	480 g L ⁻¹ SN	580		BASF Canada
Glyphosate +	Roundup WeatherMAX® +	9	540 g L ⁻¹ SN	450 +	0.5% v/v	Monsanto Canada Inc.
dicamba/diflufenzopyr	Distinct®	4/19	70% WG	75/25		BASF Canada
Glyphosate +	Roundup WeatherMAX® +	9	540 g L ⁻¹ SN	450 +	0.5% v/v	Monsanto Canada Inc.
dicamba/diflufenzopyr	Distinct®	4/19	70% WG	150/50		BASF Canada
Glyphosate +	Roundup WeatherMAX® +	9	540 g L ⁻¹ SN	450 +	0.5% v/v	Monsanto Canada Inc.
saflufenacil	Heat®	14	70% WG	18		BASF Canada
Glyphosate +	Roundup WeatherMAX® +	9	540 g L ⁻¹ SN	450 +	0.5% v/v	Monsanto Canada Inc.
saflufenacil	Heat®	14	70% WG	50		BASF Canada
Glyphosate + carfentrazone	Roundup WeatherMAX® + Aim®	9 14	540 g L ⁻¹ SN 240 g L ⁻¹ EC	450 + 18	1.0% v/v	Monsanto Canada Inc. FMC of Canada
Glyphosate + carfentrazone + sulfentrazone	Roundup WeatherMAX® + Aim® + Authority®	9 14 14	$\begin{array}{c} 540 \ {\rm g} \ {\rm L}^{-1} \ {\rm SN} \\ 240 \ {\rm g} \ {\rm L}^{-1} \ {\rm EC} \\ 480 \ {\rm g} \ {\rm L}^{-1} \ {\rm SN} \end{array}$	450 + 9 + 53	1.0% v/v	Monsanto Canada Inc. FMC of Canada FMC of Canada
Glyphosate +	Roundup WeatherMAX® +	9	540 g L ⁻¹ SN	450 +	1.0% v/v	Monsanto Canada Inc.
carfentrazone +	Aim® +	14	240 g L ⁻¹ EC	9 +		FMC of Canada
sulfentrazone	Authority®	14	480 g L ⁻¹ SN	105		FMC of Canada
Glyphosate +	Roundup WeatherMAX® +	9	540 g L ⁻¹ SN	450 +		Monsanto Canada Inc.
MCPA/dichlorprop/mecoprop-p	Optica Trio	4/4/4	600 g L ⁻¹ SN	395/765/320		Nufarm Agriculture Inc.
Glyphosate + 2,4-D ester	Roundup WeatherMAX® + 2,4-D ester LV 700	9 4	540 g L ⁻¹ SN 660 g L ⁻¹ EC	450 + 560		Monsanto Canada Inc. Nufarm Agriculture Inc.
Glyphosate +	Roundup WeatherMAX® +	9	540 g L ⁻¹ EC	450 +		Monsanto Canada Inc.
pyraflufen-ethyl/2,4-D ester	Blackhawk®	14/4	6.1/473 g L ⁻¹ EC	188/167		Nufarm Agriculture Inc.
Glyphosate +	Roundup WeatherMAX® +	9	540 g L ⁻¹ SN	450 +		Monsanto Canada Inc.
pyraflufen-ethyl/bromoxynil	Conquer® II	14/6	25/235 g L ⁻¹ EC	4.5/140		Nufarm Agriculture Inc.

Table 3-1. Herbicide treatments used at Lethbridge and Coalhurst, AB in 2014 and 2015 to manage glyphosate-resistant (GR) and glyphosate-susceptible (GS) kochia in chemical fallow.

Note: MOA, mode of action; EC, emulsifiable concentrate; SN, solution; WG, wettable granule.

locations and years with the exception of Coalhurst in 2014 where biomass was collected from a 0.45 m^2 area (2 rows by 1 m).

3.2.4. Statistical Analysis

Kochia density, visual control, and biomass data were analyzed using the GLIMMIX procedure in SAS 9.4 (SAS Institute, Inc., Cary, NC, USA). Analyses were separated by year due to the addition of two herbicide treatments in 2015 that were not present in 2014 (Table 3-2). The main and interaction effects of kochia accession (GR vs. GS), herbicide treatment, and experimental location (Lethbridge vs. Coalhurst) were considered fixed effects. Random effects included experimental replication nested within location, herbicide treatment by replication nested within location, and kochia accession by replication nested within location. Outliers were removed according to Lund's test (Lund 1975). The distribution and link functions were optimized using visual assessment of predicted vs. residual values and the within-group covariance structure of residuals was fit based on minimization of the Akaike Information Criterion (AIC). The assumption of normality was assessed using the Shapiro-Wilk test, while homoscedasticity was evaluated using visual assessment of the residual vs. predicted values. Visual control estimates for the untreated control treatment were removed from the analyses to avoid heteroscedasticity induced by lack of variation in this treatment among locations, and experimental replications.

A gaussian distribution was used with the identity link function and an unaltered covariance structure of residuals for analysis of kochia density. The same distribution and link functions were used to assess kochia visual control, but the covariance structure of residuals was adjusted based on the location main effect. For kochia biomass, the lognormal distribution was used with the identity link function and the covariance structure of residuals was adjusted based on the interaction effect of kochia accession and location. Significant main and interaction effects were **Table 3-2.** Visual control (%) of glyphosate-resistant (GR) and glyphosate-susceptible (GS) kochia 3 weeks after herbicide application in chemical fallow at Lethbridge and Coalhurst, AB, in 2014 and 2015.^a

			Ι	isual cor	ntrol in 2014				Vi	sual control i	in 2015		
	Rate	L	ethbridge	e	(Coalhurs	st	Le	ethbridge		Co	alhurst	1
				GR	. <u> </u>		GR			GR			GR
				vs.			vs.			vs.			vs.
Herbicide treatment		GR	GS	GS	GR	GS	GS	GR	GS	GS	GR	GS	GS
	g ai/ae ha ⁻¹	%	%		%	%		%	%		%	%	
Glyphosate	450	0 h	95 a	***	55 d	99	* * *	0 e	93 abc	***	0 e	89	***
Glyphosate + dicamba	450 + 290	61 f	97 a	***	94 ab	99	* *	78 abc	96 ab	* * *	94 ab	97	ns
Glyphosate + dicamba	450 + 580	80 cde	98 a	* * *	99 a	99	ns	90 a	99 a	*	95 ab	97	ns
Glyphosate + dicamba/diflufenzopyr	450 + 75/25	73 e	96 a	* * *	95 ab	98	ns	75 abc	91 abc	***	89 abc	94	*
Glyphosate + dicamba/diflufenzopyr	450 + 150/50	84 cd	95 a	* * *	98 a	99	ns	86 a	94 abc	*	92 abc	95	ns
Glyphosate + saflufenacil	450 + 18	89 bc	99 a	* * *	99 a	99	ns	68 bc	90 abc	***	91 abc	95	*
Glyphosate + saflufenacil	450 + 50	99 a	99 a	ns	95 ab	99	*	80 ab	93 abc	* * *	91 abc	96	*
Glyphosate + carfentrazone	450 + 18	85 cd	99 a	***	89 b	99	* * *	69 bc	91 abc	* * *	90 abc	97	***
Glyphosate + carfentrazone + sulfentrazone	450 + 9 + 53	95 ab	99 a	ns	96 ab	99	ns	79 ab	90 abc	**	90 abc	97	* * *
Glyphosate + carfentrazone + sulfentrazone	450 + 9 + 105	98 ab	99 a	ns	99 a	99	ns	91 a	95 abc	ns	96 a	98	ns
Glyphosate + MCPA/dichlorprop/mecoprop-p	450+395/765/320	79 de	98 a	***	96 ab	99	ns	88 a	98 a	**	95 ab	96	ns
Glyphosate + 2,4-D ester	450 + 560	36 g	78 b	* * *	76 c	98	* * *	28 d	79 с	***	78 d	95	* * *
Glyphosate + pyraflufen-ethyl/2, 4-D ester	450+188/167	_	N/A			N/A		61 c	85 abc	***	79 cd	87	* *
Glyphosate + pyraflufen-ethyl/bromoxynil	450 + 4.5/140		N/A			N/A		69 bc	80 bc	**	84 bcd	94	* * *

Note: Within columns, different letters indicate significant difference based on Tukey's honestly significant difference ($\alpha = 0.05$). GR vs. GS indicates the level of

significant difference in visual control between GR and GS kochia accessions for each herbicide treatment. *, **, and *** indicate significant difference between means at P < 0.05, 0.01, and 0.001, respectively, while NS indicates lack of significant difference ($P \ge 0.05$).

^aAnalyses were separated by year due to the addition of two herbicide treatments in 2015 (glyphosate + pyraflufen-ethyl/2,4-D ester and glyphosate + pyraflufenethyl/bromoxynil).

ddetermined according to the F test and treatment means were compared using Tukey's honestly significant difference ($\alpha = 0.05$). Kochia biomass means are presented on the original data scale following post-hoc back transformation.

3.3. Results and Discussion

3.3.1. Glyphosate-resistant Kochia

Several herbicide mixtures controlled GR kochia effectively in chemical fallow (Tables 3-2 and 3-3) despite variable precipitation among years during the month of herbicide application (June) (Figure 3-1). A greater number of treatments controlled GR kochia in Coalhurst compared with Lethbridge, based on visual assessments ($\geq 80\%$ control). These differences were likely due to the subjectivity of visual control estimates among locations and assessors, or due to environmental differences between these two locations. The Pest Management Regulatory Agency defines weed control as $\geq 80\%$ efficacy (Pest Management Regulatory Agency 2003). The best glyphosate mixture treatments that resulted in acceptable ($\geq 80\%$) control of GR kochia among all environments were glyphosate + dicamba $(450 + 580 \text{ g ae } ha^{-1})$, glyphosate + dicamba/diflufenzopyr (450 + 150/50 g ai/ae ha⁻¹), glyphosate + saflufenacil (450 + 50 g ai/ae ha⁻¹) ¹), and glyphosate + carfentrazone + sulfentrazone $(450 + 9 + 105 \text{ g ai/ae ha}^{-1})$. The treatments that showed acceptable control at the majority of environments (three out of four environments) were glyphosate + saflufenacil (450 + 18 g ai/ae ha⁻¹), glyphosate + carfentrazone (450 + 18 g ai/ae ha⁻¹) ¹), glyphosate + carfentrazone + sulfentrazone (450 + 9 + 53 g ai/ae ha⁻¹), and glyphosate + MCPA/dichlorprop/mecoprop-p (450 + 395/765/320 g ai/ae ha⁻¹). Glyphosate + pyraflufenethyl/bromoxynil $(450 + 4.5/140 \text{ g ai/ae ha}^{-1})$ (tested in 2015 only) showed acceptable control of GR kochia (84% visual control) at Coalhurst only (compared with 69% visual control at Lethbridge) (Table 3-2).

	Location ^a		Kochia accession ^b			
						GR
						VS.
Herbicide treatment	Rate	Lethbridge	Coalhurst	GR	GS	GS
	(g ai/ae ha ⁻¹)	(kg ha^{-1})	$(kg ha^{-1})$	(kg ha ⁻¹)	(kg ha^{-1})	
Untreated control		2264 a	980 a	1701 a	1304 a	ns
Glyphosate	450	607 bc	106 abc	1590 a	40 b	***
Glyphosate + dicamba	450 + 290	291 cde	34 abc	200 cde	49 ab	ns
Glyphosate + dicamba	450 + 580	219 de	8 bc	116 de	15 b	**
Glyphosate + dicamba/diflufenzopyr	450 + 75/25	467 bcd	139 abc	582 abcd	111 ab	*
Glyphosate + dicamba/diflufenzopyr	450 + 150/50	474 bcd	7 c	166 de	21 b	***
Glyphosate + saflufenacil	450 + 18	335 cde	35 bc	276 bcd	42 b	***
Glyphosate + saflufenacil	450 + 50	127 e	58 bc	165 de	45 b	*
Glyphosate + carfentrazone	450 + 18	426 bcd	146 ab	1,067 abc	58 b	***
Glyphosate + carfentrazone + sulfentrazone	450 + 9 + 53	137 e	116 abc	468 abcd	34 b	***
Glyphosate + carfentrazone + sulfentrazone	450 + 9 + 105	29 f	17 bc	36 e	13 b	ns
Glyphosate + MCPA/dichlorprop/mecoprop-p	450 + 395/765/320	278 cde	40 bc	353 abcd	31 b	***
Glyphosate $+ 2,4$ -D ester	450 + 560	1052 ab	154 ab	1337 ab	121 b	***

Table 3-3. Biomass (kg ha⁻¹) of glyphosate-resistant (GR) and glyphosate-susceptible (GS) kochia 6 weeks after herbicide application in chemical fallow at Lethbridge and Coalhurst, AB in 2014.

