DISSERTATION

GYPHOSATE-RESISTANT KOCHIA (KOCHIA SCOPARIA) MANAGEMENT IN THE CENTRAL GREAT PLAINS AND WESTERN CANADA

Submitted by

Eric P. Westra

Department of Bioagricultural Sciences and Pest Management

In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Spring 2016

Doctoral Committee:

Advisor: Scott Nissen
Co-Advisor: Andrew Kniss

Brian Olson
Dale Shaner
Ken Barbarick

ABSTRACT

GYPHOSATE-RESISTANT KOCHIA (KOCHIA SCOPARIA) MANAGEMENT IN THE CENTRAL GREAT PLAINS AND WESTERN CANADA

Glyphosate-resistant (GR) kochia (*kochia scoparia*) has become more common throughout the Western Great Plains, and has now been reported to exist as far south as Texas up to Canada. Further evolution of GR kochia threatens the utility of glyphosate and GR crops for weed control, therefore, research relating to the management of GR kochia was conducted to address this current widespread problem.

First, a four year survey study was conducted across Eastern Colorado to determine the frequency and occurrence of GR kochia in Eastern Colorado. Each year, kochia seed was collected from geo-referenced sites across Eastern Colorado for greenhouse screening to evaluate resistance to glyphosate, dicamba, and fluroxypyr. Over the four years, the occurrence of GR kochia remained fairly constant with 60, 45, 39, and 52% of populations tested categorized as GR. The same was observed for dicamba-resistant kochia over the three years with 33, 45, and 28% of populations tested categorized as dicamba-resistant. For the three years tested, no collections were deemed resistant to fluroxypyr. Populations with multiple resistance to glyphosate and dicamba increased over the three years with 14, 15, and 20% of the populations classified as resistant to both glyphosate and dicamba, which highlights the importance of fluroxypyr for control of these multiple resistant populations. Unlike resistance to acetolactate synthase (ALS), or Acetyl-CoA carboxylase (ACCase) inhibitor herbicides, the rate of evolution

for glyphosate or dicamba resistant kochia appears to be slower at the landscape level which suggests there may be a potential fitness penalty or inheritance restriction keeping the frequency and occurrence of resistance fairly stable over the four years.

Next, field studies were conducted to evaluate how treatments influence the further selection of GR kochia when starting at a targeted baseline of 10% GR kochia. For POST treatments, glyphosate was compared to glufosinate, and the inclusion of a pre-emergent herbicide (pendimethalin) with both glyphosate and glufosinate was evaluated to determine how herbicide treatments impact the further selection of GR kochia progeny. The impact of canola variety selecting for GR in the absence of herbicide applications was compared between DKL 30-42 and InVigor L150. Kochia survivors from treatments with glyphosate had progeny with higher frequencies of GR compared to kochia survivors from treatments with glufosinate. However, the advantage of reducing the frequency of GR progeny from treatments with glufosinate was reduced when the control efficacy of glufosinate decreased in the second year. The inclusion of a pre-emergent herbicide (pendimethalin) reduced the frequency of GR kochia progeny and significantly reduced the number of kochia individuals that were exposed to post-emergent applications, which is key for GR kochia management. Canola variety did contribute to kochia suppression, but both varieties appeared equivalent in their suppression, however differences in phenotypes between canola varieties impacted the frequency of GR kochia progeny that remained below the canola canopy. Management recommendations to minimize further selection and evolution of GR kochia in Canola are to incorporate an alternative mode of action (glufosinate) either in a rotation or tank mix (once varieties are available) to reduce the frequency of GR kochia progeny, and most importantly, to incorporate a pre-emergent herbicide to limit the further selection and evolution of GR kochia.

ACKNOWLEDGEMENTS

I would like to thank Monsanto for providing funding for my research, and the opportunity to work on these projects. I would like to specifically thank Brian Olson and Todd Pester from Monsanto for their collaboration and contributions to my research projects.

I am extremely grateful to have Dr. Scott Nissen as my major advisor for my PhD, and the opportunity he provided me to work on a pursuing my PhD. Having known me since I was a young rambunctious kid, it has been a pleasure to work with him on a daily basis for the past three years. I have thoroughly enjoyed our daily interactions, and I am extremely appreciative for his guidance and mentorship throughout my PhD. Thank you to my Co-Advisor Dr. Andrew Kniss and committee members Dr. Brian Olson, Dr. Dale Shaner and Dr. Ken Barbarick for their advice and guidance on my research and education. I feel blessed to have had the opportunity to work with all of these individuals, and to have them as role models in my life. I would like to give special thanks to Dale Shaner for taking a chance on a young undergraduate student, and the impact he had on my development as a weed scientist throughout graduate school, I will always appreciate the support you have provided me.

I have truly cherished the relationships I have made with fellow graduate students in the Weed Lab over the years, particularly Tom Getts, Darci Giacomini, Kallie Kessler, Curtis Hildebrandt, and Derek Sebastian. Thank you to all of those who helped me in the field, lab, or greenhouse throughout the years, especially Tyler Hicks who has spent many long days with me in the greenhouse and field assisting on research projects.

Having grown up around the Weed Lab at CSU, I will truly miss the atmosphere and daily interactions with everyone at the Weed Lab. Galen Brunk had a tremendous impact on my life from a young age and is dearly missed around the Weed Lab. I would like to thank my dad Phil Westra for being a great role model as a Weed Scientist in my life, and for providing me the distance needed to pursue a career in Weed Science at CSU. I also appreciate the exposure to weed science which my dad provided me from a young age, as these experiences initiated my interest which developed into a passion for Weed Science. I would also like to thank my mom Karen, and siblings Stephanie, Amy, and Jeff (aka Chubba) for their emotional support and encouragement throughout graduate school, I love you fam. Finally, I am beyond grateful for my amazing wife Amber who has always patiently supported me and encouraged me even when times were hard and days were long. I am lucky to have found such a tremendous spouse to go through life with, and I greatly appreciate her support and love throughout my education. Having been together since high school, I am excited for what the future holds for the two of us. Much Love!

TABLE OF CONTENTS

LIST OF TABLES

APPENDIX

LIST OF FIGURES

APPENDIX

Chapter 1: Herbicide-resistant Kochia (*Kochia scoparia)* in Eastern Colorado

Summary

Glyphosate-resistant kochia has been reported across the Western and Midwestern US. From 2011 to 2014 a roadside survey of kochia was collected from agronomic regions across eastern Colorado to evaluate the frequency (% of resistance within a population) and distribution (number of populations classified as resistant to a discriminating herbicide dose over geographical area) of glyphosate-, dicamba-, and fluroxypyr-resistant kochia. Samples were screened with glyphosate in 2011, and with glyphosate, dicamba, and fluroxypyr in 2012-2014. From each geo-referenced sample location, kochia seed was collected from around 5 to 20 individual mature kochia plants. The composite seed samples were screened in the greenhouse to evaluate the level of resistance to each herbicide, and evaluate multiple-resistance patterns. Populations were classified as susceptible (<2% survival), developing resistance (2-20% survival), or resistant (>20% survival to a respective discriminating dose for each herbicide). Developing resistance and resistant populations were grouped together for total resistant frequencies discussed below. Over the four years, the distribution of glyphosate-resistant kochia remained relatively constant with 60, 45, 39, and 52% of populations tested categorized as glyphosate-resistant. The same was observed for dicamba-resistant kochia over the three years with 33, 45, and 28% of populations tested categorized as dicamba-resistant. For the three years tested, no populations were classified as resistant to fluroxypyr. Populations with multiple resistance to glyphosate and dicamba increased over the three years with 14, 15, and 20% of the populations classified as either developing resistance or resistant to both glyphosate and dicamba within a population. Unlike resistance to acetolactate synthase (ALS), or Acetyl-CoA

carboxylase (ACCase) inhibitor herbicides, the rate of evolution for glyphosate- or dicamba-resistant kochia appears to be slower at the landscape level, suggesting there may be a potential fitness penalty or complex inheritance keeping the frequency and distribution of herbicide-resistance fairly stable over the four years. With the confirmation of both glyphosate- and dicamba-resistance in kochia from eastern Colorado, fluroxypyr represents an important herbicide for populations that have now evolved multiple resistance to triazines, ALS, glyphosate, and auxinic (dicamba) herbicides.

Introduction

Kochia (*Kochia scoparia*) is a common annual broadleaf weed that is economically important in crop production systems and non-crop areas in semiarid to arid regions of North America (Friesen et al., 2009). Kochia is an introduced C4 species that germinates at low soil temperatures, emerges early in the spring (sometimes in late February), grows rapidly, and is tolerant to heat, drought, and salinity. These attributes all contribute to its competitiveness in cropping systems (Friesen et al., 2009). Kochia is commonly found in cultivated fields, gardens, roadsides, ditch banks, and waste areas throughout the west (Whitson et al., 1991). If kochia populations are not controlled, or if resistant individuals survive herbicide applications, kochia densities can increase exponentially due to prolific seed production which can range from 2,000 to 30,000 seeds per plant (Stallings et al., 1995).

Significant outcrossing occurs in kochia due to its flower morphology, facilitating the transfer of genetic traits such as herbicide-resistance via pollen movement (Dawit et al., 1994). Seeds are physically dispersed when mature plants detach from their root systems in the fall and tumble across the landscape. Kochia populations contain high levels of genetic and phenotypic diversity (Mengistu and Messersmith, 2002). Because of pollen mediated gene flow and wind driven seed dispersal, herbicide-resistance can be easily spread within and between populations.

In a review of weed competitiveness with crops, out of 20 weed species examined, kochia had the highest competition index, according to removal and additional experiments with sugarbeet (Vilà et al., 2004). Kochia has been ranked as one of the most problematic weeds in cultivated fields including corn (*Zea mays*), sorghum (*Sorghum bicolor*), wheat (*Triticum aestivum*), and soybean (*Glycine max*) (Waite et al., 2013). Corn grain yields can decrease by 0.33 kg ha^{-1} for every 1 kg ha^{-1} of kochia biomass produced (Gail et al., 1993). Kochia can decrease wheat yields from 15 to 58% when kochia densities varied from 4 to 70 plants m^{-2} (Challaiah et al., 1983). While problematic in cultivated fields, kochia can also utilize valuable nutrients and moisture when growing in fallow fields.

In eastern Colorado, glyphosate is a key herbicide for post emergent weed control in chemical fallow, as well as for pre-plant and post-harvest weed control. Substituting chemical weed control for mechanical control has had many positive impacts in crop production such as reduced soil erosion, increase soil organic carbon (organic matter), and lower CO_2 emissions (Vencill et al., 2012). With the widespread adoption of glyphosate-resistant crops, glyphosate usage has increased significantly, resulting in immense selection pressure on weeds in these cropping systems (Duke and Powles, 2008). Applying this level of selection pressure to a weed as genetically diverse and abundant as kochia has resulted in the evolution of glyphosate-resistant (GR) kochia populations in eastern Colorado. In eastern Colorado, GR kochia has become more common in both fallow and cropping phases. Since the first discovery of GR kochia from Kansas in 2007 (Heap, 2015), GR kochia has since been reported in 10 states across the West and Midwest including Colorado, Idaho, Montana, Nebraska, North Dakota, Oklahoma, Oregon, South Dakota, Wyoming, and the prairie provinces of Canada (Heap, 2015).

Currently, kochia populations have evolved resistance to four herbicide modes of action, including acetolactate synthase (ALS) inhibitors (B/2) (HRAC/WSSA mode of action classification), 5-enolpyruvyl-shikimate-3-phosphate (EPSPS) inhibitors (G/9), photosystem II-inhibitors (PSII) (C1/5), and synthetic auxin (O/4) herbicides (Heap, 2015). Recently, a kochia population from Kansas was found to be have multiple-resistance to these four modes of action within a single population (Varanasi et al., 2015). Synthetic auxin herbicides (primarily dicamba and to a lesser extent fluroxypyr) have been used to control multiple-resistant kochia populations (Beckie et al., 2014b). Dicamba is commonly used for kochia control in small grain production (Nandula and Manthey, 2002), and as dicamba applications become more common, the frequency (percent of resistance within populations) and distribution (number of populations classified as resistant to a discriminating herbicide dose over a geographical area) of dicamba-resistant kochia populations will continue to increase. Fluroxypyr is labeled for use in small grains, corn, and non-cropland. Overall fluroxypyr use has been less than dicamba, likely a function of relative weed control spectrum and cost.

Herbicide-resistant kochia populations have been confirmed in eastern Colorado based on samples submitted by growers, but the frequency and distribution of herbicide-resistant kochia populations is unknown. Similar surveys with GR kochia have been conducted to determine the incidence of GR kochia in Alberta, Saskatchewan, and Manitoba (Beckie et al., 2013b; Beckie et al., 2014b). This paper reports the results of a four year survey conducted from 2011 to 2014 to evaluate the frequency and distribution of herbicide-resistance kochia populations in eastern Colorado. The objectives of this study were to a) evaluate the frequency and distribution of glyphosate, dicamba, and fluroxypyr-resistant kochia in eastern Colorado, b) evaluate multiple resistance patterns within kochia populations, and c) evaluate changes in the frequency and distribution of herbicide-resistant kochia over a four-year time frame.

Materials and Methods

Seed Collections

Kochia seed was collected from roadside locations across eastern Colorado in October and November each year from 2011 to 2014.These collections were conducted by driving transects throughout eastern Colorado while maintaining a minimum distance of 10 miles between sample locations. The design of sampling locations and driving transects were intended to provide separation between kochia collection sites and to capture the current status of herbicide-resistant kochia in cropping areas across eastern Colorado without biasing the collection to reported problem fields. Most of the sampling efforts were targeted in eastern Colorado: however, in 2014 a sub-set of samples was collected from the western slope of Colorado in order to evaluate the frequency of herbicide-resistant kochia in the western part of the state. The majority of kochia populations were collected from chemically fallowed fields, typically in wheat or corn cropping rotations, as samples present within cropping systems were less common given that mature kochia individuals were typically removed during crop harvest. Kochia collected from field margins that survived harvest operations were also commonly sampled during seed collections. After a collection location was selected, seed was harvested from between 5 to 20 kochia plants to create a composite seed sample for that location (referred to as the population). The number of plants, as well as the radius from which plants were collected was recorded for each site. Each sampling location was geo-referenced using a hand held GPS unit (Trimble Geo XH 2005 series) (Trimble Boulder 4730 Walnut Street Suite 201 Boulder, CO 80301).

Greenhouse Screening

Kochia seed collected in the field was cleaned from the chaff with a combination of an air-blower and sieves before being incorporated with a v-mixer in order to fully mix the composite seed sample to assure an even probability of screening progeny from each mature plant collected per location. Composite seed samples from each collection location were seeded into plug flats where individual kochia seedlings were germinated and grown in a cell that was 1.3 ×1.3 ×2.5 cm (American Clay Works, Denver CO). Plants were grown in flats until the kochia seedlings were approximately 2.5 cm tall. Seedlings were then transplanted into 3.8 cm ×3.8 cm ×5.8 cm inserts (American Clay Works, Denver, CO) where they were then grown until plants reached 10 to 15 cm. In 2014, kochia populations were screened in smaller inserts in order to minimize greenhouse space usage. In 2011-2013, 54 individual plants from each population were screened, whereas in 2014, 72 individuals were screened in smaller inserts to determine the frequency of resistance for a given herbicide. Fine grade potting mix (Fafard #2-SV) (American Clay Works, Denver, CO) was used as the growing media for both plug flats and larger inserts. Plants were grown and watered daily in a greenhouse that had a 14/10 h photoperiod with temperatures maintained between 22 and 26 C.