Note: Within columns, different letters indicate significant difference based on Tukey's honestly significant difference ($\alpha = 0.05$). *, **, and *** indicate significant difference between means at P < 0.05, 0.01, and 0.001, respectively, while NS indicates lack of significant difference (P ≥ 0.05).

^{*a*}Location main effect.

^bKochia accession main effect.

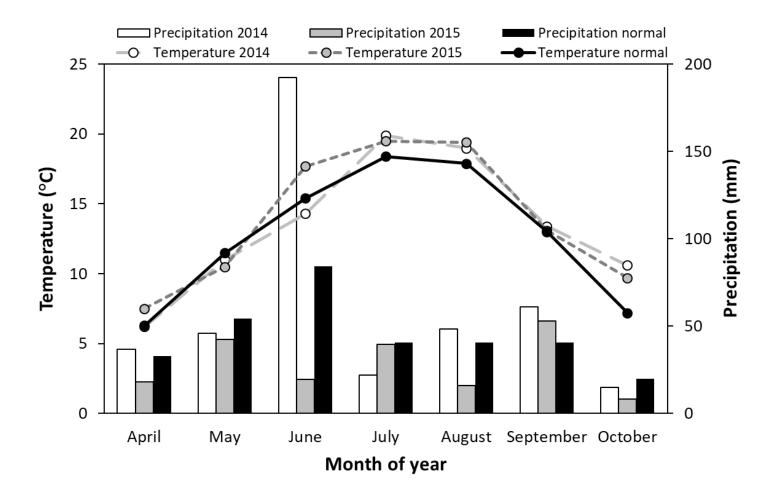


Figure 3-1. Growing season monthly average temperature and precipitation at Coalhurst and Lethbridge during 2014 and 2015 compared with the 30-year average (normal) monthly temperature and precipitation for this region. The Coalhurst site received 50 mm, and 25 mm of irrigation in June/July 2014 and May 2015, respectively. The Lethbridge site received 6 mm, 25 mm, and 25mm in May, June, and July of 2015, respectively.

In 2014, GR kochia biomass supported the visual control estimates, resulting in a biomass reduction of $\geq 80\%$ for all treatments that had acceptable visual control; with the exception of glyphosate + carfentrazone + sulfentrazone $(450 + 9 + 53 \text{ g ai/ae ha}^{-1})$ at 72% biomass reduction and glyphosate + MCPA/dichlorprop/mecoprop-p, which resulted in slightly less than acceptable control (79%) and a similar reduction in biomass (79%) (Table 3-3). One anomaly was glyphosate + carfentrazone $(450 + 18 \text{ g ai/ae ha}^{-1})$, which showed acceptable visual control at 3 WAA (in three out of four environments at 85%, 89%, and 90%), but only a 37% reduction in biomass in 2014. This likely was due to the contact nature of carfentrazone (with little-to-no systemic action) resulting in control of top growth but little plant mortality, allowing for kochia regrowth prior to the biomass assessment (Table 3-3). Assessment of visual control at multiple time points (including 6 WAA) would aid this conjecture, however, these data were collected only at a single time point in the current study. The glyphosate + dicamba $(450 + 290 \text{ g ae ha}^{-1})$ treatment reduced kochia biomass by 88% among locations in 2014, but did not result in acceptable visual control. Differences in kochia biomass among herbicide treatments were not observed in 2015 due to large variability in the biomass measurement (Table 3-4).

Dicamba is a synthetic auxin (group 4) within the benzoic acid chemical family, and a systemic herbicide that is translocated in the xylem and phloem (Hall et al. 1999). While the 290 g ae ha⁻¹ rate of dicamba (plus glyphosate at 450 g ae ha⁻¹) suppressed GR kochia (61% visual control in 2014 and 78% in 2015) at the Lethbridge location, this treatment resulted in excellent kochia control (94% visual control in 2014/2015) at Coalhurst (Table 3-2) and reduced shoot biomass (in 2014) by 88% (Table 3-3). The 2X label rate of dicamba at 580 g ae ha⁻¹ (plus glyphosate at 450 g ae ha⁻¹) was excellent (91% visual control average among locations and years) at controlling GR kochia. Lower rates of dicamba have been shown to be ineffective at controlling

Table 3-4. ANOVA table showing the significance of main and interaction effects of herbicide treatment (H), glyphosate-resistant vs. glyphosate-susceptible kochia accession (A), and experimental location (L) on kochia visual control, plant density, and aboveground biomass at Lethbridge and Coalhurst, AB, in 2014 and 2015.

		2014			2015	
Fixed effect	Visual control	Plant density	Aboveground	Visual	Plant density ^a	Aboveground
			biomass	control		biomass
	(%)	(plants m ⁻²⁾	(kg ha^{-1})	(%)	(plants m^{-2})	$(kg ha^{-1})$
Herbicide (H)	< 0.001	0.363	< 0.001	< 0.001	0.595	0.192
Accession (A)	< 0.001	0.001	< 0.001	< 0.001	< 0.001	0.054
НхА	< 0.001	0.293	0.009	< 0.001	0.895	0.418
Location (L)	< 0.001	< 0.001	< 0.001	< 0.001	N/A^{b}	0.044
HxL	< 0.001	0.347	0.001	< 0.001	N/A	0.348
A x L	< 0.001	0.097	0.024	< 0.001	N/A	0.177
H x A x L	< 0.001	0.188	0.186	< 0.001	N/A	0.588

Note: Bolded values indicate significant main or interaction effects at P < 0.05. N/A, not applicable.

^aP values for kochia density in 2015 are for the Lethbridge location only.

^bVisual differences in kochia density were absent at Coalhurst in 2015, and thus density was measured in the untreated control plots only. Coalhurst 2015 kochia density data were absent from the analysis of variance.

kochia (Burton et al. 2014). In a greenhouse study, the chemical fallow rate of dicamba (140 g ae ha⁻¹) suppressed GR kochia shoot biomass by 76% (Burton et al. 2014) and in a field study near Lethbridge, AB, dicamba at 139 g ae ha⁻¹ showed inadequate kochia control (Low 2016). Shoot and root biomass, glyphosate uptake into the leaves, and glyphosate translocation to roots can be reduced in johnsongrass (*Sorghum halepense*) when applying a mixture of glyphosate + dicamba versus glyphosate alone (Flint and Barrett 1989*a*). In field and greenhouse studies on kochia control, glyphosate + dicamba had an antagonistic effect due to reduced translocation of each active ingredient when applied in combination, where significantly better control was observed with glyphosate alone than with glyphosate + dicamba mixtures (Ou et al. 2018). In the current study, glyphosate and dicamba antagonism was not observed visually or quantitatively (in biomass estimates); however, our experiment was not designed to test this hypothesis directly (Table 3-2; Table 3-3).

Dicamba/diflufenzopyr have the combined activity of a synthetic auxin (group 4) and an auxin transport inhibitor (group 19), which focuses dicamba to the meristematic sinks thereby achieving greater kochia efficacy with a lower rate of active ingredient (Shaner 2014). Greenhouse studies have shown 82% control (biomass reduction of GR kochia) with dicamba/diflufenzopyr applied at 100 g ai ha⁻¹ (Burton et al. 2014), but in our field studies this rate was inadequate for control in 2014 causing a biomass reduction of 65% only (Table 3-3). Field studies often exhibit lower herbicide efficacy compared with greenhouse studies using similar herbicide rates because of the impact of environmental stressors (competition, weather, etc.) on herbicide availability, uptake, and translocation. In the current study, glyphosate + dicamba/diflufenzopyr at 450 + 150/50 g ai/ae ha⁻¹ (2X label rate of dicamba/diflufenzopyr) showed excellent control (90% visual control average among environments) causing a 90% reduction of GR kochia biomass in 2014.

Saflufenacil is a protoporphyrinogen oxidase (PPO) inhibitor (group 14) that is absorbed rapidly by leaves and roots and has moderate residual activity in soil (Shaner 2014). The label rate of saflufenacil (18 g ai ha⁻¹) (plus glyphosate at 450 g ae ha⁻¹) showed acceptable (\geq 80%) visual control in three out of four environments and reduced GR kochia biomass by 84%. This concurs with a similar study from Montana that showed 100% visual control and 91% biomass reduction of GR kochia in response to saflufenacil (Kumar et al. 2014). The high rate of saflufenacil (50 g ai ha⁻¹) (plus glyphosate 450 g ae ha⁻¹) showed excellent GR kochia control (91% control among environments, and a 90% reduction in biomass in 2014), and is an excellent, effective option for control of GR kochia in chemical fallow.

Glyphosate + carfentrazone + sulfentrazone at the label rate $(450 + 9 + 53 \text{ g ai/ae ha}^{-1})$ resulted in 90% visual control (average among environments) with only a 72% reduction in biomass in 2014. Increasing the rate of sulfentrazone in this mixture to 105 g ai ha⁻¹ resulted in excellent visual control of GR kochia (96% control) and a 98% reduction in kochia biomass (in 2014). Carfentrazone and sulfentrazone are both PPO inhibitors (group 14), but carfentrazone is a contact herbicide with little-to-no residual activity in soil, while sulfentrazone is systemic with moderate residual activity (half-life of 121 to 302 days) (Shaner 2014). This combination was among the best mixture options for controlling GR kochia, in part, because it included a quick (hours to days) contact herbicide resulting in rapid necrosis and plant cell death, in addition to extended residual activity to help control subsequent emergence of kochia seedlings.

3.3.2. Glyphosate-susceptible Kochia

In general, GS kochia visual control was excellent among treatments (\geq 90%), in part because all herbicide treatments were mixed with glyphosate; however, some herbicide treatments resulted in visual control that was considered acceptable only (\geq 80% but < 90%) (Table 3-2). All treatments at both Lethbridge and Coalhurst achieved $\geq 80\%$ control of GS kochia, with the exception of the glyphosate plus 2,4-D ester mixture, which resulted in just below the 80% control threshold at Lethbridge in 2014 and 2015 (Table 3-2). Glyphosate mixed with 2,4-D can result in antagonism when applied to field bindweed (*Convolvulus arvensis*) or johnsongrass because 2,4-D can affect the uptake and translocation of glyphosate (Flint and Barrett 1989*a*, 1989*b*). Perhaps this antagonism resulted in lower kochia control by the glyphosate plus 2,4-D ester mixture in the current study.

Biomass of GS kochia in 2014 supported the visual efficacy data with all treatments resulting in a biomass reduction of at least 90% compared with the untreated control (Table 3-3). Glyphosate + dicamba (450 + 580 g ae ha⁻¹), glyphosate + dicamba/diflufenzopyr (450 + 150/50 g ai/ae ha⁻¹), and glyphosate + carfentrazone + sulfentrazone (450 + 9 + 105 g ai/ae ha⁻¹) resulted in the greatest biomass reduction (98 to 99% biomass reduction compared with the untreated control) and almost eliminated the GS kochia present (Table 3-3). Even though the herbicide treatments did not result in different visual control of GS kochia at Coalhurst in either year (e.g., 98 to 99% visual control in 2014), differences in kochia biomass were observed among the herbicide treatments in 2014 (ranging from 7 to 154 kg ha⁻¹ among herbicide treatments) (Tables 3-2 and 3-3).