Herbicide Applications

In 2011, 55 kochia populations were screened with glyphosate only, whereas in 2012 to 2014 all kochia populations (2012: 42, 2013: 33, 2014: 96) were screened separately with glyphosate, dicamba, and fluroxypyr to evaluate multiple resistance within populations. When kochia plants were between 10-15 cm tall a herbicide was applied via a moving overhead single nozzle sprayer (DeVries Manufacturing Hollandale, MN) calibrated to deliver 187 L ha^{-1}. For a given population, a subset of kochia was sprayed individually with glyphosate, dicamba and fluroxypyr. For glyphosate treatments, RoundUp Weathermax (Monsanto, St. Louis, MO) was applied at 840 g ae ha^{-1} with ammonium sulfate (AMS) at 20 g L^{-1}, for dicamba treatments Clarity (BASF, Florham Park, NJ) was applied at 280 g ai ha^{-1} with nonionic surfactant (NIS) at 1% V/V, and for fluroxypyr treatments Starane Ultra (Dow AgroSciences, Indianapolis, IN) was applied at 157 g ai ha^{-1}. These discriminating rates for each herbicide were selected based on suggested labeled rates for kochia control. If kochia individuals are able to survive labeled field rates they were classified as resistant, as these populations would reduce weed control efficacies at rates which control susceptible populations. For glyphosate specifically, although plants may have survived the discriminating dose (840 g ae ha^{-1}), the presence of increased EPSPS copy number was not evaluated for surviving individuals, so we cannot conclude definitively that survival was due solely to increased EPSPS copy number, however population responses were different from susceptible populations.

After the herbicide was applied, plants were maintained in the greenhouse for 21 d before they were rated as either dead or alive on an individual plant basis. Individual plants varied in their response from herbicide applications ranging from complete control (dead) to minimal visual injury (alive). If plants showed initial herbicide symptoms (e.g., varying levels of chlorosis or epinasty), but then displayed regrowth during the 21 day time period they were rated as survivors. The frequency of resistance for a given herbicide and field population was calculated from number of survivors out of the total number of individuals screened. To categorize the level of resistance, methods were used similar to Owen et al. 2007, where kochia populations were classified as either susceptible (<2% survival), developing resistance (2-19%

survival), or resistant (>20% survival) to the respective discriminating herbicide rate for each herbicide. Geo-referenced collections sites were mapped using Arc Catalogue and ArcMap (Version 10.2.1) in order to visualize spatial patterns of resistance for a given year, as well as to compare changes between years.

Results

In 2011, kochia populations from 55 collection locations were screened for glyphosate resistance. The percent of individuals which survived a glyphosate treatment, or frequency of glyphosate resistance within populations ranged from 0 to 96%. The proportion of GR kochia populations was as follows: 11% were categorized as resistant, 49% were developing resistance, and 40% were classified as susceptible (Figure 1.1A and 1.2). Combining the number of populations that were resistant or developing resistance, 60% of populations were no longer completely susceptible to glyphosate.

Forty-two kochia populations collected in 2012 were screened for resistance to glyphosate, dicamba, and fluroxypyr to determine the frequency of resistance for all three herbicides representing two mode of actions, and to evaluate the potential for and patterns of multiple resistance within and among kochia populations. In 2012, the frequency of glyphosate resistance ranged from 0% to 98%, while the frequency of dicamba resistance ranged from 0% to 78%. The frequency of fluroxypyr resistance was 0% for all populations. For glyphosate 24% of populations were resistant, 21% were developing resistance and 55% of populations were susceptible (Figure 1.2). For dicamba, 10% of populations were resistant, 24% of populations were developing resistance, and 67% of populations were susceptible (Figure 1.3). For fluroxypyr, 100% of populations were classified as susceptible (Figure 1.4). In 2012, 45% and

33% of populations were not completely susceptible to glyphosate and dicamba, respectively. In the first year of screening for multiple resistance, 14% of the populations were classified as either resistant or developing resistance to both glyphosate and dicamba, and only 36% of populations were classified as completely susceptible to all three herbicides (Figure 1.1B).

In 2013, 33 kochia populations were screened for resistance to glyphosate, dicamba, and fluroxypyr. The frequency of glyphosate resistance ranged from 0% to 76%, while the frequency of dicamba resistance ranged from 0% to 82%. The frequency of fluroxypyr resistance was 0% for all populations. Out of the 33 populations screened with glyphosate, 12% were resistant, 27% were classified as developing resistance, and the remaining 61% of populations were classified as susceptible (Figure 1.2). For dicamba, 9% of populations were classified as resistant, 36% of populations were classified as developing resistance and the remaining 55% populations were classified as susceptible (Figure 1.3). For fluroxypyr, 100% of populations were classified as susceptible (Figure 1.4). Out of 33 populations, 39 and 45% of populations were not completely susceptible to glyphosate and dicamba, respectively. Out of 33 populations, 15% of populations had resistance to both glyphosate and dicamba, while 30% of populations were completely susceptible to all three herbicides (Figure 1.1C).

In 2014, 96 kochia populations were screened for resistance to glyphosate, dicamba, and fluroxypyr. The frequency of glyphosate resistance ranged from 0% to 67%, and the frequency of dicamba resistance ranged from 0% to 72% resistant. Similar to 2012 and 2013 data, the frequency of fluroxypyr resistance was 0% for all populations. For glyphosate 23% of populations were classified as resistant, 29% of populations were classified as developing resistance, and the remaining 47% of populations were susceptible to glyphosate (Figure 1.2). For dicamba 8% of populations were resistant, 19% of populations were classified as developing resistance, and the remaining 72% of populations were susceptible to dicamba (Figure 1.3). For fluroxypyr, 100% of populations were classified as susceptible (Figure 1.4). In 2014, 52 and 28% of populations were not completely susceptible to glyphosate and dicamba, respectively. In 2014, 20% of populations were resistant to both glyphosate and dicamba, and 40% of populations were susceptible to all three herbicides (Figure 1.1D). In 2014, 20 kochia samples were collected from the western slope of Colorado. Only one population (collected near Grand Junction, CO) was classified as resistant to glyphosate (47%), and it was susceptible to dicamba and fluroxypyr. The remaining 19 populations screened were susceptible to all three herbicides, but were not included on geo-referenced maps to maintain resolution in eastern Colorado (Figures 1.2, 1.3, and 1.4).

The occurrence of glyphosate and dicamba resistance remained relatively consistent over the four year time frame of this field survey (Figure 1.1) and there were no kochia populations identified as resistant to fluroxypyr. Based on greenhouse screening results from the populations that were collected, we did not see significant increases in the distribution of glyphosate or dicamba-resistance (Figure 1.2 and 1.3) which has been observed in kochia resistant to other modes of action such as ALS or PSII-inhibitor herbicides.

Discussion

Understanding the factors that influence herbicide-resistance evolution can help develop management recommendations to minimize resistance evolution. Whole plant screening with glyphosate, dicamba, and fluroxypyr can establish the occurrence and frequency of resistance, but does not identify the mechanism conferring resistance in these field populations of kochia. Knowledge of herbicide-resistance mechanisms and inheritance can provide information on the potential for further evolution and spread. Understanding weed biology can also provide insight into the potential for herbicide-resistance evolution in that particular species. When herbicide-resistant survey data is combined with information on herbicide-resistance mechanisms, and weed biology, we can better understand how herbicide-resistance evolves at the landscape level, and use this knowledge to minimize further evolution of herbicide-resistance.

Large scale surveys have been periodically in Australia conducted to monitor patterns of herbicide resistance eg. (Broster and Pratley, 2006; Llewellyn and Powles, 2001; Owen et al., 2007; Walsh et al., 2007). In Australia, ALS and Acetyl-CoA carboxylase (ACCase) resistance in rigid ryegrass (*Lolium rigidum*) has been monitored for several years and recent survey data indicates that as much as 90% of ryegrass populations contain individuals that are resistant to both ACCase and ALS-inhibitor herbicides to the point that resistant ryegrass is more common than susceptible populations (Boutsalis et al., 2012; Owen et al., 2014; Owen et al., 2007). For ALS resistance in ryegrass, high initial frequency of resistant alleles, or mutations conferring ALS resistance (Preston and Powles, 2002), combined with continued use of low-cost ALS herbicides has contributed to widespread resistance evolution (Owen et al., 2007). Kochia resistant to ALS-inhibitor herbicides have also become widespread in eastern Colorado, and most kochia populations are now considered resistant to ALS-inhibitor herbicides. A statewide survey of ALS resistant kochia in Colorado showed that only 1% of 6000 plants were resistant in 1991, but by 1992 and 1993 the level of resistance increased to approximately 50% (Westra and D'Amato, 1995). Compared to ALS resistance evolution in Australia or eastern Colorado, the frequency of EPSPS and auxinic herbicide resistance in kochia from eastern Colorado appears to be increasing at a much slower rate, and the distribution of resistance is much less that that observed with ALS resistance, which may be influenced by potential fitness penalties or complex inheritance of EPSPS and auxinic resistance mechanisms.

Glyphosate-resistance has currently evolved in 16 monocot and 16 dicot species, compared to ALS inhibitor resistance which has evolved in 61 monocot and 96 dicot species, or triazine resistance which has evolved in 23 monocot and 50 dicot species (Heap, 2015). Glyphosate resistance can result from increased 5-enolpyruvylshhikimate-3-phosphate synthase (EPSPS) gene copy number (Gaines et al., 2010), target site-based resistance from amino acid changes in the EPSPS gene (Baerson et al., 2002), reduced translocation to meristematic tissues (Powles and Preston, 2006), altered cellular transport (Lorraine-Colwill et al., 2002), vacuole sequestration of glyphosate (Ge et al., 2010), or other potentially undiscovered mechanisms. Based on evaluations of herbicide-resistance mechanisms in GR kochia from Eastern Colorado, glyphosate-resistance results from increased EPSPS copy number (Wiersma et al., 2015a).

The stability of increased EPSPS copy number in *A. palmeri* is unknown, as pseudo-F2 *A. palmeri* had a higher relative EPSPS copy number than the sum of copy numbers from both parents, which suggests that additional gene copies may be gained during recombination (Gaines et al., 2010). Although gene amplification is unstable during sexual recombination, apomixis may occur, which could maintain gene amplification in the population (Ribeiro et al., 2014; Trucco et al., 2007). In contrast, ALS-resistance due to a target-site mutation has been shown to be inherited as a partially dominant nuclear inherited gene (Tranel and Wright, 2009), and PSII target site resistance is inherited from mutations on maternally inherited chloroplastic genes (Powles and Yu, 2010). Inheritance restrictions of EPSPS compared to ALS-inhibitor or PSII herbicides may influence differences in the rate or resistance evolution between these MOA's.

The further evolution and spread of glyphosate resistance confirmed by increased EPSPS copy number and expression could also be influenced by a fitness penalty for resistant biotypes in the absence of glyphosate selection (Haider et al., 2007). However several studies with

Amaranthus palmeri, and recently a study with *kochia scoparia* have shown that there is not a fitness penalty associated with EPSPS gene amplification (Giacomini et al., 2014; Kumar and Jha, 2015; Vila-Aiub et al., 2014). If glyphosate-resistance due to EPSPS gene amplification in kochia was associated with a fitness penalty, this could partially explain why glyphosate resistance is not as widespread or common as ALS resistance, even though both herbicide groups have been used in a similar manner (high weed control efficacy, low cost, high adoption rates and subsequent high selection pressures). The wide spread occurrence of ALS resistance has been attributed to negligible fitness cost associated with resistance in the absence of herbicide selection (Tranel and Wright, 2002). However, when herbicide-resistance is associated with a fitness penalty, resistance can be maintained in populations if selections pressures are continually applied. For example, although there is a fitness penalty associated with PSII resistance, their persistent use on huge genetically diverse weed populations since the 1950's has led to wide spread resistance evolution (Powles and Yu, 2010). If increased EPSPS in kochia is associated with a fitness penalty, continual selection pressures from over-reliance on glyphosate could maintain resistance in populations, but limit the rate of evolution compared to ALS resistance.

Similar to eastern Colorado, the increased occurrence of GR kochia in Canada has led to surveys evaluating the frequency and distribution of GR kochia. The majority of GR kochia populations identified in a four year Canadian survey found that most GR kochia originated in chemical-fallow cropping systems, and the vast majority of GR kochia was also resistant to ALS herbicides (Beckie et al., 2014b). The GR kochia surveys in Canada showed that GR kochia populations were completely susceptible to dicamba (Beckie et al., 2014b), while our survey data from eastern Colorado demonstrated that dicamba resistance was present in some populations. In western Canada dicamba represents an important management strategy for GR kochia, while in eastern Colorado fluroxypyr appears to be the most important control option for these multiple resistant populations which are now resistant to glyphosate and dicamba, with resistance to ALS-inhibitor herbicides likely, and possible resistance to PSII herbicides.

Currently, dicamba-resistance has evolved in 6 different dicot species, and dicamba resistant kochia has been reported in 6 different states in the Midwest (Heap, 2015). Compared to herbicide classes such as ALS and PSII-inhibitors, auxinic resistance is much less common even though members of this herbicide MOA have been used for over 50 years (Cranston et al., 2001). Dicamba resistance in kochia has been suggested to be caused by mutations in the auxin receptor(s) which may affect endogenous auxin binding and alter auxin-mediated responses such as gravitropism and root growth inhibition (Goss and Dyer, 2003). The low occurrence of dicamba resistance may be due to the rare occurrence of resistant individuals (resistant alleles) in natural weed populations, or mutations that confirm resistance may be lethal (Jasieniuk et al., 1995). Recently studies with wild mustard (*Sinapsi arvensis* L.) have shown that the gene responsible for dicamba resistance may be associated with a fitness penalty, and this could explain the relatively slow occurrence and spread of auxinic herbicide-resistance (Mithila et al., 2012). Dicamba-resistance is inherited as a dominant allele (Preston et al., 2009). The low occurrence of dicamba-resistant kochia in Eastern Colorado suggests a fitness penalty could be restricting the rate of evolution as resistance is inherited as a single dominant gene.