3.3.3. Differences Between Glyphosate-resistant and –Susceptible Kochia Accessions

Many of the herbicide mixtures resulted in greater control of GS kochia compared with GR kochia accessions. Visual control ratings showed greater control of GS kochia compared with GR kochia among environments (P < 0.05 in all environments) when treated with glyphosate alone, glyphosate + 2,4-D ester (450 + 560 g ae ha⁻¹), and glyphosate + carfentrazone (450 + 18 g ai/ae ha⁻¹) (Table 3-2). Glyphosate + dicamba (450 + 290 g ae ha⁻¹), glyphosate + dicamba/diflufenzopyr

 $(450 + 75/25 \text{ g} \text{ ae/ai } \text{ha}^{-1})$, and glyphosate + saflufenacil (both rates) resulted in greater control of GS compared with GR kochia in three out of four environments. The only treatment with no difference between kochia accessions in either location or year was glyphosate + carfentrazone + sulfentrazone ($450 + 9 + 105 \text{ g} \text{ ai/ae } \text{ha}^{-1}$), as this was among the most effective treatments on GR kochia visual control (96%) and biomass reduction (98%). The remaining treatments did not show a clear trend of differences between kochia accessions based on visual control ratings.

Among herbicide treatments, the GR kochia accession had greater aboveground biomass (by about 7X; data not shown) than the GS kochia accession in 2014 (the herbicide treatments resulted in about 3X to 40X greater GR kochia biomass than the same treatments on GS kochia) (Tables 3-3 and 3-4). This was due, in part, to the greater density of GR than GS kochia present in 2014 (112 ± 4.5 GR vs. 83 ± 4.5 GS kochia plants m⁻²) and 2015 (223 ± 10.5 GR vs. 171 ± 10.5 GS kochia plants m⁻² at Lethbridge; not measured in Coalhurst); but could be due also to the lower efficacy of herbicide mixtures for GR kochia management (Tables 3-2 and 3-4). At Lethbridge in 2014, the glyphosate + carfentrazone $(450 + 18 \text{ g ai/ae ha}^{-1})$, glyphosate + dicamba (450 + 290 and)580 g ae ha⁻¹), glyphosate + saflufenacil (450 + 18 and 50 g ai/ae ha⁻¹ rate), glyphosate + carfentrazone + sulfentrazone (450 + 9 + 53 and 105 g ai/ae ha⁻¹), and glyphosate + MCPA/dichlorprop/mecoprop-p (450 + 395/765/320 g ai/ae ha⁻¹) treatments all resulted in $\ge 80\%$ reduction in kochia biomass among kochia accessions, while all treatments showed $\geq 80\%$ biomass reduction at Coalhurst (Table 3-3). Glyphosate applied alone reduced GS kochia biomass in 2014 by about 97%, while the biomass of GR kochia was reduced by 7% only. This confirms that the GR kochia accession used was rather homogeneous for the glyphosate resistance trait.

Kochia accession differences in density, visual control, and biomass among locations and years could be attributed to differences in soil, weather conditions during or after application, and weather throughout the growing season. The two experimental locations had different soil parameters including soil texture (loam vs. clay loam), organic matter (2.5 vs. 3.6% OM), and pH (8.3 vs. 7.8 pH). Weather at the time of application may have influenced herbicide efficacy because heat, cold or drought stress can impact herbicide uptake and translocation. The total accumulated precipitation at Lethbridge and Coalhurst for the 2014 growing season (April to Oct) was above average (421 mm in 2014 vs. 313 mm 30-yr average), while the precipitation in 2015 was below average (197 mm in 2015 vs. 313 mm 30-yr average) (Figure 3-1).

In conclusion, the best treatments ($\geq 80\%$ visual control in all environments and $\geq 80\%$ biomass reduction in 2014 compared with the untreated control) for controlling GR and GS kochia in chemical fallow fields in southern Alberta were glyphosate + dicamba $(450 + 580 \text{ g ae ha}^{-1})$, glyphosate + dicamba/diflufenzopyr (450 + 150/50 g ai/ae ha⁻¹), glyphosate + saflufenacil (4550 g ai/ae ha⁻¹), and glyphosate + carfentrazone + sulfentrazone (450 + 9 + 105 g ai/ae ha⁻¹); and somewhat less consistently ($\geq 80\%$ visual control three out of four environments with $\geq 80\%$ biomass reduction in 2014) glyphosate + saflufenacil (450 + 18 g ai/ae ha⁻¹). Glyphosate + carfentrazone + sulfentrazone (450 + 9 + 105 g ai/ae ha-1) was consistently one of the best treatments for kochia control among environments and kochia accessions. Due to the recent discovery of triple-resistant kochia in Alberta, resistant to ALS-inhibitors, glyphosate, and dicamba (Beckie et al. 2019), glyphosate mixtures with multiple effective modes of action are warranted for successful and sustainable kochia management. Rotating these herbicide mixtures with several effective modes of action (Beckie and Reboud 2009) on chemical fallow and subsequent crops could help mitigate the accumulation of multiple herbicide resistance traits by reducing recurrent selection pressure.

Resistance management is necessary due to the quick evolution of herbicide resistance in kochia. The first report of GR kochia in Canada was identified in 2011 in chemical fallow fields in Warner County, Alberta, and at the time (2012) only 5% of kochia populations surveyed were confirmed GR (Beckie et al. 2013; Hall et al. 2014). After only five years, the incidence of glyphosate resistance in kochia populations increased from 5% (in 2012) to 50% (in 2017) (all kochia surveyed were ALS inhibitor-resistant and 18% were dicamba-resistant) (Beckie et al. 2019). The current study revealed several effective options for control of GS kochia in chemical fallow, and that the efficacy of many herbicide mixtures can be reduced following the assimilation of the glyphosate resistance trait. It is clear that mixing and rotating multiple effective modes of action can be a valuable tool for mitigating herbicide resistance, but the effective options (and thus efficacy of control) can diminish quickly following the selection for new types of resistance. For this reason, farmers are urged to adopt a proactive approach to integrated weed management; of which herbicides should comprise an important role supported be several other non-chemical tools. The use of cover crops, strategic spot tillage, mowing, and patch management are all tools that could help prolong the efficacy of these herbicide mixtures by mitigating seed production and limiting the number of kochia seeds returned to the soil seedbank.

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Chapter Four: Herbicide Strategies for Managing Glyphosateresistant and Susceptible Kochia (*Bassia scoparia*) in Spring Wheat

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4.1. Introduction

Kochia [*Bassia scoparia* (L.) A.J. Scott] is a troublesome, summer annual C₄ tumbleweed that was introduced to North America as an ornamental garden forb in the late 1800s (Friesen et al. 2009). At present, kochia is widely disseminated among the Prairie Provinces of Canada and the Western United States. While the range of kochia in North America continues to expand northward, its current northern distribution is limited by growing season length and thermal time requirements for successful reproduction (Beckie et al. 2012*b*). Despite these limitations, kochia remains the 15th most abundant weed species among annual crops in Alberta and Saskatchewan following post-emergence herbicide application, and the most abundant weed in the mixed grassland ecoregion of Alberta (Leeson 2016; Leeson et al. 2019).

Kochia is a problematic weed in agricultural lands including annual crops, perennial forages, hay fields, and rangeland, in addition to ruderal areas such as roadsides, railways, and oil well sites (Friesen et al. 2009). Several weedy traits allow kochia to thrive in such diverse environments. Kochia is tolerant of several abiotic stresses, including drought, heat, and salinity

(Braidek et al. 1984; Friesen et al. 2009; Endo et al. 2014). In Western Canada, it is among the first weed species to emerge in the spring, but prolonged emergence periodicity can result in emergence after pre- or post-emergence herbicide applications (Schwinghamer and Van Acker 2008; Dille et al. 2017; Kumar et al. 2018). Kochia plants produce a large number of seeds (up to 120 000 seeds plant⁻¹ in non-competitive environments), and these seeds can be dispersed over long distances when the stem of the senescing plant breaks at an abscission layer and the tumbleweed is blown by prevailing winds (Becker 1978; Stallings et al. 1995; Friesen et al. 2009; Beckie et al. 2016).

Herbicide resistance in kochia can spread rapidly due, in part, to protogynous flowering where the stigmas emerge and are receptive to pollen before the anthers fully mature on the same plant – which causes initial outcrossing prior to self-pollination. Efficient pollen- and seedmediated gene flow (Beckie et al. 2016) and short seed bank longevity (1-2 years) (Beckie et al. 2018a) contribute to rapid population turnover and evolution of resistance in response to recurrent selection pressures, like herbicides. Acetolactate synthase (ALS) inhibitor-resistant kochia was reported first in Saskatchewan and Manitoba in 1988, and in Alberta in 1989 (Morrison and Devine 1994; Heap 2020). Two decades later, ALS inhibitor-resistant kochia was disseminated throughout Western Canada, and present in 85% of surveyed populations (Beckie et al. 2011). Currently, all kochia populations in Western Canada are considered ALS inhibitor-resistant (Beckie et al. 2019). Kochia was the first glyphosate-resistant (GR) weed reported in Western Canada (in Alberta in 2011) (Beckie et al. 2013), following initial reports of this biotype in Kansas in 2007 (Kumar et al. 2019). Subsequent surveys in 2013 identified GR kochia in the Prairie Provinces of Saskatchewan and Manitoba (Beckie et al. 2015). In Alberta, glyphosate resistance in kochia spread rapidly from 4% to 50% of kochia populations sampled in 2012 and 2017, respectively

(Hall et al. 2014; Beckie et al. 2019). The 2017 survey of Alberta also reported dicamba resistance in 18% of the kochia populations sampled, while 10% of the populations were triple herbicideresistant to ALS inhibitors, glyphosate, and dicamba. Rapid spread of herbicide-resistant kochia in the Canadian Prairies over the past decade warrants investigation of alternative herbicide options in many crops, including small-grain cereals, pulses, and oilseeds.

Glyphosate is a non-selective herbicide that is touted for its several favorable qualities including systemic activity on a wide range of plant species, low mammalian toxicity, minimal impact on the environment, and low herbicide cost (Duke and Powles 2008). Farmers in the Great Plains of North America rely on glyphosate as a cost-effective option for pre-plant burndown weed control in place of tillage in no-till production systems (Geddes 2019). Wide-spread adoption of no-till cropping systems and production of GR crops contribute to increased glyphosate use pre-plant and post-emergence. In addition to these windows for weed control, farmers also use glyphosate to manage weeds pre- and post-harvest. As a result, glyphosate use in the Canadian Prairies tripled in the past decade (Blackshaw and Harker 2016), which undoubtedly increased selection pressure for GR weeds (Beckie et al. 2013). Greater abundance of GR weeds, like kochia, in the Great Plains of North America threatens the sustainability of no-till production systems because farmers may consider reverting to tillage for mechanical weed control (Geddes 2019).

Spring wheat (*Triticum aestivum* L.) (including durum) is the most grown crop in the Canadian Prairies based on seeded area, where it was grown on about 9.4 million hectares in 2020 (Statistics Canada 2020). Kochia can be difficult to manage in spring wheat, especially if it is controlled inadequately by, or emerges after, the pre-plant burndown herbicide application. Based on representative herbicide programs in the United States, GR kochia control varied among experimental sites to a greater extent in wheat compared with corn (*Zea mays* L.) or soybean

[*Glycine max* (L.) Merr.] (Sbatella et al. 2019). Lower densities of glyphosate-susceptible (GS) kochia (14 and 21 plants m⁻²) reduced spring wheat yield by 10 to 33% in Manitoba, while higher densities (195 to 520 plants m⁻²) reduced yield by 40 to 73% (Friesen et al. 2009). In addition to yield loss, kochia can hinder harvest operations because indeterminate growth results in kochia plants which remain green long after spring wheat senescence (Figure 4-1).

Due to the reliance on glyphosate for pre-plant weed control before growing wheat and the increased abundance of herbicide-resistant kochia in western Canada, there is a need to determine effective herbicide options for kochia control. The objective of this study was to determine which herbicide options remain effective for management of GR and GS kochia in spring wheat in western Canada.

4.2. Materials and Methods

4.2.1. Site Description

Field experiments were conducted at the Agriculture and Agri-Food Canada Lethbridge Research and Development Centre located near Lethbridge, AB (49.69°N, -112.77°W) in 2013, 2014, and 2015, and at Hamman Ag Research Inc. located near Coalhurst, AB (49.79°N, -112.99°W) in 2013 and 2014. Soils at both locations were classified as dark brown chernozems. The soil at Lethbridge was a clay loam with 3.6% OM and pH 7.8, while the soil at Coalhurst was a loam with 2.5% OM and pH 8.3. In all years, the previous crop at Lethbridge was silage barley, while the previous crop at Coalhurst was chemical fallow.



Figure 4-1. A kochia patch during spring wheat senescence in southwestern Saskatchewan. Photo credit: Dr. Charles M. Geddes © Her Majesty the Queen in Right of Canada, 2019.

4.2.2. Experimental Design and Treatment Structure

The field experiment conducted within each of the five environments followed a split-block randomized complete block design with a two-way factorial treatment structure and four experimental replications (blocks). The main plot size was 2.5 x 7.5 m in Lethbridge and Coalhurst 2013, 3.0 x 7.5 m in Lethbridge 2014 and 2015, and 2.5 x 6.0 m in Coalhurst 2014. Each block was split in half with two kochia accessions, one GR and the other GS. The kochia accession splitblocks were randomized among the experimental replications. A Fabro double-disk drill (or Fabro hoe-drill in Coalhurst 2014) (Fabro Enterprises Ltd., Swift Current, SK) was used to seed spring wheat 'AC Lillian' and kochia simultaneously along each experimental replication, perpendicular to the direction of the herbicide treatments. Each kochia accession was seeded in a different pass with the seeder. Each seeder pass included 10 rows of wheat spaced 23 cm apart with 9 rows of kochia seeded between the wheat rows. The wheat was planted at a depth of 3.5 cm, while the kochia seed was placed 0.3 cm below the soil surface and pressed into the soil with the seeder packing tires. Both wheat and kochia were seeded at a target rate of 300 viable seeds m⁻². The wheat seed was treated with CruiserMaxx[®] Cereals (Syngenta Canada Inc., Guelph, ON), containing 2.8% thiamethoxam, 3.4% difenoconazole, and 0.6% metalaxyl-M, at 3.9 mL kg⁻¹ seed. Monoammonium phosphate or triple superphosphate fertilizer were placed within the seed-row and urea was placed in a side-row band (or ESN broadcast before seeding in Lethbridge 2015) based on soil test recommendations for spring wheat.

The kochia seed accessions were sourced from the Agriculture and Agri-Food Canada Lethbridge Research and Development Centre. The GR kochia accession was selected and maintained among successive generations following treatment with glyphosate (Roundup Transorb[®] HC, Monsanto Canada Inc., Winnipeg, MB) at 900 g ae ha⁻¹. Both kochia accessions were ALS inhibitor-resistant, and the GS accession was selected and maintained among generations using thifensulfuron-methyl + tribenuron-methyl at 10 + 5 g ai ha⁻¹ (Refine[®] SG; FMC Corporation, Philadelphia, PA).

A pre-plant burndown was conducted in each environment with chemicals used based on the weed species that were present. In Lethbridge 2013 and Coalhurst 2013 and 2014, glyphosate was applied at 900 g ae ha⁻¹. Glyphosate was applied at 1334 g ae ha⁻¹ at Lethbridge in 2014, while glyphosate + bromoxynil (Koril[®], Nufarm Canada, Calgary, AB) were used at Lethbridge in 2015 at a rate of 1334 + 348 g ae/ai ha⁻¹.

Herbicide treatments were chosen because they were registered for control of kochia in spring wheat, or because they held potential for adequate kochia control with minimal wheat injury (Table 4-1). All herbicide treatments were applied post-emergence at the 4 to 5 leaf stage of wheat with the exception of sulfentrazone which was applied pre-emergence (1 to 2 days before or after seeding). At Lethbridge, the herbicides were applied using a 2.0 m bicycle CO₂ sprayer equipped with Greenleaf Air Mix 110-010 nozzles (Greenleaf Technologies, Covington, LA) calibrated to deliver 100 L ha⁻¹ spray solution at 290 kPa when travelling at 5 km hr⁻¹. At Coalhurst, the herbicides were applied using a 2.0 m hand-held propane-propelled sprayer equipped with John Deere LDX01 nozzles (John Deere, Moline, IL) calibrated to deliver 100 L ha⁻¹ spray solution at 242 kPa when travelling at 4 km hr⁻¹.

4.2.3. Data Collection

Visible injury of wheat was assessed within each main plot as a percentage from 0% (visually similar to the untreated control) to 100% (complete necrosis) 3 wk after post-emergence herbicide application (WAA) (Canadian Weed Science Society (CWSS) 2018). Wheat grain yield was determined by harvesting each subplot separately using a Wintersteiger Delta (Wintersteiger

Herbicide common name ^a	Herbicide trade name	Herbicide group	Concentration (g ae/ai L ⁻¹)	Formulation	Rate (g ae/ai ha ⁻¹)	Company
Dicamba + 2,4-D	Banvel [®] II + 2,4-D amine 600	4 + 4	480 + 560	SN + EC	110 + 420	BASF + Nufarm Agriculture
Bromoxynil/2,4-D	Thumper [®]	6/4	280/280	EC	280/280	Bayer CropScience
Fluroxypyr/2,4-D	OcTTain [™] XL	4/4	90/360	EC	100/400	Corteva AgriScience
Florasulam/Fluroxypyr+MCPA	Stellar TM A + Stellar TM B	2/4 + 4	2.5/100 + 600	SC + EC	2.5/100 + 350	Corteva AgriScience
Dicamba/ Fluroxypyr	Pulsar®	4/4	87/113	EC	80/104	Syngenta
Fluroxypyr + Clopyralid/MCPA	Prestige TM XCA + Prestige TM XCB	4 + 4/4	333 + 50/280	EC + EC	100 + 75/420	Corteva Agriscience
Fluroxypyr/Bromoxynil/2,4-D	Enforcer [®] D	4/6/4	80/190/240	EC	48/114/144	Nufarm Agriculture
Fluroxypyr/ Bromoxynil/2,4-D	Enforcer [®] D	4/6/4	80/190/240	EC	96/228/288	Nufarm Agriculture
MCPA/ Diclorprop-P/Mecoprop-P	Optica TM Trio	4/4/4	160/310/130	SN	395/765/320	Nufarm Agriculture
MCPA/ Mecoprop-P/Dicamba	Target [®]	4/4/4	275/62.5/62.5	SN	275/62.5//62.5	Syngenta
Pyrasulfotole/Bromoxynil	Infinity ^{®,c}	27/6	37.5/210	EC	30/170	Bayer CropScience
Dicamba/2,4-D/ Mecoprop-P	$\mathrm{DyVel}^{\circledast} \mathrm{DS}_{\mathrm{P}}$	4/4/4	110/295/80	SN	93/251/68	BASF
Dicamba/2,4-D/ Mecoprop-P	$\mathrm{DyVel}^{\circledast} \mathrm{DS}_{\mathrm{P}}$	4/4/4	110/295/80	SN	124/331/90	BASF
Dichlorprop-P/2,4-D	Estaprop [®] XT	4/4	210/400	EC	368/702	Nufarm Agriculture
Sulfentrazone‡	Authority [®] 480	14	480	SC	105	FMC Corporation
Fluroxypyr/Halauxifen+MCPA	$Pixxaro^{TM} A + Pixxaro^{TM} B$	4/4 + 4	250/16.5 + 600	EC + EC	77/5 + 350	Corteva Agriscience
Fluroxypyr/Halauxifen+MCPA	$Pixxaro^{TM} A + Pixxaro^{TM} B$	4/4 + 4	250/16.5 + 600	EC + EC	100/6.5+455	Corteva Agriscience
Dicamba	Banvel [®] II	4	480	SN	300	BASF
Dicamba	Banvel [®] II	4	480	SN	600	BASF

 Table 4-1. Herbicide treatments assessed based on control of glyphosate-resistant and glyphosate-susceptible kochia in spring wheat near Lethbridge and Coalhurst, AB, in 2013 and 2014, and Lethbridge, AB, in 2015.

Note: EC, emulsifiable concentrate; SC, suspension concentrate; SN, solution.

^aAll herbicides were applied post-emergence at wheat 4–5 leaf stage with the exception of sulfentrazone, which was applied pre-emergence.

^bApplied with ammonium sulfate at 1% v/v.

Inc., Saskatoon, SK) or Zürn 150 plot combine (Zürn Harvesting GmbH & Co. KG, Schöntal-Westernhausen, Germany), cleaning the seed using a clipper seed cleaner, and adjusting the clean seed weight to 14.5% moisture.

Kochia plant density was determined for each kochia accession two weeks after emergence by counting all seedlings within a 0.25 m² quadrat placed randomly within each subplot. Visible control of kochia was assessed within each subplot as a percentage from 0 (visually similar to the untreated control) to 100% (complete necrosis) 3 WAA (CWSS 2018). Kochia shoot biomass fresh weight was determined 6 WAA by removing and weighing all kochia from a 0.5 m² area (3 rows by 0.71 m) within each subplot.

4.2.4. Statistical Analysis

All data were analyzed using the GLIMMIX procedure of SAS 9.4 (SAS Institute Inc., Cary, NC). The wheat response variables included visible injury 3 WAA, and grain yield, while the kochia response variables included plant density, visible control 3 WAA, and shoot biomass. The analyses were separated by year due to the addition of two herbicide treatments in 2014 which were not present in 2013, and three treatments in 2015 which were not present in 2014. The main and interaction effects of herbicide treatment, kochia accession, and environment were considered fixed effects, while experimental replication nested within environment, the interaction of herbicide treatment and experimental replication nested within environment, and the interaction of kochia accession and experimental replication nested within environment were considered random effects.

Residual normality was assessed using the Shapiro-Wilk statistic, while homoscedasticity was assessed visually by plotting the residuals against the predicted values (Littell et al. 2006). Extreme outliers were removed based on Lund's test (Lund 1975). The covariance structure of

residuals was adjusted to correct for heteroscedasticity based on minimization of the Akaike Information Criterion and visual assessment of the residuals vs. predicted values (Littell et al. 2006). For analyses of kochia visible control, the residual group option was set to environment, while the group option was set to the kochia accession by environment interaction for the other response variables. For kochia biomass, a lognormal distribution was fit with the identity link function to meet the assumptions of normality and homoscedasiticity. The F-test was used to determine significant main and interaction effects, and means were compared using Tukey's HSD ($\alpha = 0.05$).

4.3. Results and Discussion

4.3.1 Annual Weather Variation

Temperatures during the growing season among the three years of field experimentation in Lethbridge ranged on average from 0.6 to 1.1°C warmer than the 30-yr climactic normal for this region (Figure 4-2). The summers (July through September) were consistently warmer than normal, however, conditions during the spring months varied around climatic normal temperatures (Figure 4-2). Cumulative growing season precipitation varied among years and ranged from about one-third greater than normal in 2013 and 2014 to one-third less than normal in 2015 (418, 421 and 197 mm of precipitation received from April through October in 2013, 2014, and 2015, respectively, compared with the 30-yr climatic normal of 313 mm). The greatest variability in precipitation was experienced in June of each year, the month during which post-emergence herbicides were applied. During the month of June, precipitation was equivalent to 184%, 228%, and 23% of the climatic normal in 2013, 2014, and 2015, respectively (Figure 4-2). Weather data were compiled for the Lethbridge site only due to lack of a weather station near the Coalhurst site, and the proximity of these two locations.