Recently there have been reports of fluroxypyr resistant kochia where R/S (resistant/susceptible) ratios based on I_{50} (Herbicide rate which causes 50% injury) values range from 1.4 to 5.7 (Jha et al., 2015). Dose-response studies have demonstrated that kochia biotypes form North Dakota had up to a six-fold resistance to fluroxypyr relative to a susceptible population, and fluroxypyr doses > 1120 g ha^{-1} were needed for 90% control of those populations

(Howatt and Ciernia, 2014). Although there have been reports of fluroxypyr resistant kochia dating back to 1994 in Montana (Heap, 2015), currently there are no studies which have evaluated physiological, biochemical, or molecular aspects of fluroxypyr-resistant kochia. Fluroxypyr-resistant kochia has yet to be identified in eastern Colorado. Fluroxypyr use patterns including restrictions to in-crop applications may limit selection pressure relative to dicamba, which can be used for in-crop, chemical fallow, and post-harvest applications resulting in greater selection pressure for resistance evolution (Jha et al., 2015). The lower use of fluroxypyr in eastern Colorado may also be influenced by differences in price, as the current price of dicamba (Clarity®, BASF, RTP, NC) is about one-third that of fluroxypyr (Starane Ultra®, Dow AgroSciences, Indianapolis, IN) (Thompson et al., 2015).

Based on results from this four-year survey, it is evident that both glyphosate and dicamba-resistant kochia populations are present in eastern Colorado, and there are several populations that contain multiple resistance to both glyphosate and dicamba. The frequency and distribution of kochia populations resistant to glyphosate and dicamba have remained fairly steady over this four year period in eastern Colorado (Figure 1.5). Compared to ALS or triazine-resistance where resistance has quickly increased to fixation in kochia populations, the evolution of glyphosate and dicamba resistance appears to be occurring at a slower rate which may be influenced by fitness penalties or inheritance of resistance mechanisms. Monitoring the frequency and distribution of herbicide-resistance in eastern Colorado can help develop management recommendations to minimize the further evolution of resistant kochia populations. Combining survey data with evaluations of resistance mechanisms, and weed biology, we can gain insight into the factors influence herbicide-resistance evolution and utilize this information to implement resistance management plans.

With the detection of both glyphosate and dicamba resistance in kochia from eastern Colorado, fluroxypyr represents an important herbicide for populations that have now developed multiple resistance to ALS-inhibitors, glyphosate, and the auxinic herbicide dicamba. Further research evaluating the inheritance and fitness penalties associated with glyphosate and auxinic resistance in kochia can provide insight into how these factors influence herbicide-resistance evolution and use this knowledge to provide management recommendations aimed at minimizing herbicide-resistant kochia evolution in eastern Colorado. Over-reliance on glyphosate and auxinic herbicides for kochia control should be avoided to maintain the utility of these important kochia herbicides where susceptibility remains, and proactive stewardship strategies such as tank mixing or rotating MOA's should be utilized to minimize further herbicide-resistance evolution in kochia.

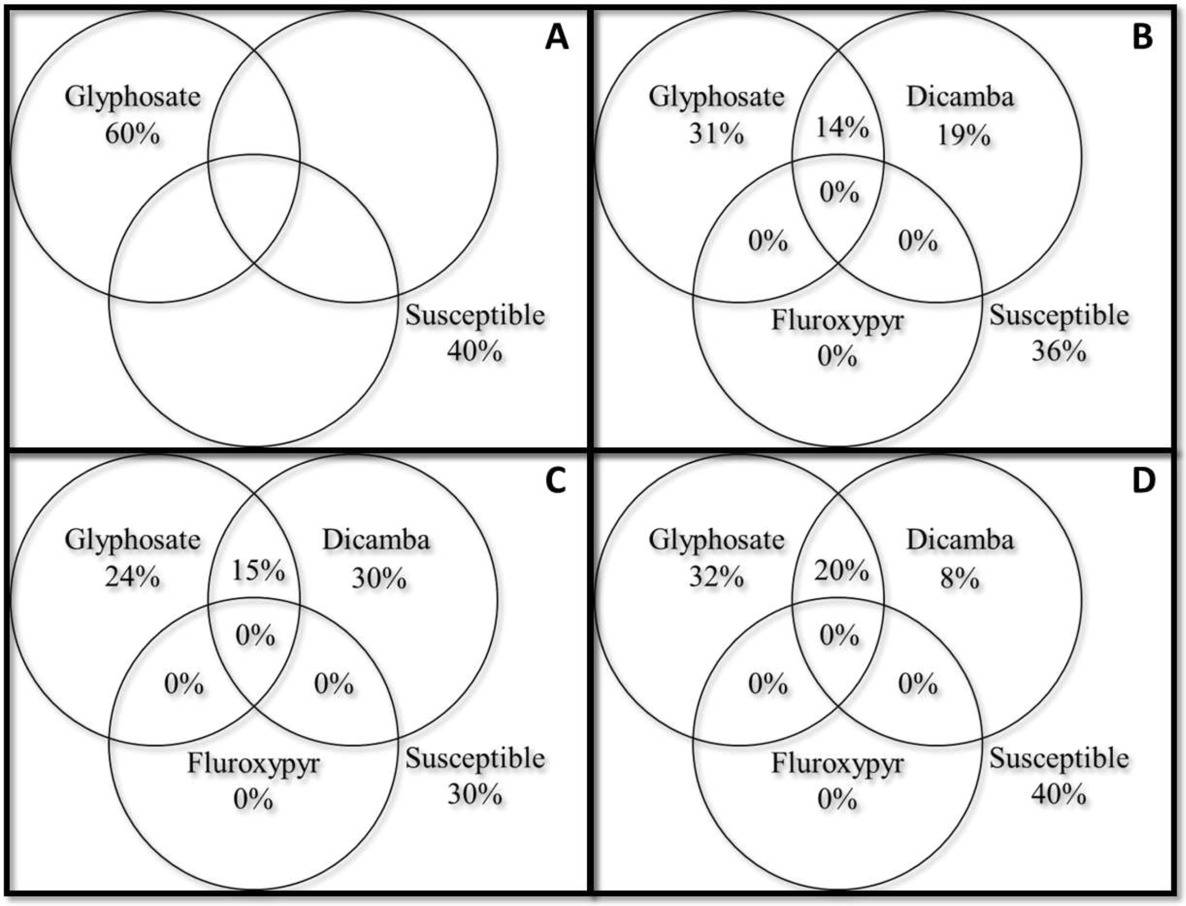

Figure 1.1 Proportion of populations that were characterized as resistant or developing resistance to glyphosate, dicamba, and fluroxypyr, and the proportion of populations that were completely susceptible to all three herbicides from A, 2011; B, 2012; C, 2013; and D, 2014.

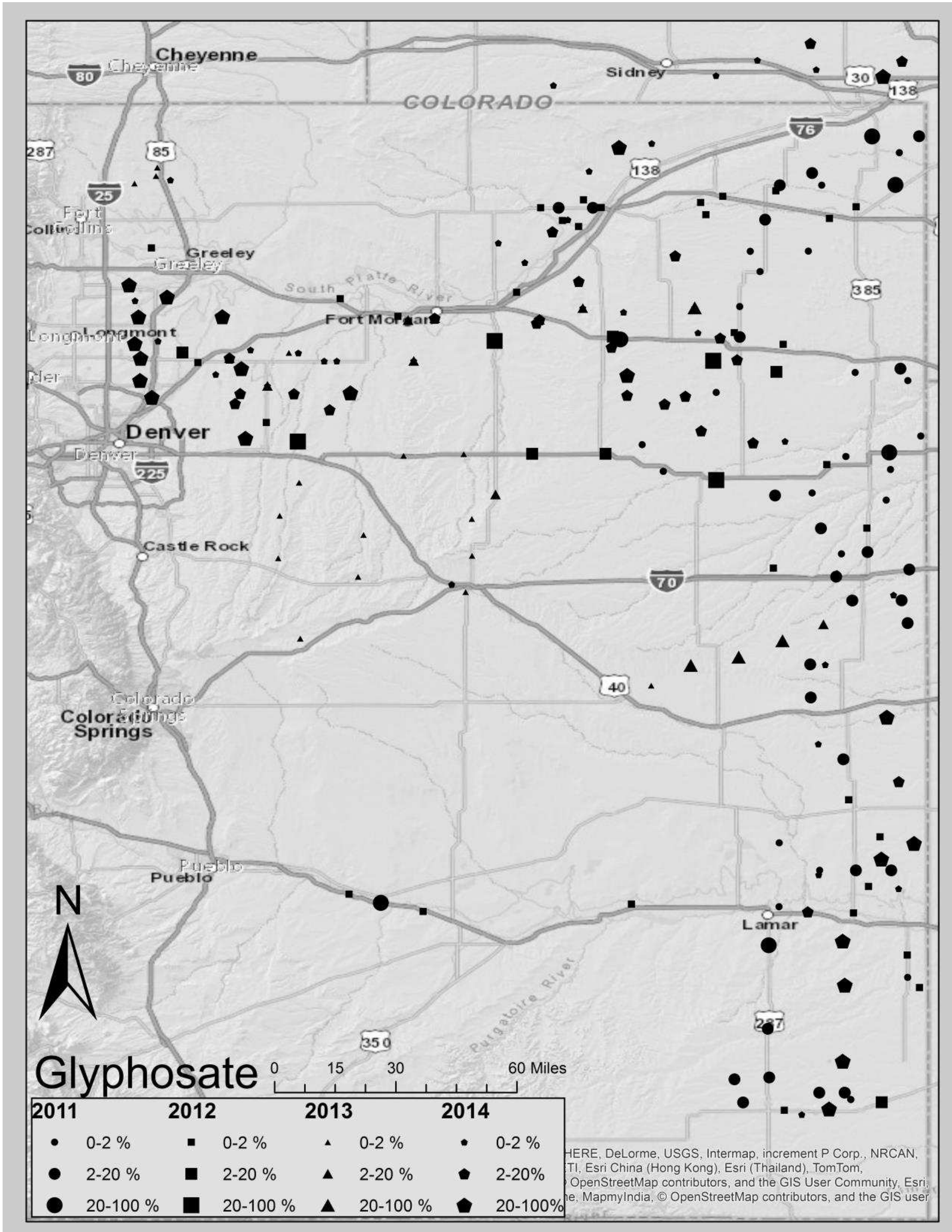

Figure 1.2 Geo-referenced GR kochia populations over time (2011-2014). Level of resistance is broken into three categories; 0-2% susceptible, 2-20% developing resistance, and 20-100% resistant.

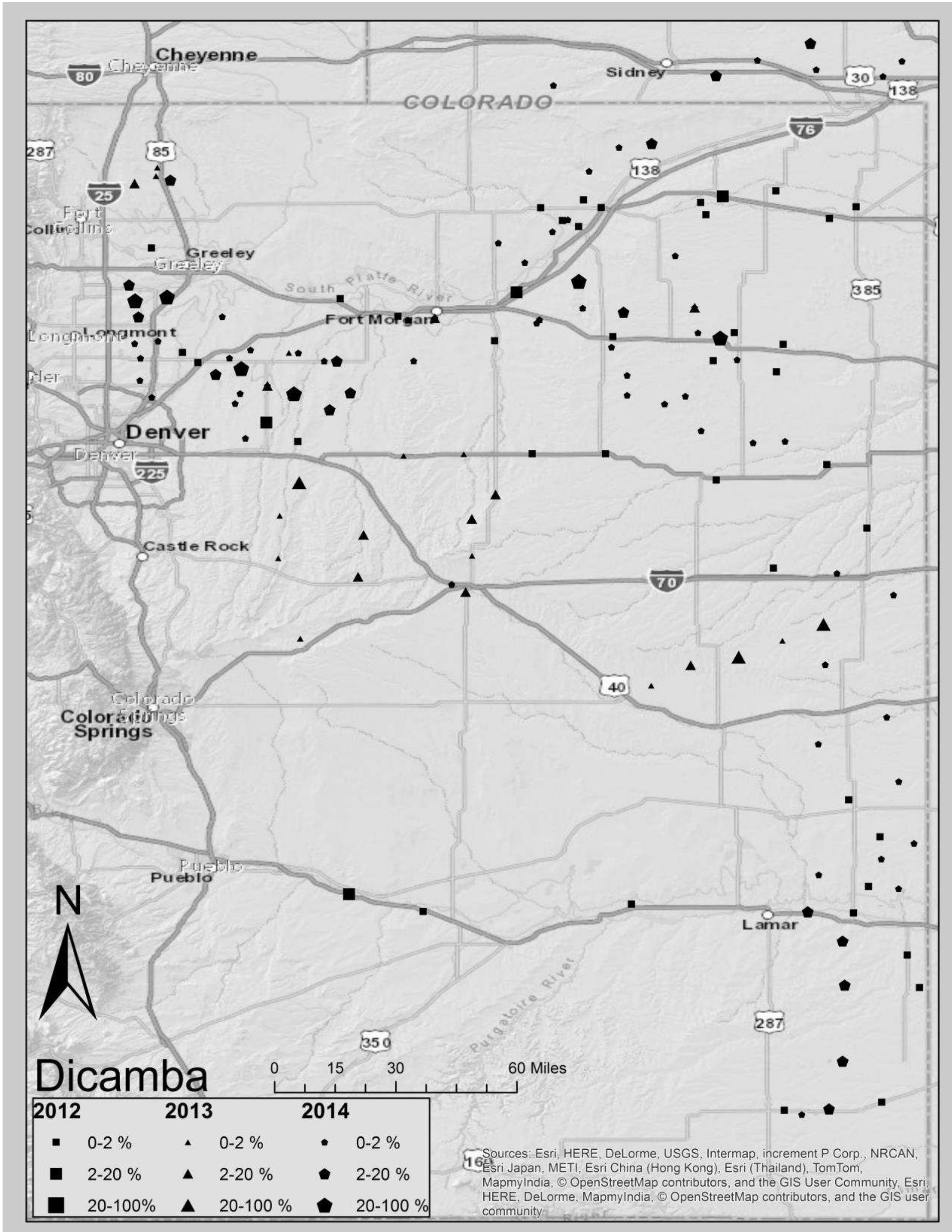

Figure 1.3 Geo-referenced dicamba-resistant kochia populations over time (2012-2014). Level of resistance is broken into three categories; 0-2% susceptible, 2-20% developing resistance, and 20-100% resistant.