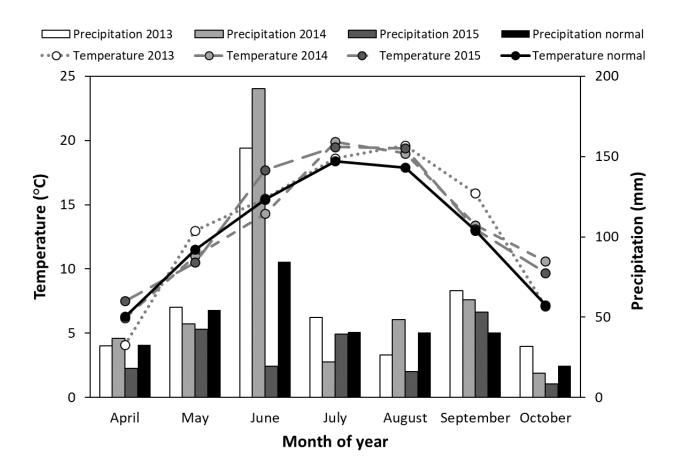


Figure 4-2. Growing season monthly average temperature and precipitation at Lethbridge AB, in 2013, 2014, and 2015 compared with the 30-yr average (normal) monthly temperature and precipitation for this region. In 2014, Coalhurst received 50 mm of supplemental irrigation in July and August. In 2015, Lethbridge received 6 mm, 25 mm, and 25mm of supplemental irrigation in May, June and July, respectively.

4.3.2. Herbicide Treatments

4.3.2.1. Wheat Injury and Grain Yield

Visible injury data did not conform to the assumptions of ANOVA due to an abundance of zero values and were therefore presented as simple means (Table 4-2). Wheat visible injury was considered minor among the herbicide treatments in the majority of environments. Injury ratings from 0 to 10% were considered acceptable because the crop generally outgrows minor injury absent of yield penalty (Pest Management Regulatory Agency (PMRA) 2016). Based on PMRA standards, wheat visible injury was not acceptable in Coalhurst 2014 and Lethbridge 2015 for certain treatments where dicamba was applied alone or in mixture with other synthetic auxin active ingredients (Table 4-2). Visible injury ranged from 11% to 21% in Coalhurst 2014 for dicamba + 2,4-D (110 + 420 g ae ha⁻¹), dicamba/fluroxypyr (80/104 g ae ha⁻¹), MCPA/mecoprop-p/dicamba (275/62.5/62.5 g ae ha⁻¹), and both high and low rates of dicamba/2,4-D/mecoprop-p (93/251/68 and 124/331/90 g ae ha⁻¹). Treatments including higher rates of dicamba applied alone were tested in 2015 only, and the highest rate (600 g ae ha⁻¹) was the only herbicide treatment in this environment that resulted in crop injury considered unacceptable (21% injury), while injury from dicamba applied at 300 g ae ha⁻¹ was considered just acceptable (10% injury).

Wheat yield remained the same among the kochia accessions, herbicide treatments, and the untreated weedy control in each of the environments tested (Table 4-3; Table 4-4). Lack of spring wheat yield response suggested that the densities of kochia present in the current study were too low to result in considerable yield reduction. This is unlikely considering the spring wheat yield loss values in response to low densities of GS kochia reported previously (Friesen et al. 2009). Alternatively, lack of yield difference following a range of herbicide treatments compared with

		Wheat injury ^{<i>a</i>} (% \pm SE)						
	Rate	20	13	20	14	2015		
Herbicide treatment ^b	(g ae/ai ha ⁻¹)	Lethbridge	Coalhurst	Lethbridge	Coalhurst	Lethbridge		
Dicamba+2,4-D	110 + 420	0 + 0.0	1 + 1.0	5 ± 0.0	18 ± 3.9	8 ± 1.4		
Bromoxynil/2,4-D	280/280	0 + 0.0	1 + 1.2	0 ± 0.0	0 ± 0.0	0 ± 0.0		
Fluroxypyr/2,4-D	40/160	0 + 0.0	2 + 1.2	0 ± 0.0	1 ± 1.3	0 ± 0.0		
Florasulam/Fluroxypyr + MCPA	2.5/100 + 350	0 + 0.0	0 + 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0		
Dicamba/Fluroxypyr	80/104	0 + 0.0	1 + 1.2	4 ± 1.3	13 ± 1.4	5 ± 2.0		
Fluroxypyr + Clopyralid/MCPA	100 + 75/420	0 + 0.0	1 + 1.2	0 ± 0.0	0 ± 0.0	0 ± 0.0		
Fluroxypyr/Bromoxynil/2,4-D	48/114/144	0 + 0.0	2 + 1.4	0 ± 0.0	1 ± 1.3	0 ± 0.0		
Fluroxypyr/Bromoxynil/2,4-D	96/228/288	0 + 0.0	2 + 1.4	4 ± 2.4	0 ± 0.0	3 ± 1.4		
MCPA/Dichlorprop-P/Mecoprop-P	395/765/320	0 + 0.0	5 + 2.5	5 ± 0.0	6 ± 2.4	9 ± 1.3		
MCPA/Mecoprop-P/Dicamba	275/62.5/62.5	0 + 0.0	0 + 0.0	0 ± 0.0	11 ± 0.5	0 ± 0.0		
Pyrasulfotole/Bromoxynil	30/170	0 + 0.0	0 + 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0		
Dicamba/2,4-D/Mecoprop-P	93/251/68	0 + 0.0	3 + 2.0	3 ± 1.4	13 ± 1.2	4 ± 1.3		
Dicamba/2,4-D/Mecoprop-P	124/331/90	0 + 0.0	3 + 1.5	8 ± 1.4	21 ± 3.1	9 ± 2.4		
Dichlorprop-P/2,4-D	368/702	0 + 0.0	3 + 2.0	5 ± 2.0	0 ± 0.0	5 ± 0.0		
Sulfentrazone ^c	105	<i>c</i>		5 ± 2.0	0 ± 0.0	0 ± 0.0		
Fluroxypyr/Halauxifen+MCPA	77/5 + 350			0 ± 0.0	0 ± 0.0	0 ± 0.0		
Fluroxypyr/Halauxifen + MCPA	100/6.5 + 455	_		—		0 ± 0.0		
Dicamba	300	—		—	_	10 ± 0.0		
Dicamba	600			—	—	21 ± 1.3		

Table 4-2. Wheat visible injury in response to herbicide treatments evaluated three weeks after post-emergence herbicide application in five environments near Lethbridge (2013-2015) and Coalhurst, AB (2013-2014).

^{*a*} Values are simple means \pm SE ^{*b*} All herbicides were applied post-emergence at wheat 4-5 leaf stage except for sulfentrazone which was applied pre-emergence ^{*c*} — indicates absence of the herbicide from testing in 2013 or 2014

		201	3			2014				201	5 ^{<i>a</i>}	
	Kochia	Kochia	Kochia	Wheat	Kochia	Kochia density	Kochia	Wheat	Kochia	Kochia	Kochia	Wheat
	control	density ^b	biomass ^s	yield	control	(plants m ⁻²)	biomass	yield	control	density	biomass	yield
Fixed effect	(%)	(plants m ⁻²)	$(g m^{-2})$	(kgha ⁻¹)	(%)		$(g m^{-2})$	(kgha ⁻¹)	(%)	(plants m ⁻²)	$(g m^{-2})$	(kgha ⁻¹)
Herbicide (H)	< 0.001	0.824		0.289	< 0.001	< 0.001	< 0.001	0.885	< 0.001	< 0.001	< 0.001	0.164
Accession(A)	0.207	0.318		0.477	< 0.001	< 0.001	0.002	0.405	0.321	0.007	0.039	0.897
Environment (E)	0.942	—§	—	0.130	< 0.001	< 0.001	0.650	< 0.001	_	_	—	—
НхА	0.026	0.112		0.366	0.456	0.432	0.423	0.712	0.151	0.079	0.065	0.385
НxЕ	< 0.001	_	—	0.950	< 0.001	< 0.001	0.359	0.174	_	_	—	—
A x E	0.669	_	—	0.982	0.100	< 0.001	0.962	0.289	_	_	—	—
HxAxE	0.174	—	—	0.111	0.489	0.519	0.328	0.774	_	—	—	_

Table 4-3. ANOVA table showing the significance of main and interaction effects of herbicide treatment (H), glyphosate-resistant vs. glyphosate-susceptible kochia accession (A), and environment (E) on kochia visible control, density, biomass, and wheat yield in 2013, 2014 (Lethbridge and Coalhurst, AB) and 2015 (Lethbridge only).

Note: Boldface text indicates significant effects at $P \le 0.05$; dash (--) indicates absence of the effect from the ANOVA.

^{*a*}The experiment in 2015 was conducted near Lethbridge only.

^bVisible differences in kochia density were absent at Lethbridge in 2013, and thus density was measured in the untreated control treatment only; Lethbridge 2013 was excluded from the kochia density ANOVA.

^cKochia biomass was not sampled in 2013.

	,	Wheat grain yield ^{<i>ab</i>} (kg ha ⁻¹)							
	Rate	201	13	201	4	2015			
Herbicide treatment ^c	(g ae/ai ha ⁻¹)	Lethbridge	Coalhurst	Lethbridge	Coalhurst	Lethbridge			
Untreated		4240	4360	4294	2938	4195			
Dicamba + 2,4-D	110 + 420	4315	4454	4246	3141	4359			
Bromoxynil/2,4-D	280/280	4330	4655	4355	3278	4261			
Fluroxypyr/2,4-D	40/160	4359	4565	4479	2814	4663			
Florasulam/Fluroxypyr + MCPA	2.5/100 + 350	4528	4728	4380	2941	4441			
Dicamba/Fluroxypyr	80/104	4230	4549	4343	3188	4278			
Fluroxypyr + Clopyralid/MCPA	100 + 75/420	4313	4646	4711	2948	4296			
Fluroxypyr/Bromoxynil/2,4-D	48/114/144	4361	4605	4324	3069	4534			
Fluroxypyr/Bromoxynil/2,4-D	96/228/288	4229	4333	4393	2973	4671			
MCPA/Dichlorprop-P/Mecoprop-P	395/765/320	4581	4518	4583	2898	4304			
MCPA/Mecoprop-P/Dicamba	275/62.5/62.5	4393	4840	4564	2930	4496			
Pyrasulfotole/Bromoxynil	30/170	4421	4728	4268	2878	4533			
Dicamba/2,4-D/Mecoprop-P	93/251/68	4271	3328	4286	3268	4679			
Dicamba/2,4-D/Mecoprop-P	124/331/90	4359	4316	4676	2956	4401			
Dichlorprop-P/2,4-D	368/702	4211	4463	4336	3008	4705			
Sulfentrazone	105	d	—	4310	3261	4626			
Fluroxypyr/Halauxifen + MCPA	77/5 + 350	—	—	4625	3105	4610			
Fluroxypyr/Halauxifen + MCPA	100/6.5 + 455	—	—	—	—	4759			
Dicamba	300	—	—	—	—	4623			
Dicamba	600	—	—	—	—	3734			
\pm SEM		(± 162)	(± 175)	(± 194)	(± 194)	(± 254)			

Table 4-4. Wheat grain yield in response to kochia management with herbicide treatments in five environments near Lethbridge (2013-2015) and Coalhurst, AB (2013-2014).

^{*a*} Values are means, while parenthetical values indicate SEM

^b Within columns, significant differences were absent based on the F-test and Tukey's HSD ($\alpha = 0.05$)

^c All herbicides were applied post-emergence at wheat 4-5 leaf stage except for sulfentrazone which was applied pre-emergence d — indicates absence of the herbicide from testing in 2013 or 2014

that of the untreated weedy control could suggest that spring wheat yield loss manifests in response to kochia interference prior to the wheat 4-5 leaf stage. A true untreated weed-free control treatment would aid in this conjecture; however, this treatment was not present in the current study. The sulfentrazone (105 g ai ha⁻¹) treatment applied pre-emergence could serve a similar purpose as the weed-free control due to minimal wheat visible injury (0 to 5% among environments) and the very low density of kochia present in this treatment prior to the timing of the post-emergence herbicide treatments (6 plants and 1 plant m⁻² in 2014 and 2015, respectively; treatment not present in 2013). However, since kochia density was evaluated prior to the post-emergence herbicide timing only, we cannot rule out the possibility that late emerging kochia caused wheat yield loss following the period of residual activity provided by sulfentrazone applied pre-emergence. Despite the lack of wheat yield response to herbicide treatments in the current study, the true benefit of herbicide treatment in the wheat phase of the crop rotation could manifest as a reduction in kochia biomass inhibiting harvest operations, and likely also reduced seed production and return to the soil seedbank.

4.3.2.2. Kochia Plant Density, Visible Control, and Biomass

Kochia densities evaluated before post-emergence herbicide treatment remained the same among all post-emergence herbicide treatments in each environment (Tables 4-5, 4-6, and 4-7). At Lethbridge 2013, kochia density was evaluated in the untreated control treatment only (85 ± 6.1 plants m⁻²) due to visual observation of consistent densities among plots. Sulfentrazone (105 g ai ha⁻¹) applied pre-emergence reduced kochia density by 96% and 99% in Lethbridge 2014 and 2015, respectively; however, this effect was not observed in Coalhurst 2014 due to the low kochia densities present in this environment overall (27 ± 3.8 plants m⁻² in Coalhurst 2014 compared with

Table 4-5. Visible control of glyphosate-resistant (GR) and glyphosate-susceptible (GS) kochia three weeks after post-emergence herbicide application in wheat in two environments near Lethbridge and Coalhurst, AB in 2013.

		Density ^b			Visil	ole control		
		(plants m ⁻²)				(%)		
	Rate	Coalhurst	Lethbridge	Coalhurst		1	Among environn	nents
Herbicide treatment ^a	(g ae/ai ha ⁻¹)				GS	GR	GS vs. GRI	Among accessions
Untreated		131						
Dicamba + 2,4-D	110 + 420	133	78 e	79 b	78 d	78 d	0.884	78 g
Bromoxynil/2,4-D	280/280	153	81 de	91 ab	84 cd	87 b-d	0.052	86 e-g
Fluroxypyr/2,4-D	40/160	125	97 ab	90 ab	91 a-c	96 ab	0.005	93 a-d
Florasulam/Fluroxypyr + MCPA	2.5/100 + 350	190	88 a-e	88 ab	87 b-d	89 a-c	0.128	88 c-f
Dicamba/Fluroxypyr	80/104	147	96 ab	90 ab	93 a-c	94 ab	0.559	93 a-d
Fluroxypyr + Clopyralid/MCPA	100 + 75/420	173	93 a-c	88 ab	92 a-c	89 a-c	0.083	91 a-f
Fluroxypyr/Bromoxynil/2,4-D	48/114/144	118	95 ab	84 ab	91 a-c	88 a-c	0.061	90 a-f
Fluroxypyr/Bromoxynil/2,4-D	96/228/288	191	99 a	96 a	97 a	97 a	0.826	97 a
MCPA/Dichlorprop-P/Mecoprop-P	395/765/320	179	98 a	93 a	96 ab	96 ab	0.826	96 ab
MCPA/Mecoprop-P/Dicamba	275/62.5/62.5	124	83 c-e	85 ab	84 cd	84 cd	0.715	84 fg
Pyrasulfotole/Bromoxynil	30/170	172	90 a-d	96 a	92 a-c	93 a-c	0.559	93 a-e
Dicamba/2,4-D/Mecoprop-P	93/251/68	164	86 b-e	91 ab	89 a-c	88 a-d	0.308	88 b-f
Dicamba/2,4-D/Mecoprop-P	124/331/90	140	95 ab	94 a	94 a-c	95 ab	0.466	94 a-c
Dichlorprop-P/2,4-D	368/702	168	80 de	92 a	84 cd	88 a-d	0.027	86 d-f

Note: Values are LS means. Within columns, different letters indicate significant differences based on Tukey's HSD ($\alpha = 0.05$).

^aHerbicides were applied post-emergence at wheat 4-5 leaf stage.

^bVisual differences in kochia density were absent among treatments in Lethbridge 2013, and therefore density was evaluated in the untreated control only (data not shown).

^cP value indicating significant difference in visible control between GS and GR kochia accessions.

			Lethbridge			Coalhurst		Aı	nong environm	ents
		Visible			Visible			Visible		
	Rate	control	Density	Biomass	control	Density	Biomass	control	Density	Biomass
Herbicide treatment ^a	(g ae/ai ha ⁻¹)	(%)	(plants m ⁻²)	$(g m^{-2})$	(%)	(plants m ⁻²)	$(g m^{-2})$	(%)	(plants m ⁻²)	$(g m^{-2})$
Untreated			129 a	138 a		27	45		78 a	78 a
Dicamba + 2,4-D	110 + 420	67 gh	140 a	18 a-c	91 ab	24	26	79 fg	82 a	21 а-с
Bromoxynil/2,4-D	280/280	63 h	148 a	5 b-e	83 b	36	12	73 g	92 a	8 b-d
Fluroxypyr/2,4-D	40/160	83 с-е	136 a	48 ab	90 ab	21	13	86 c-e	79 a	25 ab
Florasulam/Fluroxypyr + MCPA	2.5/100 + 350	78 d-f	140 a	23 ab	90 ab	22	14	84 d-f	81 a	18 a-c
Dicamba/Fluroxypyr	80/104	83 с-е	112 a	11 b-d	89 ab	26	13	86 c-e	69 a	12 bc
Fluroxypyr + Clopyralid/MCPA	100 + 75/420	74 e-g	130 a	43 ab	89 ab	34	32	81 ef	82 a	37 ab
Fluroxypyr/Bromoxynil/2,4-D	48/114/144	68 f-h	111 a	8 b-d	94 a	39	19	81 ef	75 a	13 a-c
Fluroxypyr/Bromoxynil/2,4-D	96/228/288	92 a-c	129 a	1 c-e	93 a	28	7	92 a-c	78 a	3 cd
MCPA/Dichlorprop-P/Mecoprop-P	395/765/320	84 b-d	121 a	12 b-d	93 a	39	11	89 b-d	80 a	11 bc
MCPA/Mecoprop-P/Dicamba	275/62.5/62.5	71 f-h	144 a	20 а-с	90 ab	26	26	80 ef	85 a	23 а-с
Pyrasulfotole/Bromoxynil	30/170	94 ab	140 a	1 de	94 a	37	0	94 ab	88 a	0^b
Dicamba/2,4-D/Mecoprop-P	93/251/68	76 d-g	127 a	10 b-d	89 ab	22	21	83 d-f	74 a	15 a-c
Dicamba/2,4-D/Mecoprop-P	124/331/90	83 с-е	145 a	13 a-d	89 ab	22	12	86 c-e	83 a	13 bc
Dichlorprop-P/2,4-D	368/702	71 f-h	135 a	21 а-с	88 ab	25	14	80 f	80 a	17 a-c
Sulfentrazone	105	98 a	5 b	1 e	95 a	8	2	96 a	6 b	1 d
Fluroxypyr/Halauxifen + MCPA	77/5 + 350	77 d-g	146 a	38 ab	86 ab	22	25	82 ef	84 a	30 ab

Table 4-6. Visible control three weeks after post-emergence herbicide application, density, and aboveground biomass of glyphosate-resistant (GR) and glyphosate-susceptible (GS) kochia in wheat in two environments near Lethbridge and Coalhurst, AB in 2014.

Note: Values are LS means. Within columns, different letters indicate significant differences based on Tukey's HSD ($\alpha = 0.05$).

 a All herbicides were applied post-emergence at wheat 4–5 leaf stage except for sulfentrazone, which was applied pre-emergence. b Non-estimable.

		Lethbridge				
		Visible				
	Rate	control	Density	Biomass		
Herbicide treatment ^a	(g ae/ai ha ⁻¹)	(%)	(plants m ⁻²)	$(g m^{-2})$		
Untreated			246 a	208 a		
Dicamba + 2,4-D	110 + 420	68 g	210 a	23 b-d		
Bromoxynil/2,4-D	280/280	71 fg	235 а	29 bc		
Fluroxypyr/2,4-D	40/160	79 d-f	210 a	33 ab		
Florasulam/Fluroxypyr + MCPA	2.5/100 + 350	79 d-f	230 a	27 bc		
Dicamba/Fluroxypyr	80/104	86 b-d	219 a	12 b-g		
Fluroxypyr + Clopyralid/MCPA	100 + 75/420	79 d-f	217 a	42 ab		
Fluroxypyr/Bromoxynil/2,4-D	48/114/144	73 e-g	188 a	18 b-e		
Fluroxypyr/Bromoxynil/2,4-D	96/228/288	94 ab	212 a	3 e-g		
MCPA/Dichlorprop-P/Mecoprop-P	395/765/320	89 bc	198 a	1 g		
MCPA/Mecoprop-P/Dicamba	275/62.5/62.5	72 fg	227 а	15 b-f		
Pyrasulfotole/Bromoxynil	30/170	92 ab	216 a	2 fg		
Dicamba/2,4-D/Mecoprop-P	93/251/68	78 d-f	243 a	2 g		
Dicamba/2,4-D/Mecoprop-P	124/331/90	89 bc	227 а	5 b-g		
Dichlorprop-P/2,4-D	368/702	71 fg	177 a	11 b-g		
Sulfentrazone	105	99 a	1 b	1 d-g		
Fluroxypyr/Halauxifen + MCPA	77/5 + 350	71 fg	199 a	42 ab		
Fluroxypyr/Halauxifen + MCPA	100/6.5 + 455	81 de	198 a	37 ab		
Dicamba	300	83 cd	231 a	5 c-g		
Dicamba	600	94 ab	221 a	2 d-g		

Table 4-7. Visible control three weeks after post-emergence herbicide application, density, and aboveground biomass of glyphosate-resistant (GR) and glyphosate-susceptible (GS) kochia in wheat in one environment near Lethbridge, AB in 2015.

Note: Values are LS means. Within columns, different letters indicate significant differences based on Tukey's HSD ($\alpha = 0.05$).

^{*a*}All herbicides were applied post-emergence at wheat 4–5 leaf stage except for sulfentrazone, which was applied preemergence.

85 to 205 plants m⁻² among kochia accessions and herbicide treatments in the other environments) (Tables 4-6 and 4-7).

Several herbicide treatments controlled GR and GS kochia accessions effectively in spring wheat. The PMRA defines weed control as a visible control rating of \geq 80% (PMRA 2016). Several herbicide treatments achieved $\geq 80\%$ visible control in all environments in which they were tested, including dicamba/fluroxypyr (80/104 g ae ha⁻¹), fluroxypyr/bromoxynil/2,4-D (96/288/288 g ae/ai ha⁻¹), MCPA/dichlorprop-p/mecoprop-p (395/765/320 g ae ha⁻¹), pyrasulfotole/bromoxynil (30/170 g ai ha⁻¹), dicamba/2,4-D/mecoprop-p (124/331/90 g ae ha⁻¹), sulfentrazone (105 g ai ha⁻¹) ¹) applied pre-emergence, fluroxypyr/halauxifen + MCPA (100/6.5 + 455 g ae ha⁻¹), and both rates of dicamba (300 or 600 g ae ha^{-1}); although the latter four treatments were only tested in 3, 1, 1, and 1 environment(s), respectively (Tables 4-5, 4-6, and 4-7). Among these treatments, sulfentrazone (105 g ai ha⁻¹) applied pre-emergence, and fluroxypyr/bromoxynil/2,4-D (96/288/288 g ae/ai ha⁻¹), pyrasulfotole/bromoxynil (30/170 g ai ha⁻¹), and the highest rate of dicamba (600 g ae ha⁻¹) applied post-emergence resulted in excellent visible control (\geq 90% visible control) in all environments tested. While excellent kochia control was achieved by the high rate of dicamba (600 g ae ha⁻¹) applied alone (Table 4-7), it also resulted in unacceptable wheat visible injury (21% visible injury) (Table 4-2), and therefore should not be considered for this purpose.