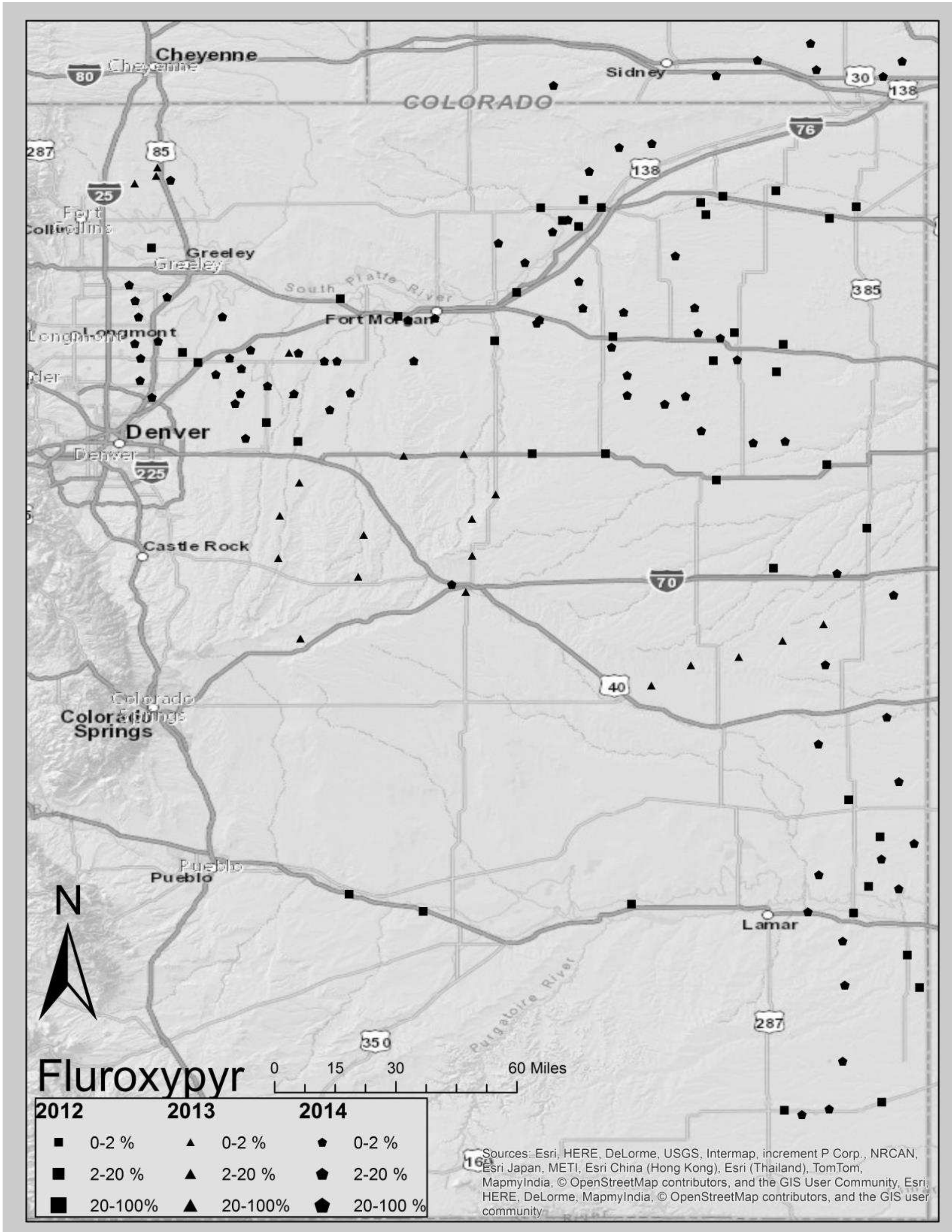

Figure 1.4 Geo-referenced fluroxypyr-resistant kochia populations over time (2012-2014). Level of resistance is broken into three categories; 0-2% susceptible, 2-20% developing resistance, and 20-100% resistant.

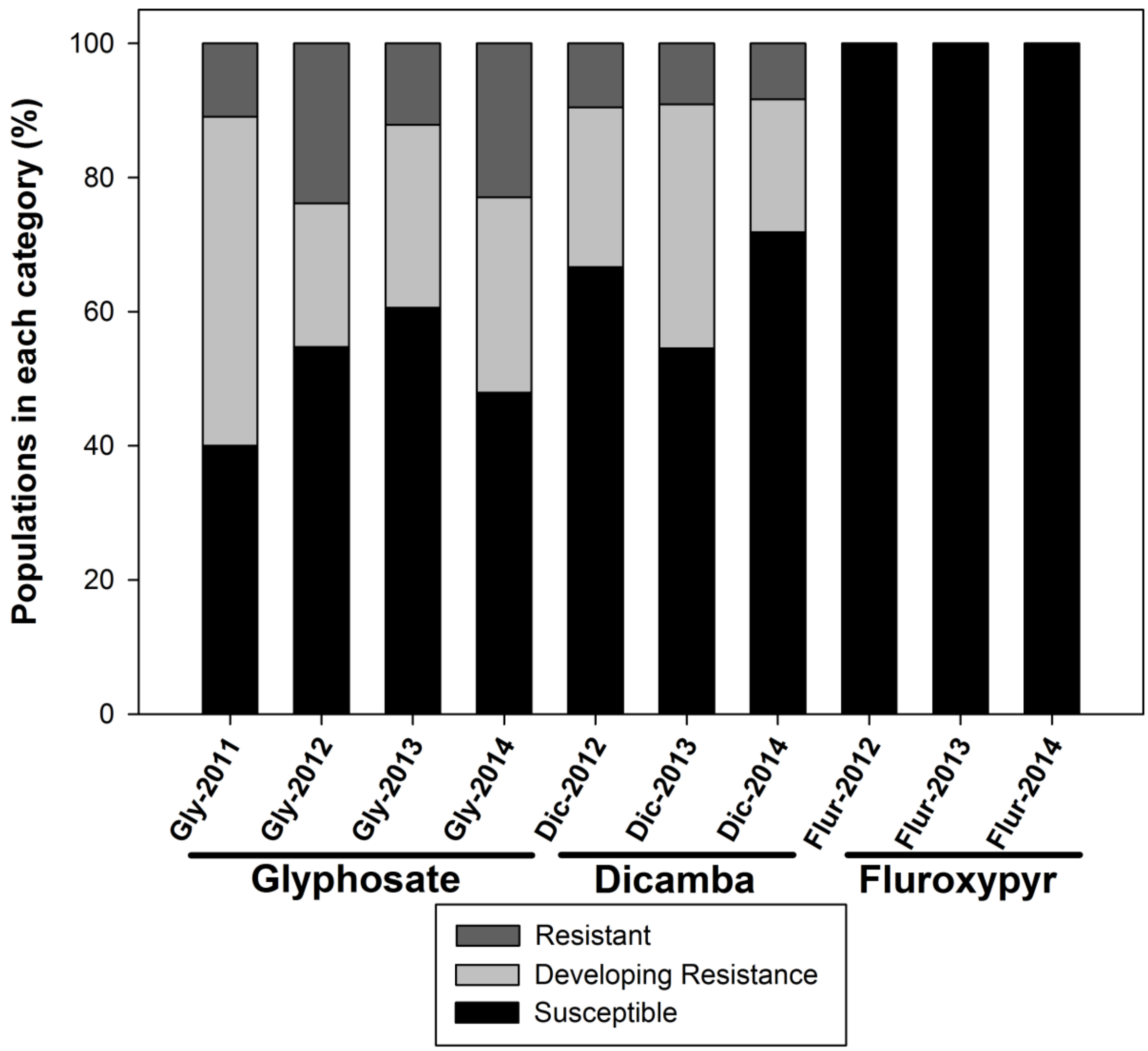

Figure 1.5 Percent of populations classified as susceptible (<2% survival), developing-resistance (2-20% survival), and resistant (>20% survival) to a discriminating rate for glyphosate, dicamba, and fluroxypyr over the four-year survey.

References

Baerson SR, Rodriguez DJ, Tran M, Feng Y, Biest NA, Dill GM (2002) Glyphosate-resistant goosegrass. Identification of a mutation in the target enzyme 5-enolpyruvylshikimate-3-phosphate synthase. Plant physiology 129:1265-1275

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

Beckie HJ, Gulden RH, Shaikh N, Johnson EN, Willenborg CJ, Brenzil CA, Shirriff SW, Lozinski C, Ford G (2014) Glyphosate-resistant kochia (Kochia scoparia L. Schrad.) in Saskatchewan and Manitoba. Canadian J of Plant Sci 95:345-349

Boutsalis P, Gill GS, Preston C (2012) Incidence of herbicide resistance in rigid ryegrass (Lolium rigidum) across southeastern Australia. Weed Technol 26:391-398

Broster J, Pratley J (2006) A decade of monitoring herbicide resistance in Lolium rigidum in Australia. Animal Production Sci 46:1151-1160

Challaiah A, Burnside O, Johnson V, Roeth F, Schimidt J (1983) Identifying weed competitiveness of winter wheat cultivars. Proceedings. North Central Weed Control Conference, Urbana

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

Dawit M, Maxwell BD, Peter KF, Dyer WE (1994) Kochia (Kochia scoparia) Pollen Dispersion, Viability and Germination. Weed Sci 42:548-552

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

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

Gail AW, Alex RM, Garold WM (1993) Control of Triazine-Resistant Kochia (Kochia scoparia) in Conservation Tillage Corn (Zea mays). Weed Sci 41:225-231

Gaines TA, Zhang W, Wang D, Bukun B, Chisholm ST, Shaner DL, Nissen SJ, Patzoldt WL, Tranel PJ, Culpepper AS, Grey TL, Webster TM, Vencill WK, Sammons RD, Jiang J, Preston C, Leach JE, Westra P (2010) Gene amplification confers glyphosate resistance in Amaranthus palmeri. Proceedings of the National Academy of Sciences 107:1029-1034

Ge X, Avignon DAd, Ackerman JJH, Sammons RD (2010) Rapid vacuolar sequestration: the horseweed glyphosate resistance mechanism. Pest Management Sci 66:345-348

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

Goss GA, Dyer WE (2003) Physiological characterization of auxinic herbicide-resistant biotypes of kochia (Kochia scoparia). Weed Sci 51:839-844

Haider J, Vencill W, Culpepper A, Grey T (2007) Physiological response of glyphosate-resistant Palmer amaranth. Proc South Weed Sci Soc 60:180

Heap I (2015) International Survey of Herbicide Resistant Weeds. www.weedscience.org

Howatt KA, Ciernia M (2014) Kochia samples from North Dakota with variable response to fluroxypyr. Proceedings of the Western Soc of Weed Sci:67: 79

Jasieniuk M, Morrison IN, Brûlé-Babel AL (1995) Inheritance of dicamba resistance in wild mustard (Brassica kaber). Weed Sci:192-195

Jha P, Kumar V, Lim CA (2015) Variable response of kochia [Kochia scoparia (L.) Schrad.] to auxinic herbicides dicamba and fluroxypyr in Montana. Canadian J of Plant Sci 95:965-972

Kumar V, Jha P, (2015) Growth and reproduction of glyphosate-resistant and susceptible populations of kochia scoparia. PloS one 10.11 e0142675

Llewellyn RS, Powles SB (2001) High Levels of Herbicide Resistance in Rigid Ryegrass (Lolium rigidum) in the Wheat Belt of Western Australia. Weed Technol 15:242-248

Lorraine-Colwill DF, Powles SB, Hawkes TR, Hollinshead PH, Warner SAJ, Preston C (2002) Investigations into the mechanism of glyphosate resistance in Lolium rigidum. Pesticide Biochemistry and Physiology 74:62-72

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

Mithila J, McLean MD, Chen S, Christopher Hall J (2012) Development of near-isogenic lines and identification of markers linked to auxinic herbicide resistance in wild mustard (Sinapis arvensis L.). Pest Management Sci 68:548-556

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

Owen M, Martinez N, Powles S, Iannetta P (2014) Multiple herbicide-resistant Lolium rigidum (annual ryegrass) now dominates across the Western Australian grain belt. Weed Research 54:314-324

Owen MJ, Walsh MJ, Llewellyn RS, Powles SB (2007) Widespread occurrence of multiple herbicide resistance in Western Australian annual ryegrass (Lolium rigidum) populations. Crop and Pasture Sci 58:711-718

Powles SB, Preston C (2006) Evolved Glyphosate Resistance in Plants: Biochemical and Genetic Basis of Resistance. Weed Technol 20:282-289

Powles SB, Yu Q (2010) Evolution in Action: Plants Resistant to Herbicides. Annual Review of Plant Biology 61:317-347

Preston C, Belles DS, Westra PH, Nissen SJ, Ward SM (2009) Inheritance of Resistance to the Auxinic Herbicide Dicamba in Kochia (Kochia scoparia). Weed Sci 57:43-47

Preston C, Powles S (2002) Evolution of herbicide resistance in weeds: initial frequency of target site-based resistance to acetolactate synthase-inhibiting herbicides in Lolium rigidum. Heredity 88:8-13

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

Stallings GP, Thill DC, Mallory-Smith CA, Shafii B (1995) Pollen-Mediated Gene Flow of Sulfonylurea-Resistant Kochia (Kochia scoparia). Weed Sci 43:95-102

Thompson CR, Peterson DE, Fick WH, Stahlman PW, Slocombe JW (2015) Chemical Weed Control for Field Crops, Pastures, Rangeland, and Noncropland. K-State Research and Extension 15-16

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

Trucco F, Zheng D, Woodyard AJ, Walter JR, Tatum TC, Lane Rayburn A, Tranel PJ (2007) Nonhybrid progeny from crosses of dioecious amaranths: implications for gene-flow research. Weed Sci 55:119-122

Varanasi VK, Godar AS, Currie RS, Dille AJ, Thompson CR, Stahlman PW, Jugulam M (2015) Field-evolved resistance to four modes of action of herbicides in a single kochia (Kochia scoparia L. Schrad.) population. Pest Management Sci 71:1207-1212

Vencill WK, Nichols RL, Webster TM, Soteres JK, Mallory-Smith C, Burgos NR, Johnson WG, McClelland MR (2012) Herbicide Resistance: Toward an Understanding of Resistance Development and the Impact of Herbicide-Resistant Crops. Weed Sci 60:2-30

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

Vilà M, Williamson M, Lonsdale M (2004) Competition experiments on alien weeds with crops: lessons for measuring plant invasion impact? Biological Invasions 6:59-69

Waite J, Thompson CR, Peterson DE, Currie RS, Olson BLS, Stahlman PW, Al-Khatib K (2013) Differential Kochia (Kochia scoparia) Populations Response to Glyphosate. Weed Sci 61:193-200

Walsh M, Owen M, Powles S (2007) Frequency and distribution of herbicide resistance in Raphanus raphanistrum populations randomly collected across the Western Australian wheatbelt. Weed Res 47:542-550

Westra P, D'Amato T (1995) Issues Related to Kochia (Kochia scoparia L.) Management in Western Agriculture. Proceedings of the Western Soc of Weed Sci:39

Whitson TD, Burrill LC, Dewey SA, Cudney DW, Nelson B, Lee RD, Parker R (1991) Weeds of the West.

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

Chapter 2: Managing Glyphosate-resistant Kochia (*Kochia scoparia*) in Canola

Summary

There are a limited number of post-emergent (POST) herbicides for broadleaf control in canola, and over reliance on herbicide-tolerant canola varieties, specifically RoundUp Ready, has resulted in the evolution of glyphosate-resistant (GR) kochia which threatens the utility of glyphosate for weed control in canola. Information on how different herbicide treatments influence the percentage of subsequent GR kochia progeny is needed to provide management recommendations to minimize further evolution of GR kochia. Kochia at a ratio of 10% GR: 90% glyphosate-susceptible was established in plots with RoundUp Ready and Liberty Link canola to evaluate how herbicide treatments impact changes in the frequency of GR kochia. Field studies were conducted in 2014 and 2015 to a) compare the selection for GR kochia progeny between POST applications of glyphosate and glufosinate b) evaluate how including a pre-emergent herbicide (pendimethalin) along with glyphosate or glufosinate POST impacts the selection for GR kochia progeny and c) evaluate the impact of canola variety selecting for GR kochia progeny in the absence of herbicide applications. Results showed that a) kochia survivors from glyphosate applications had higher frequencies of GR progeny compared to survivors from glufosinate applications, although differences in GR progeny between POST herbicides were less pronounced when glufosinate efficacy was reduced in the second year of the study b) the inclusion of a pre-emergent (PRE) herbicide (pendimethalin) significantly reduced the number of kochia individuals that were exposed to POST applications, and reduced the frequency of GR progeny, and c) canola variety did contribute to kochia suppression but both varieties appeared equivalent in their suppression, differences in phenotypes between canola varieties impacted the frequency of GR kochia progeny that grew below the canola canopy. Management

recommendations to minimize the further GR kochia evolution are to incorporate an alternative mode of action (glufosinate) either in a rotation or tank mix to reduce the frequency of GR kochia progeny, and most importantly, to incorporate a pre-emergent herbicide to limit the evolution and selection of GR kochia.

Introduction

There were a limited number of post-emergent (POST) herbicides for broadleaf weed control in canola before the introduction of herbicide-tolerant (HT) canola varieties. Following the introduction of glyphosate and glufosinate tolerant canola, the adoption rate for herbicide tolerant canola varieties RoundUp Ready (Monsanto, St. Louis, MO) and Liberty Link (Syngenta, Greensboro, NC) was very rapid. Herbicide tolerant varieties now comprise the majority of canola acres in the USA and Canada.

Weed management options for conventional canola were limited to trifluralin pre-plant incorporated (PPI) and quizalofop combined with either clopyralid or ethametsulfuron (POST) at a cost of $86 per hectare. The introduction of glyphosate-tolerant canola varieties represented a $32 per hectare cost saving compared to conventional canola systems (Gianessi, 2005). With widespread resistance to sulfonylurea herbicides (ethametsulfuron) (Beckie et al., 2011), and limited kochia activity with clopyralid (Lloyd et al., 2011), options for broadleaf weed control were limited in conventional canola, until HT canola varieties were introduced for use with glufosinate, triazine, imidazolinone herbicides, or glyphosate (Beckie et al., 2006).

One of the main drivers for such rapid adoption of glyphosate tolerant canola was the very limited number of PPI or PRE herbicides options available for weed management, combined with limited herbicides available for POST applications. Soil applied herbicides labeled in canola (trifluralin or ethafluralin) have limited weed spectrums, require soil incorporation which restricts the use of no-till, and soil residuals can adversely impact subsequent crop rotations (Beckie et al., 2006). Beckie et al. 2006 also showed that in canola, available POST herbicides such as ethametsulfuron have limited activity on broadleaf weed species to the point where weed competition resulted in extensive yield losses, limiting the use of this herbicide to fields with minimal weed pressure. The adoption of HT canola varieties has significantly improved weed control and allowed for no-till practices without rotational restrictions that were required with conventional canola herbicides. In the USA and Canada, growers rapidly adopted herbicide-tolerant canola because of more effective weed control, higher yield, and higher net returns based primarily on the higher yield, reduced dockage, and lower herbicide costs (Devine and Buth, 2001). In 2014, about half of the 8.1 million hectares of canola harvested in Canada were glyphosate tolerant (Canola Council of Canada); however, glyphosate use is not restricted to in-crop applications. Glyphosate is also used for weed control in no-till, and chemical fallow systems (Beckie et al., 2014a). This results in several glyphosate applications per year when growing canola (pre-plant, potential for multiple in crop applications).

The rapid, wide-spread adoption of GT-canola and the continued use of glyphosate as the primary herbicide for chemical fallow and pre-plant weed management has had unintended consequences, the most important being the evolution of glyphosate resistant (GR) kochia. GR kochia was first reported in USA in 2007 (Kansas) (Heap, 2015) and in western Canada in 2011 (Beckie et al., 2013a). Glyphosate-resistant kochia threatens the long-term sustainability of RoundUp Ready canola production because there are a limited number of POST herbicides for broadleaf weed control, and the loss of glyphosate would remove an essential weed management component in canola and make it very difficult to practice no-till production.

Kochia is an introduced, annual broadleaf that germinates in early spring and is tolerant to cold, heat, drought, and saline conditions (Friesen et al., 2009). Kochia's protogynous flowers ensure a high degree of out crossing (Stallings et al. 1995), which results in high levels of genetic diversity within and between populations (Mengistu and Messersmith 2002). Mature kochia plants also produce copious amounts of pollen for extended periods, which is typically an indication that the species is naturally highly outcrossing (Friesen et al., 2009). Kochia has the ability to transfer genetic traits such as herbicide-resistance through pollen movement (Dawit et al., 1994), as well as physical distribution when mature plants detach from their root systems in the fall and tumble across the landscape, dispersing seed. Among 40 non-native weed species in the northwestern United States, kochia was reported to have the highest rate of spread in this region (Forcella, 1985). During the past 40 years, kochia has extended northward in the Canadian prairies (Beckie et al., 2012), and is one of the top 10 most abundant agricultural weeds in the Canadian prairies (Leeson, 2005). Beckie et al. (2013) showed that within the grassland region of Canadian prairies, kochia had the highest risk for development of glyphosate resistance out of all present weeds. Therefore it should not be a surprise that GR kochia has evolved in RoundUp Ready canola systems in Canada.

Previous studies investigated the selection of herbicide-resistant weeds (Beckie et al., 2014c; Beckie and Reboud, 2009); however, most of these studies involved target-site resistance for either acetolactate synthase (ALS)-inhibitor or acetyl-CoA carboxylase (ACC) inhibitor resistance, where inheritance patterns are understood (Murray et al., 1995; Shaner, 1999; Tranel and Wright, 2009), and resistance was typically governed by a single gene that displays partial dominance.

Beckie et al. 2009 demonstrated that starting with a 5% ALS-inhibitor resistant kochia, after one application of ethametsulfuron, the level of ALS resistance in field pennycress (*Thlaspi arvense*) increased to 29%, and up to 85% after four applications. If an alternative mode of action (bromoxynil/MCPA) was included along with ethametsulfuron, the resistance level was similar to the treatment with no ALS-inhibitor herbicide was applied (approximately 3% resistant) (Beckie et al. 2009).

Long-term studies in Canada between 1979 and 1998 examined the frequency of herbicide use on resistance evolution in wild oat (*Avena fatua*). Triallate resistance occurred after 18 years when the herbicide was applied annually in continuous spring wheat, but resistance did not develop when triallate was applied 10 times in the wheat phase of a wheat-fallow rotation over the same period (Beckie and Reboud, 2009). Although studies have evaluated how herbicide treatments influence herbicide-resistance evolution, there are no studies which have evaluated how different modes of action influence GR evolution when compared to glyphosate.

Glyphosate resistance in kochia has been associated with increase copy number of the EPSPS gene. Resistant plants contain 3 to 9 times more copies of the gene (Godar et al., 2015; Kumar et al., 2015; Wiersma et al., 2015b) compared to susceptible kochia. The stability and inheritance of the increased EPSPS copy number has been evaluated. In Palmer amaranth a pseudo-F2 population had a higher relative EPSPS copy number than the sum of copy numbers from both parents, suggesting that additional gene copies may be gained during recombination (Gaines et al., 2010). Although gene amplification appears to be unstable during sexual recombination, apomixis may provide a mechanism that could maintain the EPSPS gene amplification in the population (Ribeiro et al., 2014; Trucco et al., 2007). Detection of EPSPS genes on distal ends of homologous chromosomes suggests that increase in EPSPS gene copies in GR kochia occurred as a result of unequal crossover during meiosis resulting in tandem gene duplication (Jugulam et al., 2014). Glyphosate resistance is due to increased EPSPS gene copy number, and the mechanism of inheritance is still relatively unknown; however, knowing the potential for further resistance selection and how herbicide treatments might impact this selection is critical to minimizing the further evolution of GR kochia.

This study was conducted to evaluate how herbicide treatments influence the frequency of GR kochia progeny when starting at a targeted baseline level of 10% GR kochia in a manner similar to that described by Beckie et al. (Beckie and Reboud, 2009). The objectives of this study were to a) compare the selection for GR kochia progeny between POST applications of glyphosate and glufosinate b) evaluate how including a pre-emergent herbicide (pendimethalin) along with glyphosate or glufosinate POST impacts the selection for GR kochia progeny and c) evaluate the impact of canola variety selecting for GR kochia progeny from kochia grown in the absence of herbicide applications.

Materials and Methods

Field Site

Field experiments were conducted in 2014 and 2015 at the Colorado State University Agricultural Research, Development, and Education Center (ARDEC) located just north of Fort Collins Colorado at 40.652° N, -105.000° W. The field site was in corn for the previous two years, and native kochia seed was minimal to nonexistent in the seed bank at the beginning of the study. The soil type was a Fort Collins clay (Fine-loamy, mixed, superactive, mesic Aridic

Haplustalfs) with 32% sand 25% silt and 43% clay, with a CEC of 36.6, 2.35 % organic matter, and pH of 7.6. The field site was under linear irrigation for both years. In 2014, spring time temperatures and precipitation were similar to 10 year averages, while in 2015, spring time temperatures were cooler than normal and precipitation in May was 79 mm higher than ten year averages which delayed planting in 2015.

Experimental Design

The experiment was arranged in a split-plot design with four replications, where canola variety, RoundUp Ready ((DKL 30-42) Monsanto, St. Louis, MO) or Liberty Link ((InVigor L150) Syngenta, Greensboro, NC), was the whole plot factor, and weed management treatment was the sub-plot factor. Weed management treatments consisted of a control (Weedy check-no management), hand weeded control (Non-weedy check), PRE plus POST herbicide treatment, and a POST only treatment. POST treatments were glyphosate or glufosinate. Between years, both canola variety and herbicide treatments were re-randomized and the study was conducted at the same location in 2014 and 2015. In 2015, delayed planting allowed for an application of diquat to remove germinated kochia and volunteer canola from 2014 study. Before planting in 2015 all plots were scouted to ensure that kochia carryover from 2014 was minimal to non-existent.

Planting

Canola was planted using a six row cone seeder with two passes (1.5m) for each plot. Each canola variety was planted based on 100 seed weights for a target density of 3000 plants per plot or 107 plants m^{-2}. Canola was seeded at a target depth of 2 cm, and was planted on May 27^{th} and June 9^{th} in 2014 and 2015, respectively.

After planting canola, kochia seed was mixed into a vermiculite carrier at a ratio of 10% GR seed: 90% glyphosate-susceptible. Based on 100 seed weights, approximately 11 grams of susceptible kochia seed and 0.7 grams of GR seed were seeded into each plot for a target of 750 resistant seeds and 6750 susceptible seeds per plot, based on a target kochia density of 270 seeds m^{-2}. The amount of seed planted for both kochia accessions were adjusted based on percent resistance, as well as germination percentage. Once weighed out, both glyphosate-susceptible and resistant kochia seed were incorporated into 1000 ml of medium textured vermiculite using a V-mixer for 5 min per batch to ensure that kochia seed was evenly distributed throughout the vermiculite carrier. Kochia seed and the vermiculite carrier were then seeded using a 0.76 m wide drop spreader with 4 passes for each plot. Kochia seed was incorporated with 10 mm of irrigation immediately after planting to incorporate seeds into the soil. Because GR kochia seed was used in the experiment, Sudangrass (2014) or corn (2015) was used as a crop border row surrounding the entire study, as well as around individual plots in order to minimize wind mediated kochia pollen flow, and off site kochia movement.

Kochia Biotypes

The susceptible kochia population originated from Northern Colorado, and the presence and absence of resistance was confirmed by screening a subset of each population with glyphosate at 840 g ae ha^{-1}. The resistant population was collected from Eastern Colorado and was on average 98% resistant to the field rate of glyphosate (840 g ae ha^{-1}) and had a GR_{50} value of 807 g ae ha^{-1}, plants did show stunting at the field rate compared to an untreated check. The susceptible population had a GR_{50} value of 145 g ae ha^{-1} with no survivors at the field rate of glyphosate.

Herbicide Applications

Pendimethalin (Prowl H_2O®, BASF) was used as the pre-emergent herbicide for both canola varieties at a rate of 1120 g ai ha^{-1}. Pendimethalin was selected as the pre-emergent herbicide because it can be applied PRE and incorporated with irrigation, compared to ethafluralin or trifluralin which require physical incorporation (PPI) in the top 5 to 8 cm of soil. POST herbicide applications consisted of glufosinate (Group 10) or glyphosate (Group 9), depending on the canola variety. Glyphosate (RoundUp WeatherMax®, Monsanto, St. Louis) and glufosinate (Liberty 280®, Syngenta, Greensboro, NC) were both applied at 1.61 L ha^{-1}, or 840 g ae ha^{-1} and 450 g ai ha^{-1} for glyphosate and glufosinate, respectively. Ammonium sulfate (AMS) was added to both glyphosate and glufosinate spray solutions at a rate of 20 g L^{-1}. POST herbicides were applied when kochia seedlings were 5 to 10 cm tall, and when the canola was in the 2-3 leaf growth stage. Herbicides were applied using CO_2 backpack sprayer and hand–held boom calibrated to deliver 187 L ha^{-1} at 275 kPa. For glyphosate and pendimethalin field applications, and for glyphosate greenhouse screening, wide angle flat spray tips (Turbo Teejet 11002VS, Spraying Systems Co., Wheaton, IL) were used. For glufosinate field applications, extended range flat fan spray tips (Teejet XR11002VS, Spraying Systems Co., Wheaton, IL) were used for herbicide applications.

Data Collection

Plant Densities

Initial plant densities for canola and kochia were measured on June 18th in 2014 and on July 1st in 2015. Plant densities were evaluated prior to POST herbicide applications (0 days after treatment (DAT)) using four $1m^2$ quadrats per plot. Initial plant densities evaluated the impact of a pre-emergent herbicide application on canola and kochia densities. Sampling locations within the plot were staked so that application plant densities could be evaluated from the same locations within the plot. Three weeks (21 DAT) after POST herbicides were applied, kochia and canola densities were evaluated using the same procedure in order to evaluate the impact of POST herbicide applications on plant densities.

Canola and Kochia Harvest

On September 8th (104 days after planting (DAP)) and September 17th (100 DAP) both the canola and kochia were harvested from all plots in 2014 and 2015, respectively. Canola was harvested by hand cutting all plants from each plot with hand-clippers, plants were then placed on tarps in which they were transported outside of the plot area and then hand fed into a Wintersteiger (Wintersteiger Inc., Salt Lake City, UT) combine. While hand-harvesting canola, kochia individuals that grew above the canola canopy were harvested and combined into grain totes. Once the canola and above canopy kochia were removed, kochia individuals that remained below the canola canopy were collected and maintained separately from above canopy kochia for biomass evaluation and screening purposes. During harvest, the total number of kochia plants that grew above and remained below the canola canopy were recorded, and dry weight biomass was recorded for all kochia plants present in plots at the time of harvest.