All of the herbicide treatments resulted in acceptable visible control of GR and GS kochia in Coalhurst, with the exception of dicamba + 2,4-D (110 + 420 g ae ha⁻¹) in Coalhurst 2013, while greater variation in visible control was observed among treatments in Lethbridge. Variability in visible control estimates among experimental locations is common in herbicide research due to the subjectivity of visual ratings among different assessors (Duddu et al. 2019). While visible control estimates are subject to personal standards of herbicide efficacy, weed biomass estimates do not share these same biases.

Similar to the estimates of kochia visible control, several herbicide treatments resulted in acceptable kochia control based on aboveground shoot biomass evaluated 6 WAA. In particular, sulfentrazone (105 g ai ha⁻¹) applied pre-emergence, and pyrasulfotole/bromoxynil (30/170 g ai ha⁻¹), or both rates of dicamba (300 or 600 g ae ha⁻¹) applied post-emergence reduced kochia biomass by \ge 90% compared with the untreated control among the environments in 2014 and 2015 (although the dicamba-only treatments were only tested in 2015) (Tables 4-6 and 4-7). Fluroxypyr/bromoxynil/2,4-D (96/288/288 g ae ha⁻¹) reduced kochia biomass by \geq 90% in Lethbridge 2014 and 2015, and 84% in Coalhurst 2014. The high rate of fluroxypyr/halauxifen + MCPA (100/6.5 + 455 g ae ha⁻¹) reduced kochia biomass by 82% in Lethbridge 2015, the only environment in which it was tested (Table 4-7). It is important to note, however, that statistical differences in kochia biomass among herbicide treatments were absent in Coalhurst 2014 due to large variability in the biomass estimates likely as a result of lower kochia population densities (27 \pm 3.8 plants m⁻²) (Table 4-6). Excluding the Coalhurst 2014 environment from consideration (due to lack of statistical difference), \geq 90% reduction in kochia biomass in Lethbridge 2014 and 2015 was achieved also by dicamba/fluroxypyr (80/104 g ae ha⁻¹), fluroxypyr/bromoxynil/2,4-D (48/114/114 g ae/ai ha⁻¹), MCPA/dichlorprop-p/mecoprop-p (395/765/320 g ae ha⁻¹), and both rates of dicamba/2,4-D/mecoprop-p (93/251/68 and 124/331/90 g ae ha⁻¹). Dicamba + 2,4-D (110 +420 g ae ha⁻¹), bromoxynil/2,4-D (280/280 g ai/ae ha⁻¹), florasulam/fluroxypyr + MCPA (2.5/110 +350 g ai/ae ha⁻¹), MCPA/mecoprop-p/dicamba (275/62.5/62.5 g ae ha⁻¹), and dichlorpop-p/2,4-D (368/702 g ae ha⁻¹) resulted in acceptable control and reduced kochia biomass by $\geq 80\%$ in the

Lethbridge 2014 and 2015 environments (Tables 4-6 and 4-7). Kochia shoot biomass was not evaluated in 2013.

The majority of herbicides evaluated in the current study were mixtures of synthetic auxins. Dicamba, fluroxypyr, 2,4-D, MCPA, clopyralid, dichlorprop-p, mecoprop-p, and halauxifen are synthetic auxins used (among other purposes) to manage broadleaf weeds selectively in smallgrain cereal crops (Hall et al. 1999; Epp et al. 2016). The mechanism of weed control by synthetic auxin herbicides remains elusive, however, recent reports suggest that synthetic auxin herbicides result in a rapid increase in abscisic acid (ABA) through up-regulation of the rate-limiting step for ABA production which causes down-regulation of photosynthesis-related genes and a loss of photosynthesis (Gaines 2020). Some auxinic herbicides have soil residual activity which can be limited by rapid microbial degradation (Hall et al. 1999). For example, dicamba/2,4-D/mecopropp $(124/331/90 \text{ g ae } ha^{-1})$ and MCPA/dichlorprop-p/mecoprop-p $(395/765/320 \text{ g ae } ha^{-1})$ are synthetic auxin mixtures with little soil residual activity (Shaner 2014). Dicamba has low persistence in soil with a half-life of \leq 14 days, while fluroxypyr persistence in soil can vary from a half-life of 11 to 68 days depending on whether it is present in formulation as an ester or acid (Shaner 2014). Fluroxypyr (70 g ae ha⁻¹) alone or in combination with 2,4-D (70 + 560 g ae ha⁻¹) resulted in excellent control of sulfonylurea-resistant kochia in Manitoba (92 to 96% reduction in biomass 60 days after application) (Friesen et al. 2013), while dicamba (140 g ae ha⁻¹) alone or in combination with fluroxypyr (53/69 g ae ha⁻¹) reduced biomass of GR and GS kochia accessions by 76% and 82% 3 WAA in a controlled-environment study (Burton et al. 2014). A somewhat higher rate of dicamba/fluroxypyr (80/104 g ae ha⁻¹) in the current study resulted in \geq 83% visible control in all environments, and $\geq 92\%$ kochia biomass reduction in Lethbridge 2014 and 2015

compared with the untreated control (Tables 4-5, 4-6 and 4-7), and therefore correspond with previous observations under controlled-environment (Burton et al. 2014).

Herbicide mixtures with multiple modes of effective action are favorable because they can help mitigate or delay the development and spread of herbicide resistance (Beckie and Reboud 2009; Evans et al. 2016). Fluroxypyr/bromoxynil/2,4-D (96/228/288 g ae/ai ha⁻¹) includes a combination of rapid uptake of 2,4-D, slight soil residual activity of fluroxypyr, and contact activity of the photosystem II (PSII) inhibitor bromoxynil. Bromoxynil is readily absorbed into leaves (with little to no translocation) resulting in chlorosis within 1 to 2 days and necrosis within 3 to 6 days after foliar application (Shaner 2014). In a greenhouse study, Burton et al. (2014) showed excellent control of GR and GS kochia in response to MCPA/bromoxynil (275/275 g ae/ai ha⁻¹) (99% biomass reduction compared with the untreated control 3 WAA). However, due to the contact activity of bromoxynil, kochia control can diminish over time because of partial plant recovery or seedling recruitment following herbicide application (Kumar and Jha 2015). Thus, an effective strategy could be to mix bromoxynil with another active ingredient which provides more sustained control. Pyrasulfotole/bromoxynil (30/170 g ai ha⁻¹) provides the contact activity of a PSII inhibitor (bromoxynil) with pyrasulfotole, which inhibits 4-hydroxyphenylpyruvatedioxygenase (HPPD). Pyrasulfotole causes tissue bleaching by inhibiting pigment biosynthesis, and remains active in the soil often for the duration of the growing season (van Almsick 2009). This is an important advantage for kochia management because prolonged emergence periodicity can result in flushes of emerged seedlings after treatment with a pre- or post-emergence herbicide (Schwinghamer and Van Acker 2008; Dille et al. 2017; Kumar et al. 2018).

Layering of effective herbicide modes of action pre- and post-emergence can be another way to mitigate or delay the selection for herbicide resistance. Sulfentrazone is a soil-applied

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herbicide that can be applied pre-plant or pre-emergence. Sulfentrazone is a protoporphyrinogen oxidase (PPO) inhibitor that is systemic and has moderate soil residual activity with a half-life ranging between 121 to 302 days (Shaner 2014). When applied 1 to 2 days before or after planting, sulfentrazone (105 g ai ha⁻¹) resulted in excellent kochia management with almost no wheat visible injury (Tables 4-2, 4-5, 4-6 and 4-7). Sulfentrazone provided excellent kochia control in the absence of crop competition when applied alone in Montana ($\geq 91\%$ visible control when applied at 210 g ai ha⁻¹) or with glyphosate and carfentrazone in Alberta (\geq 91% visible control of GR kochia when applied at 105 g ai ha⁻¹) (Kumar and Jha 2015; Torbiak et al. 2021). When applied prior to sunflower (Helianthus annuus L.) in Kansas, sulfentrazone alone (at 90 to 140 g ai ha⁻¹) or mixed with S-metolachlor showed excellent kochia control (Reddy et al. 2012). Carfentrazone is another PPO inhibitor, and unlike sulfentrazone, it is registered for use prior to spring wheat in Western Canada (Anonymous 2020). Carfentrazone is a contact herbicide with almost no residual activity in soil. Rapid necrosis and plant cell death can be observed within hours following carfentrazone application, however, little residual activity offered by carfentrazone can result in kochia regrowth (Torbiak et al. 2021). Consistent kochia control (Tables 4-5, 4-6 and 4-7) and almost no wheat visible injury in response to sulfenrazone (Table 4-2) suggest that this herbicide should be considered for registration prior to wheat in Western Canada.

4.3.3. Differences Among Kochia Accessions

In general, the GR kochia accession was present at a greater density than the GS accession in all environments in 2014 and 2015 [GR kochia densities of 33 (\pm 4.3), 153 (\pm 7.1), and 248 (\pm 14.6) in Coalhurst 2014, Lethbridge 2014, and Lethbridge 2015, respectively, compared with GS kochia densities of 21 (\pm 4.0), 98 (\pm 6.3), and 162 (\pm 13.9) in these same environments]. A similar trend was observed in Coalhurst 2013 [159 (\pm 14.9) vs. 149 (\pm 15.9) plants m⁻² for GR and GS kochia, respectively], however, these kochia densities were not statistically different (P = 0.318) (Table 4-3).

There were differences in visible control among the kochia accessions in 2014 (P < 0.001), where the herbicide treatments overall resulted in greater control of the GS compared with the GR kochia accession (85 ± 0.5 vs. $83 \pm 0.5\%$ visible control of GS vs. GR kochia, respectively) (Table 4-3). It is likely that these differences were caused by the greater density of GR compared with GS kochia present in 2014, and not due to the presence vs. absence of the glyphosate resistance trait. The opposite was observed for some herbicide treatments in 2013, where fluroxypyr/2,4-D (40/160 g ae ha⁻¹) (96 vs. 91% visible control of GR vs. GS kochia, respectively) and dichlorprop-p/2,4-D (368/702 g ae ha⁻¹) (88 vs. 84% visible control of GR vs. GS kochia, respectively) resulted in slightly greater control of the GR compared with GS kochia accessions (Table 4-5), while differences in kochia density were absent (Table 4-3). This could be due to negative crossresistance similar to that reported for ALS inhibitor-resistant kochia and PPO- or HPPD-inhibiting herbicides (Beckie et al. 2012a), or more likely statistical difference absent of biological significance since the differences observed were minimal (Table 4-5). Similar to visible control, greater density of GR compared with GS kochia in 2014 and 2015 resulted in greater biomass of GR than GS kochia among herbicide treatments. In 2014, GR kochia biomass averaged 19 (3.00 0.16; natural logarithm-transformed mean \pm SE) g m⁻² among herbicide treatments ± compared with 10 (2.3 \pm 0.20) g m⁻² for GS kochia (P = 0.002) (Table 4-3). Likewise, GR kochia biomass in 2015 averaged 17 (2.9 \pm 0.26) g m⁻² compared with 6 (1.8 \pm 0.30) g m⁻² for GS kochia (P = 0.039). Despite these minor differences in visible control and biomass among kochia accessions, general observations from the current study agree with previous greenhouse research

which showed similar response of GR and GS kochia to a range of alternative herbicide treatments (Burton et al. 2014).