Kochia Greenhouse Screening with Glyphosate

Once harvested and air dried, seed from harvested kochia plants were hand stripped to form a composite seed sample from each plot for glyphosate-resistance screening in the greenhouse. For the weedy check plots, kochia seed from above and below the canola canopy were screened separately in order to evaluate differences in the frequency of GR kochia. For plots that received a herbicide application (POST, PRE, or both), kochia seed from plants that survived a herbicide application (both below and above canopy) were combined for greenhouse glyphosate screening as the amount of seed required for greenhouse screening was often limited.

Collected kochia seed was planted into plug flats where individual kochia seedlings were germinated and grown in a cell that was 1.27 by 1.27 by 2.54 cm (American Clay Works, Denver CO). Fine grade potting soil (Fafard #2-SV) (American Clay Works, Denver, CO) was used as the growing media for greenhouse screenings. Plants were grown in plug flats until kochia seedlings were approximately 2.5 cm tall. Kochia seedlings were then transplanted and grown in inserts that were 3.8 cm by 3.8 cm by 5.8 cm deep (American Clay Works, Denver, CO) until plants were 5 to 10 cm tall. At this stage, kochia was treated with glyphosate (RoundUp WeatherMax) at 840 g ae ha^{-1} with AMS at 20 g L^{-1} via a moving overhead single nozzle sprayer (DeVries Manufacturing Hollandale, MN) calibrated to deliver 187 L ha^{-1}. Plants were grown and maintained in a greenhouse at a 14/10 photoperiod with temperatures maintained between 22 and 26 °C. Plant were watered daily to field capacity until 21 days after treatment (DAT) when they were evaluated. Individual kochia plants were rated as either dead or alive three weeks after application, and the percentage of glyphosate-resistance was determined by dividing the number of survivors by the total number of individuals screened from a given plot. To determine the frequency of resistant from seed for a given plot, 72 individual kochia plants were screened with glyphosate in the greenhouse. In order to evaluate changes in the frequency of GR kochia progeny from different herbicide treatments, the percentage of GR kochia progeny from field studies were compared to targeted planting ratios of ten percent GR kochia to evaluate changes in the frequency of resistance.

Statistical analysis

Plant Densities, and Kochia Counts and Biomass at Harvest

To determine impact of herbicide treatments on canola densities, and kochia density and kochia biomass at time of harvest, a split-plot analysis of variance (ANOVA) was conducted using the Proc Glimmix method in SAS 9.3 (SAS Institute, Cary, NC). Factors included in the model were the whole plot factor of canola variety, subplot factor-treatment. For plant densities taken 0 and 21 DAT, DAT was included along with canola variety and treatment in the model. For kochia density and biomass at harvest, kochia growth in relation to the canola canopy (below or above) was also included along with canola variety and treatment in the model. All interactions of the factors above were included in the model. The random factors were year, block, and interaction terms with year and block. Interaction terms from the model with P values < 0.05 were considered significant, and significant interactions were further analyzed by comparisons between of all pairs of least square means, and comparing respective p-values to a significance level of 0.05.

Canola Yields

Canola yields were analyzed using the Proc GLM method in SAS 9.3 (SAS Institute, Cary, NC). Fisher's Protected LSD test was conducted on treatments and year.

Results and Discussion

Canola Response

For canola yield, only the main effects of treatment and year were significant. Canola yields were significantly higher in 2014 compared to 2015, 2,698±78 kg/ha compared to 2,224±72, respectively. In addition, canola yields from the hand weeded check (2,704±121, mean ± SE), POST (2,644 ± 118), and PRE + POST herbicide (2,489 ± 80) treatments were not significantly different from each other, but were significantly different from the weedy check (2007±83). Irrigation was used to provide supplemental moisture; however, canola yields from this study were similar to the average yields of 2120 kg ha^{-1} reported for all provinces in Canada for 2014 (http://www.canolacouncil.org/markets-stats/statistics/bushelsacre/), and also similar to average yields of 2007 kg ha^{-1} in the USA (http://www.uscanola.com/crop-production/). Lower canola yields in 2015 were lower most likely due to later planting date and cooler growing season compared to 2014.

Although greenhouse screening prior to field studies showed acceptable canola tolerance to pendimethalin, when applied at 1120 g ai ha^{-1} in field studies, canola injury was observed in both years. Pendimethalin was selected as the PRE herbicide for field studies because it can be incorporated with moisture and does not require physical incorporation (5-8 cm) needed for ethafluralin and trifluralin. Kochia seed was planted on soil surfaces, and physical incorporation of a herbicide would have buried kochia seed below a depth of typical germination.

Initial canola stand counts (0 DAT) did not show a negative impact from pendimethalin in 2014 (Table 2.1), primarily because all canola plants were counted even those displaying injury symptoms. However, canola stand counts 21 DAT showed the effect of pendimethalin injury on canola stand reduction in 2014 (Table 2.1). In 2015, pendimethalin injury to canola was observed for both 0 and 21 DAT stand counts (Table 2.2). Both varieties displayed some stunting and stand reduction. Canola's phenotypic plasticity compensated for this stand reduction and yields were maintained. With adequate moisture canola can compensate for lower stand counts by increasing pods per plant and increasing pod retention at each node (Angadi et al., 2003). So, even though there was stand reduction with pendimethalin treatments, yields were not significantly lower.

Kochia Response

Overall kochia densities were lower in 2015 compared to 2014 (Table 2.3 & 2.4). Kochia control from POST only treatments was greater in 2014 compared to 2015 for both glyphosate and glufosinate (Table 2.5 & 2.6 and 2.7 & 2.8). Based on kochia densities from weedy check plots in 2014, glyphosate and glufosinate provided 99.3 and 98.3 percent kochia control, respectively. In 2015, glyphosate only provided 62% kochia control, while glufosinate treated plots had more kochia plants per square meter than the weedy check (Table 2.6). This lack of kochia control was most likely the result unfavorable weather conditions following the glufosinate application. Five hours after application, 0.84 cm of rainfall was recorded at the field site, and for three days after application the weather was mostly overcast. Glufosinate efficacy can decrease under low light intensity (Petersen and Hurle, 2001) and rainfall within 5 or 6 hours after treatment has been shown to decrease glufosinate efficacy (Langelüddeke et al., 1988). The reduction in kochia control observed with glyphosate was not attributed to weather because the rainfast period for the glyphosate formulation used was only 30 mins.

One of the best ways to minimize further evolution of GR kochia is to reduce the number of individuals that are exposed to glyphosate in-crop. The combination of PRE + POST herbicides can be an effective strategy for resistance management. In this study, the majority of kochia individuals were controlled by pendimethalin (2014-94.7%, 2015-87.9%), leaving a relatively small number of plants exposed to glyphosate. The probability that a single kochia plant will survive both a PRE herbicide plus a POST glyphosate application is much lower than a plant surviving a POST application of glyphosate. The probability of developing multiple resistance (eg glyphosate and glufosinate or glyphosate and pendimethalin) is the product of the two individual probabilities for resistance development, which is much less likely to occur (Mithila and Godar, 2013). Modeling studies have shown that herbicide mixtures can delay resistance longer than rotations if both herbicides are effective in controlling a targeted weed species (Diggle et al., 2003; Lagator et al., 2013; Powles et al., 1997).

Changes in the Frequency of Glyphosate-resistant Kochia

The targeted ratio for the seeded kochia in this experiment was 10% GR to 90% glyphosate-susceptible; however, additional screening of seed lots prepared for field plantings showed that 6.6% ± 1.2 (mean ± SE) and 6.2% ± 1.3 of the kochia seeded survived a glyphosate application in the greenhouse for 2014 and 2015, respectively (Table 2.9). This initial ratio of R/S kochia was used to study the impact weed management on the frequency of glyphosate resistance.

From the weedy-check plots, the kochia that grew above the canola canopy, and remained below the canola canopy was harvested separately to screen in the greenhouse for glyphosate resistance. We hypothesized that there could be a fitness penalty associated with glyphosate resistance and that the most competitive kochia (above the canola canopy) was less likely to be GR. The percent GR kochia for above canopy kochia in 2014 was 1.2 ± 0.2 and 1.8 ± 1.1 for RoundUp Ready and Liberty Link varieties, respectively (Table 2.10). Screening of above canopy kochia from both varieties showed that the percent of GR kochia in 2015 was 14.2 ± 4.1 and 23.3± 5.6 for RoundUp Ready and Liberty Link varieties, respectively (Table 2.10). For 2014, the GR frequencies were less than the ratio that was planted, but frequencies were higher than the ratio planted in 2015. Reduced kochia densities in 2015 compared to 2014 resulted in less intraspecific competition with kochia, which may have allowed for a greater proportion of GR kochia to grow above the canola canopy and cross with other kochia increasing the frequency of GR progeny in 2015.

In 2014, kochia that remained below the canola canopy in weedy-check plots had a resistance frequency of 24.0% ± 1.8 and 33.7% ± 4.3 for RoundUp Ready and Liberty Link plots, respectively (Table 2.10). In 2015, the percent of GR for below canopy kochia was 11.3% ± 4.1 and 38.9% ± 7.9 for RoundUp Ready and Liberty Link plots, respectively (Table 2.10). If greater amount of GR grew above the canola canopy in RoundUp Ready plots in 2015, this could explain the reduction in GR kochia from below the canola canopy. These GR frequencies from below canopy kochia were greater than the ratio that was planted for both years.

Although the percentage of GR kochia was much lower for above canopy kochia compared to below canopy kochia, seed production (using biomass as a proxy) was much greater for plants that grew above the canola canopy (Table 2.7 & 2.8), which made the contributions of resistance progeny to the seed bank similar between below and above canopy kochia (eg higher resistance and less amounts of seed versus lower resistance and greater amounts of seed). Kochia density and biomass at harvest suggests that canola variety did not have a significant impact on kochia suppression in the absence of herbicides (Table 2.6 & 2.8). However, the GR frequency in below canopy kochia was consistently higher for Liberty Link compared to RoundUp Ready (Table 2.10) which could be influenced by phenotypic differences between the two varieties. The Liberty Link variety had a single stem plant architecture with an average canopy height of 135 cm, compared to RoundUp Ready which displayed multiple branches per plant and had a an average canopy height of 95 cm.

Differences in the resistance frequency based on canopy position from weedy check plots could have been influenced by the relative competitiveness between the two kochia biotypes used in this study (resistant and susceptible). It is important to point out that the two kochia accessions used in this study were not isogenic for glyphosate-resistance. The two biotypes had different genetic backgrounds which could have influenced the relative fitness in the absence of glyphosate applications. Since there were differences in genetics besides the resistance trait, we cannot conclude that differences in resistance frequency between canopy position was caused by a fitness penalty in the GR kochia biotype in the absence of glyphosate.

Seed collected from kochia that survived herbicide applications were screened to determine the frequency of GR progeny. At the beginning of the study we hypothesized that kochia progeny from individuals that survived glyphosate treatment would have a higher percentage of GR progeny compared to individuals that survived glufosinate, as glyphosate would preferentially select for kochia survivors that possess glyphosate-resistance. Screening of progeny from kochia that survived herbicide treatments in 2014 showed that plants that survived a POST application of glyphosate had an average GR percentage of 33.9 ±13.9, while progeny from plants that survived glufosinate were only 1.85% ±0.37 GR (Table 2.11). In 2014, there were no survivors for pendimethalin (PRE) + glyphosate (POST) treatments; however, there were survivors from plots treated with pendimethalin (PRE) + glufosinate (POST). Seeds from these plants were less than 1% GR resistant (Table 2.11). In 2015, plants that survived glyphosate were 58.0% ± 10.3 GR, while progeny from plants that survived glufosinate were 21.5% ± 3.3 GR (Table 2.11). In 2015, plants that survived (PRE) + (POST) treatments were 30.6% ± 14.0 and 12.2% ± 3.7 GR for glyphosate and glufosinate, respectively (Table 2.11).

Kochia that survived treatments with glyphosate had higher frequencies of GR progeny were compared to kochia that survived glufosinate, although the difference were less when glufosinate control efficacy failed in 2015. Herbicides with different modes of action, like pendimethalin or glufosinate, do not preferentially select for kochia survivors with glyphosate-resistance, and it would be a random probability that survivors would contain the GR trait (eg percent of survivors * initial percentage of GR kochia).

The variability in kochia control from glufosinate between years showed the risk of utilizing glufosinate as an alternative control option for GR kochia. In 2014, excellent kochia control with glufosinate reduced the frequency of GR kochia compared to the ratio planted. However when glufosinate failed in 2015 due to weather conditions, the frequency of GR kochia from treatments with glufosinate was greater than the ratio that was planted. Compared to glyphosate, glufosinate had a reduced frequency of GR kochia, but was variable between years.

Results from this study highlight the importance of utilizing an alternative mode of action (glufosinate) for post emergent weed control which can reduce the frequency of GR kochia progeny if adequate weed control is achieved, and most importantly, the inclusion of a pre-emergent herbicide can greatly reduce the number of kochia individuals exposed to post-emergent herbicide applications, which is key to limit the evolution and selection for GR kochia.

As alternative post-emergent broadleaf herbicides are limited in canola (glufosinate), the best management practice recommendations would be to utilize an alternative mode of action (glufosinate) POST in rotation, or in a tank mix once HR varieties are available, to minimize the further evolution of GR kochia. Results from the two years show that relying solely on glufosinate for GR kochia control should be avoided as a single failure in a given year can greatly increase the frequency of GR kochia to populations that are developing GR (2-20% survival). Glyphosate only and glufosinate treatments with poor efficacy both increased the frequency of GR kochia which highlights the importance of including a pre-emergent herbicide with along with POST herbicides to minimize the evolution or further selection of GR kochia.

Regardless of whether GR kochia is present, growers should utilize a pre-emergent herbicide to reduce the probability for GR evolution or selection.

Implications for Glyphosate-resistant Kochia Management in Canola

The GR kochia biotype used in this study were selected for resistance in chemical fallowed fields in the absence of crop competition. Increased EPSPS copy number was confirmed as the resistance mechanism in the biotype used in this study, and average EPSPS copy numbers were between 6 and 8 copies. Suspected GR kochia seed obtained from Alberta were found to have EPSPS copy numbers that ranged from 7 to 25 copies. The sample with 25 copies of EPSPS was the highest copy number observed to date in kochia. Glyphosate-resistant kochia evolved in GR canola in Canada was exposed to multiple glyphosate-applications per year (burn down, potential for multiple in-crop applications) which could explain the increased EPSPS copy number. These GR kochia populations from Canada were also selected typically with crop competition, which was very different from the GR kochia biotype used in this study (absence of crop competition and typically exposed to a single glyphosate application per year). These differences between GR kochia biotypes suggests that results of this study could be exacerbated if a more competitive GR kochia line with higher levels of GR were used in a similar study. This would increase the importance of incorporating an alternative mode of action, especially a pre-emergent herbicide, on limiting the evolution or further selection of GR kochia.

Table 2.1 Canola densities for 2014 before (0 DAT) and after (21 DAT) POST herbicide applications. Different letters indicate significant differences among treatments across timing (P<0.05).

	0 DAT		21 DAT	
Treatment	Avg	SE	Avg	SE
Weedy Check	56.6 A	1.8	44.6 C	1.4
Non-Weedy Check	55.1 AB	2.2	43.9 C	2.3
POST	51.2 B	2.4	45.3 C	2.4
PRE + POST	52.7 AB	2.8	32.6 D	1.7

Table 2.2 Liberty Link and RoundUp Ready canola densities for 2015. Different letters indicate significant differences among treatments across canola variety ($P<0.05$).

	Liberty Link		RoundUp Ready	
Treatment	Avg.	SE	Avg.	SE
Weedy Check	44.9 B	3.8	33.7 BC	2.6
Non-Weedy Check	55.9 A	3.2	34.6 BC	2.7
POST	46.2 B	3.9	31.2 C	3.1
PRE + POST	29.6 CD	3.1	24 D	1.7

Table 2.3 Kochia densities for 2014 before (0 DAT) and after (21 DAT) POST herbicide applications. Different letters indicate significant differences among treatments across timing (P<0.05).

	0 DAT		21 DAT	
Treatment	Avg.	SE	Avg.	SE
Weedy Check	59.1 A	4.0	34.9 C	3.4
Non-Weedy Check	0 D	0.0	0.6 D	0.6
POST	46.5 B	2.3	0.4 D	0.3
PRE + POST	3.1 D	0.4	0.1 D	0.1

Table 2.4 Kochia densities for 2015 before (0 DAT), and after (21 DAT) post-emergent herbicide applications. Upper case letters indicate differences among treatments across initial plant densities (0 DAT), and lowercase letters indicate differences among treatments across canola variety for plant densities 21 DAT (P<0.05).

			21 DAT			
	0 DAT		RoundUp Ready		Liberty Link	
Treatment	Avg.	SE	Avg.	SE	Avg.	SE
Weedy Check	16.8 A	1.6	16.4 a	1.4	13.6 a	1.4
Non-Weedy Check	0.1 B	0.1	0.0 c	0.0	0.0 c	0.0
POST	15.6 A	1.6	6.3 b	1.0	14.1 a	2.7
PRE + POST	2.2 B	0.3	0.6 c	0.3	1.3 c	0.3

Table 2.5 Kochia densities (plant m^{-2}) at the time of harvest for 2014. Different letters indicate differences among treatments across canola variety and canopy level (P<0.05).

	Above		Below	
Treatment	Avg	SE	Avg	SE
Weedy Check	9.4 B	0.8	18.7 A	1.1
Non-Weedy Check	0.0 C	0.0	0.0 C	0.0
Post	0.0 C	0.0	0.6 C	0.3
Pre + Post	0.0 C	0.0	0.0 C	0.0

Table 2.6 Kochia densities (plants m^{-2}) at the time of harvest in 2015. Different letters indicate differences among treatments across canola variety and canopy level (P<0.05).

	Above				Below			
	RoundUp Ready		Liberty Link		RoundUp Ready		Liberty Link	
Treatment	Avg.	SE	Avg.	SE	Avg.	SE	Avg.	SE
Weedy Check	6.3 C	0.3	5.8 CD	0.2	6.7 BC	0.8	7.9 B	1.1
Non-Weedy Check	0.0 F	0.0	0.0 F	0.0	0.0 F	0.0	0.0 F	0.0
POST	0.6 F	0.1	2.4 E	0.2	4.9 D	0.2	10.5 A	0.4
PRE + POST	0.1 F	0.0	0.6 F	0.1	0.9 F	0.1	1.0 F	0.1

Table 2.7 Kochia biomass (g m^{-1}) at the time of harvest in 2014 for above and below canopy kochia. Different letters indicate significant differences among treatments across canopy level (P<0.05).

	Above		Below	
Treatment	Avg.	SE	Avg.	SE
Weedy Check	403.6 A	41.3	46.9 B	8.0
Non-Weedy Check	0.0 C	0.0	0.0 C	0.0
POST	8.4 BC	3.6	4.0 C	2.4
PRE + POST	0.0 C	0.0	0.9 C	0.6

Table 2.8 Kochia biomass (g m^{-1}) at the time of harvest in 2014 for above and below canopy kochia. Different letters indicate significant differences among treatments across canopy level and canola variety (P<0.05).

	Above				Below			
	RoundUp Ready		Liberty Link		RoundUp Ready		Liberty Link	
Treatment	Avg.	SE	Avg.	SE	Avg.	SE	Avg.	SE
Weedy Check	328.9 B	31.3	403.7 A	17.4	23.3 DEF	2.9	65.4 D	6.0
Non-Weedy Check	0.0 F	0.0	0.0 F	0.0	0.0 F	0.0	0.0 F	0.0
POST	44.0 D	3.7	170.9 C	19.0	7.9 EF	1.4	47.0 D	4.1
PRE + POST	4.9 EF	2.1	37.4 DE	7.9	0.4 F	0.4	7.8 EF	2.4

Table 2.9 Percent of glyphosate-resistant kochia seed planted into plots for 2014 and 2015 field studies (Targeted ratio of 10% GR kochia seed).

Year	Avg.	SE
2014	6.6	1.2
2015	6.2	1.3

Table 2.10 Percent of glyphosate-resistant kochia progeny from Weedy Check plots (no herbicide applied) for above and below canopy kochia from RoundUp Ready and Liberty Link plots.

	RoundUp Ready		Liberty Link	
Treatment	Avg.	SE	Avg.	SE
Above 2014	1.2	0.2	1.8	1.1
Above 2015	14.2	4.1	23.3	5.6
Below 2014	24.0	1.8	33.7	4.3
Below 2015	11.3	4.1	38.9	7.9

Table 2.11 Percent of glyphosate-resistant kochia progeny from kochia that survived a herbicide application (POST or PRE+POST) from RoundUp Ready and Liberty Link plots. In 2014 there were no survivors for PRE+POST treatment from RoundUp Ready blocks.

	2014				2015			
	RoundUp Ready		Liberty Link		RoundUp Ready		Liberty Link	
Treatment	Avg.	SE	Avg.	SE	Avg.	SE	Avg.	SE
POST	33.9	13.9	1.85	0.37	58.0	10.3	21.5	3.3
PRE + POST	na	na	0.56	0.56	30.6	14	12.2	3.7

References

Angadi S, Cutforth H, McConkey B, Gan Y, 2003 Yield adjustment by canola grown at different plant populations under semiarid conditions. Crop Sci 43:1358-1366

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

Beckie H, Weiss R, Leeson J, Olfert O, Ivany J, Blackshaw R, (2012) Range expansion of kochia (Kochia scoparia) in North America under a changing climate. Climate Change and the Canadian Agricultural Environment. Topics in Canadian Weed Sci 8:33-46

Beckie HJ, Blackshaw RE, Low R, Hall LM, Sauder CA, Martin S, Brandt RN, Shirriff SW, (2013) Glyphosate- and Acetolactate Synthase Inhibitor–Resistant Kochia (Kochia scoparia) in Western Canada. Weed Sci 61:310-318

Beckie HJ, Gulden RH, Shaikh N, Johnson EN, Willenborg CJ, Brenzil CA, Shirriff SW, Lozinski C, Ford G, (2014a) Glyphosate-resistant kochia (Kochia scoparia L. Schrad.) in Saskatchewan and Manitoba. Canadian J of Plant Sci 95:345-349

Beckie HJ, Johnson EN, Leeson JY, Shirriff SW, Kapiniak A, (2014b) Selection and evolution of acetyl-CoA carboxylase (ACC)-inhibitor resistance in wild oat (Avena fatua L.) in a long-term alternative cropping systems study. Canadian J of Plant Sci 94:727-731

Beckie HJ, Reboud X, (2009) Selecting for weed resistance: herbicide rotation and mixture. Weed Technol 23:363-370

Beckie HJ, Warwick SI, Sauder CA, Lozinski C, Shirriff S, (2011) Occurrence and Molecular Characterization of Acetolactate Synthase (ALS) Inhibitor–Resistant Kochia (Kochia scoparia) in Western Canada. Weed Technol 25:170-175

Dawit M, Maxwell BD, Peter KF, Dyer WE, (1994) Kochia (Kochia scoparia) Pollen Dispersion, Viability and Germination. Weed Sci 42:548-552

Devine M, Buth J, (2001) Advantages of genetically modified canola: a Canadian perspective. Pages 367-372 *in* the Proceedings of a BCPC Conference. Brighton, UK, British Crop Protection Council

Diggle A, Neve P, Smith F, (2003) Herbicides used in combination can reduce the probability of herbicide resistance in finite weed populations. Weed Res 43:371-382

Forcella F, (1985) Final distribution is related to rate of spread in alien weeds. Weed Res 25:181-191

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

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

Gianessi LP, (2005) Economic and herbicide use impacts of glyphosate-resistant crops. Pest Management Sci 61:241-245

Godar AS, Stahlman PW, Jugulam M, Dille JA, (2015) Glyphosate-resistant Kochia in Kansas: EPSPS Gene Copy Number in Relation to Resistance Levels. Weed Sci 63: 587-595

Jugulam M, Niehues K, Godar AS, Koo DH, Danilova T, Friebe B, Sehgal S, Varanasi VK, Wiersma A, Westra P, Stahlman PW, Gill BS, (2014) Tandem amplification of a chromosomal segment harboring EPSPS locus confers glyphosate resistance in Kochia scoparia. Plant Physiology 166:1200-1207

Kumar V, Jha P, Giacomini D, Westra EP, Westra P, (2015) Molecular Basis of Evolved Resistance to Glyphosate and Acetolactate Synthase-Inhibitor Herbicides in Kochia (Kochia scoparia) Accessions from Montana. Weed Sci 64: 758-769

Lagator M, Vogwill T, Mead A, Colegrave N, Neve P, (2013) Herbicide mixtures at high doses slow the evolution of resistance in experimentally evolving populations of Chlamydomonas reinhardtii. New Phytologist 198:938-945

Langelüddeke P, Baedelt H, Bieringer H, (1988) Trials on the influence of air humidity and rainfall on the efficacy of glufosinate-ammonium. Pages 227-232 in the Proceedings of the EWRS Symposium: Factors Influencing Herbicidal Activity and Selectivity

Leeson JY, (2005) Prairie weed surveys of cereal, oilseed and pulse crops from the 1970s to the 2000s

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

Mithila J, Godar A, (2013) Understanding Genetics of Herbicide Resistance in Weeds: Implications for Weed Management. Adv Crop Sci Tech 1:115

Murray BG, Morrison IN, Brûlé-Babel AL, (1995) Inheritance of acetyl-CoA carboxylase inhibitor resistance in wild oat (Avena fatua). Weed Sci 43:233-238

Petersen J, Hurle K, (2001) Influence of climatic conditions and plant physiology on glufosinate-ammonium efficacy. Weed Res 41:31-39

Powles S, Preston C, Bryan I, Jutsum A, (1997) Herbicide resistance: impact and management. Adv. Agron. 58:57-93

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

Shaner DL, (1999) Crop modified to resist amino acid biosynthesis inhibitors. In: Singh BK (ed). Plant Amino Acides: Biochemistry and Biotechnology, Marcel Dekker: New York 465-485

Tranel PJ, Wright TR, (2009) Resistance of weeds to ALS-inhibiting herbicides: what have we learned? Weed Sci 50:700-712

Trucco F, Zheng D, Woodyard AJ, Walter JR, Tatum TC, Lane Rayburn A, Tranel PJ, (2007) Nonhybrid progeny from crosses of dioecious amaranths: implications for gene-flow research. Weed Sci 55:119-122

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

Appendix 1

Canola Tolerance to Pendimethalin

Introduction

Field studies which evaluated the impact of a pre-emergent herbicide on selecting for glyphosate-resistant kochia progeny were conducted in 2014 and 2015. For these field studies, kochia seed was planted on the surface of plots and irrigation was applied to incorporated kochia seed in the top 1 cm of soil for germination. Physical incorporation of pre-emergent herbicides (PPI) labeled in canola would have buried kochia seeds below a depth of typical germination, and collapsed canola furrows, burying canola seed below the target planting depth.

Therefore, prior to establishing field studies, canola was screened with pendimethalin (Prowl $H_2O^{®}$, BASF, RTP, NC) to determine the level of crop tolerance. Pendimethalin was evaluated for use in canola-kochia field studies based on the fact that in can be applied pre-emergent (PRE) and incorporated with irrigation, compared to ethafluralin or trifluralin which are labeled in canola but require pre-plant incorporation (PPI) to a depth of 5 to 8 cm. For this initial greenhouse screening, soil from the site where field studies were planned to be established was brought back to Colorado State University where several rates of pendimethalin were applied and incorporated with 1 cm of simulated rainfall to evaluate canola tolerance. Canola treated with 2240 g ai ha^{-1} (2x rate used in field studies) showed similar levels of tolerance compared to ethafluralin (data not shown), and based off of greenhouse screening, pendimethalin was selected as the pre-emergent herbicide for field studies. When pendimethalin was applied at 1120 g ai ha^{-1} in field studies, canola injury in the form of plant stunting and stand reduction was observed for both RoundUp Ready and Liberty Link canola varieties which resulted in reduction in canola densities and yields. Therefore, in the fall of 2014, and spring of 2015, canola emergence studies were conducted to evaluate the impact of pendimethalin rate on canola injury in the field to evaluate the potential for pendimethalin use as a pre-emergent herbicide in canola. The objectives of this study were to a) evaluate the impact of pendimethalin rate on canola injury compared to an untreated check and industry standard (ethafluralin), and b) evaluate the effect of planting depth on canola tolerance to pendimethalin.

Materials and Methods

For canola emergence studies in both years, two separate studies were conducted at 1.9 and 3.2 cm planting depths in order to evaluate the impact of planting depth on canola injury from pendimethalin. Since similar injury from pendimethalin was observed with Liberty Link and RoundUp Ready canola varieties in field studies, we used RoundUp Ready (DKL 30-42) as the canola variety in emergence studies for both years. Within the studies at different planting depths, treatments consisted of an untreated check, pendimethalin at 560, 840, and 1120 g ai ha^{-1}, and sonalan at 840 g ai ha^{-1}. Treatments were set up in a RCB design with three replicates and individual plot sizes were 3 by 9 meters.

Both stand reduction and plant stunting were evaluated by taking normalized difference vegetation index (NDVI) readings at several time points during canola emergence from each plot. NDVI data was collected using a GreenSeeker (Trimble GreenSeeker Crop Sensing System, Trimble, Boulder, CO) connected to a Timble Nomad (Trimble, Boulder, CO) handheld computer. For each time point, NDVI was recorded at 5 Hz for two passes per plot. All NDVI readings were averaged to create a mean NDVI reading for each plot. Average NDVI values and standard errors were calculated from the three replicates for each treatment.