4.3.4. Management Implications

In addition to glyphosate and ALS inhibitor resistance in kochia, auxinic herbicide resistance is a major threat to small-grain cereal crops. Dicamba- and/or fluroxypyr-resistant kochia were reported first in the United States in 1994/1995 (Cranston et al. 2001; Goss and Dyer 2003; Kumar et al. 2019). In Canada, auxinic herbicide-resistant kochia was reported first in 2015 in a spring wheat field in Saskatchewan (Heap 2020). A subsequent 2017 Alberta survey reported that 18% of the kochia populations tested were dicamba-resistant, and 10% were triple herbicideresistant (resistant to ALS inhibitors, glyphosate and dicamba) (Beckie et al. 2019). While synthetic auxin herbicides continue to play an important role in control of GR kochia in spring wheat, farmers must remain diligent and include alternative modes of action in their herbicide programs like PPO inhibitors applied pre-emergence, or pyrasulfotole (a HPPD inhibitor) and bromoxynil (a PSII inhibitor) applied post-emergence. The current research suggests that optimal control of glyphosate and ALS inhibitor-resistant kochia in spring wheat may be achieved by a combination of sulfentrazone (105)ai ha^{-1}) applied g pre-emergence with fluroxypyr/bromoxynil/2,4-D (96/228/288 g ae/ai ha⁻¹) or pyrasulfotole/bromoxynil (30/170 g ai ha⁻¹) applied post-emergence.

The sustainability of remaining herbicides for kochia control will depend on the successful implementation of integrated weed management; of which, a key foundational principle is crop diversity (integrated herbicide management is simply not enough) (Beckie and Harker 2017). Other potential tools for integrated management of kochia include: alternative crop life cycles (e.g., winter-annuals or perennials), competitive crop cultivars, cover crops, field scouting,

resistance diagnostic testing, strategic and site-specific tillage, and potentially also harvest weed seed control (Beckie and Harker 2017; Beckie et al. 2018*b*; Kumar et al. 2019). If implemented alone, integrated herbicide strategies like those identified in the current research will remain a short-term solution at risk of resistance development, and for this reason, improved understanding of non-chemical weed control is required for sustainable kochia management in wheat production systems.

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Chapter Five: General Discussion and Conclusions

This M.Sc. thesis research furthered knowledge of effective chemical control options for glyphosate-resistant (GR) and glyphosate-susceptible (GS) kochia [*Bassia scoparia* (L.) A.J. Scott] in southern Alberta and western Canada. This research filled some of the most pertinent knowledge gaps and identified which herbicides and herbicide mixtures were effective for control of GR and GS kochia in chemical fallow and spring wheat (*Triticum aestivum* L.). The overall objectives of the research studies were to:

- Determine which herbicides and herbicide mixtures remain effective for control of GR and GS kochia in chemical fallow fields in western Canada (Chapter 3)
- Determine which herbicide options remain effective for control of GR and GS kochia in spring wheat in western Canada (Chapter 4)
- Determine whether herbicide efficacy differs among GS and GR kochia (Chapters 3 and 4)

5.1. A Guide to Management of GR and GS Kochia in Western Canada

My research identified several effective herbicide mixture options for control of GR and GS kochia in chemical fallow (Chapter 3) and identified herbicides that remain effective for management of GR and GS kochia in spring wheat (Chapter 4). Historically, kochia management in fallow and prior to wheat seeding relied on conventional tillage, but in recent decades reduced-till and no-till cropping systems have been practiced on most cropland in the Canadian prairies (Geddes 2019). Glyphosate, an affordable non-selective herbicide, is relied upon in westem Canada for weed control in lieu of tillage for chemical fallow and no-till wheat, but its overuse is

resulting in high selection pressure for GR weeds, like kochia. To sustain herbicide efficacy moving forward and to manage kochia effectively, a multi-faceted weed management approach is warranted. The crop production industry is in need of long-term approaches to manage kochia while minimizing grower dependence on herbicides.

5.1.1. Crop Rotation Management Practices Effective for Kochia Control

Crop rotation management can be a huge factor in the success of kochia management. Crops of lesser competitive ability, and with few in-crop herbicide options, such as lentils, or periods of chemical fallow, provide GR kochia with a nutrient-rich and low-competitive environment, allowing the weed to thrive. Typically, chemical fallow is a weak link due to high selection pressure on weeds; for example, the selection of GR kochia in chemical fallow using several applications of glyphosate alone for weed control. Kochia that is able to establish and set seed in crop rotation phases with reduced competitive ability will contribute to replenishment of the kochia seedbank. There are several aspects of the kochia life cycle that can be exploited for improved management, including its early emergence timing, late maturation date, and low seed dormancy. Planning crop rotations to take advantage of these characteristics could go a long way to reducing kochia infestations in croplands.

5.1.1.1. Kochia Early Season Control

Growing a spring wheat crop, and using the herbicide options that were determined to be effective for GR and GS kochia control in my research, could help with kochia management following chemical fallow or crops with reduced competitive ability. Spring wheat can be seeded early in the spring to provide a highly competitive environment (Menalled and Smith 2007) with rapid crop canopy closure helping to reduce the number of kochia seedlings that survive herbicide applications. A well-established wheat crop could effectively compete with later emerging kochia cohorts, thereby reducing kochia seed production and return to the soil seedbank.

In a study by Mosqueda et al. (2020), spring wheat had the greatest ability to reduce kochia establishment compared with sugar beet (*Beta vulgaris* L.), dry bean (*Phaseolus vulgaris* L.), and corn (*Zea mays* L.). The interaction of crop management practices on kochia survival and seed production was studied in Wyoming, Montana, and Nebraska. Crop canopies can limit light available to weeds depending on their leaf size, height, and developmental rate (Seavers and Wright 1999). Herbicide choice affected kochia mid-season density more than the crop species, while crop species affected seed production to a greater extent (Mosqueda et al. 2020). Spring wheat resulted in the lowest germinable kochia seed production per unit area, and hence the greatest reduction of kochia in the seedbank among crops. Mosqueda et al. (2020) suggested that the long-term management of weed seedbanks could be achieved through the combination of spring grains and effective herbicides, thereby reducing kochia establishment and seed production.

5.1.1.2. Herbicide-resistant Crop Technologies

Glyphosate-resistant kochia management could be improved by utilizing herbicideresistant crop technologies that take advantage of alternative modes of action. For example, growing glufosinsate-resistant canola instead of GR canola can go a long way to reducing GR kochia infestations. Burton et al. (2014) showed excellent control of GR kochia 3 WAA of glufosinate under controlled environment. In Montana, glufosinate (0.593 kg ai ha⁻¹) resulted in 87% control of kochia 1 WAA, but control declined to 73% 3 WAA and to 52% 5 WAA (Kumar and Jha 2015*a*). This can be due to environment or weather conditions at spraying, and likely also the non-residual activity of glufosinate. Glufosinate has rapid activity in the plant, and quickly disrupts photosynthesis and cell integrity, thereby limiting its own translocation (Hall et al. 2014). Kochia can recover from the "contact" nature of glufosinate (quick leaf necrosis) and regrow under certain environmental conditions. Multiple in-crop applications may be required to control kochia due to its extended emergence timing and ability to recover following glufosinate application.

5.1.1.3. Alternate Crop Life Cycles – Winter Annual/Perennial Crop

Kochia management can include changing the crop life cycle from a summer- to winterannual crop or by using a perennial forage crop. A winter cereal crop seeded in the fall will already be established in the spring when kochia emerges, providing good competition and canopy cover (Boerboom 1993). Winter cereals also mature in the late summer and can be harvested before kochia is mature and produces viable seed (Geddes and Davis 2021). Since the majority of kochia seed generally does not persist for more than a year or two (Burnside et al. 1981; Schwinghamer and Van Acker 2008), and kochia seed viability can decline to 5% of seeds after a year in the soil (Dille et al. 2017), a single successful season of kochia management can reduce the vast majority of the kochia seedbank for the next season (Geddes and Davis 2021). Early harvest dates could result in post-harvest regrowth of kochia plants that are capable of producing seed before the end of the growing season. Post-harvest tillage or herbicide application may be warranted to mitigate the production of viable seed on the lateral branches of kochia that remain after crop harvest and on post-harvest regrowth (Kumar and Jha 2015*b*).

5.1.1.4. Kochia Late Season Control

5.1.1.4.1. Post-harvest Herbicide Control

Decreasing the number of viable seeds on the lateral branches of kochia plants that pass under the combine pickup during harvest could further reduce the quantity of kochia seeds added to the soil seedbank, thereby reducing kochia establishment in subsequent years. Low (2016) examined the possibility of kochia control post-harvest and its effect on seed set and viability in central Alberta. Soil applied and foliar herbicides known to be effective on kochia were applied immediately after harvest (IAH) and three weeks after harvest (WAH) of wheat and peas. Postharvest seed production on the remaining kochia plants was high and variable. Treatments showed visual control damage on kochia plants, but kochia seed viability for all treatments was similar to the untreated check. While harvest decapitated the kochia plants and reduced the number of seeds left on the plant, still about 5,000 seeds plant⁻¹ remained after harvest; which is enough to replenish the kochia population. However, a previous study in Montana that applied glyphosate and paraquat post-harvest (late-August to mid-September) reduced kochia seed production by 92% or greater, but efficacy was dependent on application timing (Mickelson et al. 2004). Kumar and Jha (2015b) conducted similar studies in Montana on late-season kochia control in wheat stubble. They found that several herbicides applied post-harvest resulted in excellent kochia visual control, and reduced biomass and seed rain, thereby reducing seedbank replenishment. One difference in this study, as pointed out by Low (2016), is that kochia in Alberta at the post-harvest timing in this study was mature but kochia in the study by Kumar and Jha (2015b) was at vegetative growth stages. This highlights the importance of application timing and developmental stage for successful kochia management post-harvest.

5.1.1.4.2. Harvest Weed Seed Control

If spring wheat (or another annual spring cereal) is not able to be harvested before kochia maturity, another option could be to use a mechanical harvest weed seed control method. The recent development of seed destructor technologies offer farmers the ability to devitalize weed seeds during the harvest operation, thereby reducing the amount of weed seed returned to the soil seedbank. Stationary experiments using the Harrington Seed Destructor showed 99.8% control of

kochia seed that entered the machine (Tidemann et al. 2017). Kochia plants retain most of their seed during typical harvest of a summer-annual crop in western Canada, and seed destructors offer high potential to mitigate kochia seed return to the soil seedbank. Thus, harvest weed seed control may offer an effective alternative to facilitate kochia management. However, one limitation in need of further research is how to dry down green kochia plants at harvest so that the tissue moves freely through the combine and destructor unit.

5.1.2. Coordinated and Community-Based Approach to Kochia Management

A community-based approach to manage kochia would make management efforts more effective since the tumbleweed dispersal mechanism can result in long-distance seed dispersal (Beckie et al. 2016). A significant benefit could be realized if neighbors united and all committed to control kochia plants in their fields, thereby preventing tumbleweed movement. Suspected herbicide-resistant kochia patches in field borders could be treated, tilled, or mowed to manage the plants before seed set. While a community-based strategy for kochia control could be difficult to implement logistically, the biology of this weed suggests that this could be among the most effective tools to mitigate further spread of this problematic herbicide-resistant weed.

5.2. Future Research

Future successful kochia management will require the multidisciplinary and combined efforts of educational institutions, agriculture chemical companies, government policy, and growers (Shaner and Beckie 2014). Future work on how to manage GR kochia should aim at biological, cultural, and mechanical weed management strategies to use in conjunction with existing chemical control options. More research into the phenology of kochia (such as seedbank dynamics, emergence timings, or timing of seed production) could help customize these strategies since a "one size fits all" approach is seldom optimal when it comes to weed management. Future research areas could include:

- Effective herbicide options for GR and GS kochia in other crops.
- Biological controls (e.g., an insect the feeds on kochia and/or kochia seeds).
- Biological soil dynamics and how these dynamics affect kochia seed viability in the soil seedbank.
- Kochia seedbank and perennial forage crops [the competitiveness of the forage crops and their (often) earlier harvest dates could reduce the number of kochia seeds produced resulting in lower population densities in subsequent crops].
- Establishment of saline tolerant perennial forage crops in areas with high kochia infestations, e.g., AC Saltlander (a saline-tolerant hybrid wheat-grass cultivar).
- The competitive relationship between kochia and crops seeded at higher densities.
- Strategic tillage strategies to reduce the kochia seedbank while also mitigating potential detrimental effects on soil health.

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