Statistical Analysis

For pendimethalin emergence studies, Fisher's Protected LSD test was conducted across treatments for each planting depth and DAT, and a Paired t-test was conducted on NDVI values from both planting depths across both years and all DAT.

Results

2014-1.9 cm Planting Depth

For the 2014 fall canola emergence study, NDVI data was collected 32 and 46 DAT for both planting depths. For NDVI values 32 DAT at the 1.9 cm planting depth showed that all rates of pendimethalin had reduced NDVI values compared to the untreated check and sonalan treatments in a dose dependent manner (Table A1.1). However, by 46 DAT, average NDVI for pendimethalin at 560 g ai/ha was statistically similar to the untreated check and sonalan treatments. Pendimethalin at 840 and 1120 g ai ha^{-1} were statistically similar, and had the lowest NDVI values. 46 DAT there was still a dose dependent response on NDVI values with pendimethalin treatments. Pendimethalin at 560 g ai ha^{-1} had NDVI values that were statistically similar to the untreated check and sonalan treatments. Pendimethalin at 840 and 1120 g ai ha^{-1} were statistically similar with the lowest NDVI values.

2014-3.2 cm Planting Depth

For the 3.2 cm planting depth 32 DAT, NDVI data showed that the untreated check, sonalan, and pendimethalin at 840 g ai ha^{-1} were statistically similar with the highest NDVI values. Pendimethalin at 560 g ai ha^{-1} was statistically similar to sonalan and pendimethalin at 840 g ai ha^{-1}. Pendimethalin at 560 g ai ha^{-1} was also statistically similar to pendimethalin at 1120 g ai ha^{-1} which had the lowest average NDVI value (Table A1.1). 46 DAT NDVI data showed that all treatments besides pendimethalin at 1120 g ai ha^{-1} were statistically similar. All three rates of pendimethalin were statistically similar, however there was a dose dependent effect on average NDVI for pendimethalin applications, where NDVI values decreased as pendimethalin rate increased (Table A1.1).

2015-1.9 cm Planting Depth

For the 2015 spring canola emergence study, NDVI data was collected 20, 29, and 48 DAT for both planting depths. For the 1.9 cm planting depth, NDVI data was statistically similar across all treatments 20 DAT (Table A1.2), which was not observed for the deeper planting depth of 3.2 cm. For 29 DAT NDVI values, the untreated check, sonalan, and pendimethalin at 560 g ai ha^{-1} were statistically similar, while pendimethalin at 840 and 1120 g ai ha^{-1} were statistically similar with the lowest NDVI (Table A1.2). By 49 DAT, pendimethalin at 560 g ai ha^{-1} had the highest NDVI value and was statistically similar to sonalan. Sonalan was also statistically similar to the untreated check and pendimethalin at 1120 g ai ha^{-1}, which were statistically similar to pendimethalin at 840 g ai ha^{-1} which had the lowest NDVI value.

2015-3.2 cm Planting Depth

NDVI 20 DAT showed that the untreated check had the highest NDVI valued followed by pendimethalin at 560 g ai ha^{-1} and Sonalan, while pendimethalin at 840 and 1120 g ai ha^{-1} had the lowest NDVI (Table A1.2). 29 DAT, the untreated check and pendimethalin at 560 g ai ha^{-1} had the highest NDVI followed by sonalan, then pendimethalin at 840 g ai ha^{-1} and last pendimethalin at 1120 g ai ha^{-1} had the lowest NDVI (Table A1.2). By 48 DAT, the untreated check and pendimethalin at 560 g ai ha^{-1} had statistically similar NDVI. Pendimethalin at 560 g ai ha^{-1} was statistically similar to pendimethalin at 840 g ai ha^{-1} which was statistically similar to sonalan, which was statistically similar to pendimethalin at 1120 g ai ha^{-1} which had the lowest

NDVI (Table A1.2). Injury was most pronounced at 29 DAT, where a dose response effect with pendimethalin rate on NDVI was most apparent. By 48 DAT, differences in NDVI were less pronounced, but the relative order of NDVI was the same as for 29 DAT. For the 1.9 cm planting depth, dose response effects with pendimethalin were also most apparent 29 DAT, but relative order of NDVI values changed by 48 DAT (Table A1.2).

Conclusion

In general, pendimethalin at 1120 g ai ha^{-1} had the lowest NDVI data and it wasn't until pendimethalin rates were reduced to 840 and 560 g ai ha^{-1} that NDVI values were similar to sonalan and untreated check NDVI values, regardless of planting depth. For a given treatment, there was a significant effect of planting depth on NDVI values for pendimethalin treatments, with the 1.9 cm planting depth typically having higher average NDVI values compared to 3.2 cm planting depth. Paired t-test showed that NDVI mean values were not equal for 1.9 vs 3.2 cm planting depth across both years and DAT (p value =0.018). Averaged across both years at all DAT, the mean NDVI values were 0.575 and 0.552 for 1.9 and 3.2 cm planting depth, respectively. We can hypothesize that a deeper planting depth utilized greater amounts of seed resources for germination that had an influence on canola injury once it reached the zone of soil where pendimethalin was present.

Kochia control with pendimethalin at 1120 g ai ha^{-1} in the field study was around 91%, and reducing rates to 840 or 560 g ai ha^{-1} would significantly reduce kochia control efficacy. Data from canola emergence studies suggests that there would be acceptable canola tolerance at lower rates of pendimethalin, but that kochia control would drop off with rate reductions. Pendimethalin would have a better fit in direct seed or no-till operations applied as a PRE, but at the sacrifice of crop safety compared to ethafluralin or trifluralin. Based on control efficacies and canola injury, pendimethalin would not be a suitable alternative pre-emergent option in canola as rates required for adequate kochia control would result in canola injury.

Table A1.1 NDVI data from fall 2014 canola emergence study. NDVI means ± standard error of means are displayed for both planting depths at 32 and 46 DAT. Herbicide rates are in g ai ha^{-1}. Different letters within columns indicate differences in mean NDVI values among treatments within a planting depth and DAT (P<0.05).

	32 DAT		46 DAT	
Treatment	1.9 cm depth	3.2 cm depth	1.9 cm depth	3.2 cm depth
Untreated	0.5078 A ± 0.0129	0.5329 A ± 0.0298	0.6968 A ± 0.014	0.6946 A ± .0204
Pendimethalin 560	0.4446 B ± 0.0128	0.4585 BC ± 0.033	0.6587 A ± 0.0203	0.6189 AB ± 0.0358
Pendimethalin 840	0.4092 BC ± 0.0231	0.4808 AB ± 0.0183	0.6027 B ± 0.0204	0.5975 AB ± 0.0233
Pendimethalin 1120	0.3709 C ± 0.0328	0.3970 C ± 0.0112	0.5650 B ± 0.0227	0.5586 B ± 0.0223
Sonalan 840	0.5059 A ± 0.0462	0.5114 AB ± 0.0199	0.6931 A ± 0.0129	0.6716 A ± 0.0195

Table A1.2 NDVI data from spring 2015 canola emergence study. NDVI means ± standard error of means are displayed for both planting depths at 20, 29, and 48 DAT. Herbicide rates are in g ai ha^{-1}. Different letters within columns indicate differences in mean NDVI values among treatments within a planting depth and DAT (P<0.05).

	20 DAT		29 DAT		48 DAT	
Treatment	1.9 cm depth	3.2 cm depth	1.9 cm depth	3.2 cm depth	1.9 cm depth	3.2 cm depth
Untreated	0.1656 A ± 0.0071	0.1793 A ± 0.0078	0.5534 A ± 0.0265	0.5683 A ± 0.0201	0.6716 BC ± 0.0117	0.6825 A ± 0.0107
Prowl 560	0.1573 A ± 0.0067	0.1589 B ± 0.0075	0.5518 A ± 0.0264	0.5512 A ± 0.0211	0.7255 A ± 0.007	0.6774 AB ± 0.0092
Prowl 840	0.1441 A ± 0.0050	0.1342 C ± 0.0035	0.4488 B ± 0.0262	0.3859 C ± 0.0211	0.6698 C ± 0.0157	0.6454 BC ± 0.0126
Prowl 1120	0.1447 A ± 0.0067	0.1250 C ± 0.0031	0.4347 B ± 0.0241	0.3068 D ± 0.0195	0.6872 BC ± 0.0117	0.6076 D ± 0.0145
Sonalan 840	0.1594 A ± 0.0078	0.1581 B ± 0.0070	0.5683 A ± 0.0220	0.4729 B ± 0.0281	0.7004 AB ± 0.0089	0.6202 CD ± 0.0132

Appendix 2

Alternative Control Options for Glyphosate-resistant Kochia

Introduction

Since first reported in Kansas in 2007 (Heap, 2015), glyphosate-resistant (GR) kochia has increased in both frequency and distribution throughout the central great plains from Texas to the Prairie Provinces in Canada. Glyphosate-resistant weeds such as kochia threaten the long term sustainability of glyphosate for weed management in no-till crop production. Glyphosate has been called the most important herbicide globally (Powles and Preston, 2006), and the adoption of GR crops beginning around 1995 rapidly increased overall use of glyphosate for weed control. Glyphosate applications can include burndown or pre-plant applications, in crop applications (can be applied multiple times within crop), pre-harvest (typically restricted to cereal crops), and for weed control in fallow settings. The potential for multiple glyphosate applications per year, combined with widespread use on large geographic areas has resulted in tremendous selection pressures for glyphosate-resistance evolution in kochia.

Currently there are 16 monocot and 16 dicot weed species which have evolved resistance to glyphosate worldwide. Known glyphosate resistance mechanisms exceed those reported for any other herbicide and include target-site mutations, target-site gene duplications, active vacuole sequestration, limited cellular uptake, and rapid necrosis response (Sammons and Gaines, 2013). Glyphosate resistance in kochia is due to gene amplification, where resistant plants contain additional functional copies of the gene encoding 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) (Wiersma et al., 2015b).

Glyphosate-resistant kochia has become more common and can reduce weed control efficacy for glyphosate used in GR cropping systems. As glyphosate becomes less effective on GR weed species, alternative chemical options are needed to control these populations and preserve the utility of glyphosate which provides control of a wide spectrum of weed species. Glyphosate-resistant species can have a significant impact on crop yields in systems that solely rely on glyphosate as was seen with GR palmer amaranth in GR soybeans (Norsworthy et al., 2008; Tracy and Lawrence, 1994). The evolution of GR weeds necessitates the incorporation of alternative chemistries for control of GR weeds to maintain effective weed control programs and preserve crop yields. Information on how alternative modes of action perform on known GR kochia populations is needed to maintain effective weed control programs in the presence of GR weeds.

Studies were conducted with field and greenhouse trials to evaluate the control of GR kochia with alternative modes of actions in corn, wheat, soybeans, canola, and sugarbeet. The objective of this study was to evaluate the weed control efficacy of alternative herbicides in these five crops in order to provide management recommendations for the control of GR kochia populations.

Materials and Methods

Field studies were conducted in 2014 in grower fields near Yuma, Colorado (40.08936°, -102.70976°), to evaluate alternative modes of action for GR kochia control. Field protocols were established where GR kochia was suspected to exist based on reduced efficacies from glyphosate applications. Studies were established on the borders of crop fields where there were essentially monocultures of kochia. Screening was conducted in the absence of crops to evaluate kochia control efficacies. Herbicides for a given crop were selected from products that were labeled in respective crops, and generally herbicides with poor kochia control efficacy were excluded from evaluations.

Field studies were conducted to evaluate alternative modes of action for both corn and wheat, while for the remaining crops (canola, soybeans, and sugarbeet), alternative herbicides were evaluated with greenhouse screenings using a known GR kochia population collected from Eastern Colorado.

For post-emergent field studies with corn, glyphosate (RoundUp WeatherMax) was our baseline treatment for comparisons of alternative herbicide performance, and also served as a check to confirm that there was GR kochia present in the fields where studies were conducted. For wheat alternative evaluations, glyphosate (RoundUp WeatherMax) was included in all treatments to evaluate control of alternative herbicides tank mixed with glyphosate compared to glyphosate applied alone. Field studies were set up in a RCB design with three replications, and individual plots sizes were three by nine meters wide. Plots were sprayed with a CO_2 pressurized backpack sprayer calibrated to deliver 20 gallons acre^{-1}. Herbicide treatments were applied to kochia that was approximately 4 to 6 inches tall. Visual control evaluations were conducted around between 15-17 days after treatment (DAT). In addition to corn and wheat field trials, in the fall of 2014, a study was conducted in Yuma Colorado to evaluate the efficacy of fall-applied pre-emergent herbicides on kochia control in the spring of 2015. The soil type at this field site was a Kuma-Keith silt loam, fine-silty, mixed superactive, mesic Aridic Argiustoll. Fall pre-emergent treatments were sprayed on October 16, 2014, and kochia control was rated on May 21, 2015.

For studies conducted in the greenhouse, a known GR kochia population collected from Eastern Colorado herbicide-resistant kochia surveys was used to evaluate alternative herbicide control efficacy. The GR_{50} for the resistant kochia population was 2.03 lb ae acre^{-1} (Figure A2.1), and previous greenhouse screening showed that ~98% of individuals survived the field rate (0.75 lb ae acre^{-1}) of glyphosate (RoundUp WeatherMax). The kochia line utilized for greenhouse screening also was resistant to dicamba, and previous greenhouse screening with dicamba (Clarity) at 8 fl oz acre^{-1} showed that there was around 50% control based on visual evaluations compared to an untreated check.

For greenhouse screening, herbicides were applied with a moving overhead single nozzle sprayer (DeVries Manufacturing Hollandale, MN) calibrated to deliver 20 gal acre^{-1}. Evaluation of pre-emergent herbicides was conducted with field soil (Fort Collins clay (Fine-loamy, mixed, superactive, mesic Aridic Haplustalfs) with 32% sand 25% silt and 43% clay, with a CEC of 36.6, 2.35 % organic matter, and pH of 7.6). Pre-emergent herbicides were applied to inserts planted with kochia in field soil that was air dried and sieved through a 0.08 inch screen. Herbicides were applied to dry soil surfaces, and after application 0.5 inches of simulated rainfall was applied using the same overhead single nozzle sprayer in order to activate pre-emergent herbicides. After herbicide application, and until treatments were evaluated 21 DAT, field soil inserts were misted twice a day to maintain soil moisture levels. For post emergent herbicide evaluations, kochia was grown with a fine grade potting soil (Fafard #2-SV) (American Clay Works, Denver, CO). Kochia was grown until it was around 8 cm tall before post-emergent herbicides were applied. After herbicide treatment, plants were watered daily to field capacity, and visual evaluations were conducted 21 DAT. Greenhouse studies were conducted in a RCB design with three replicates, an untreated check and a glyphosate treatment were included as checks to compare alternative herbicide treatments.

For commercial relevance, weed control efficacies typically need to be greater than 95%. The objectives of this study were to find herbicides that fit this criteria with known GR kochia biotypes. Direct comparisons between treatments were not necessarily desired as long as control for that herbicide was greater than 95%. Data is presented as the average percent visual control with error bars representing the standard error of the mean.

Results

For relative comparison of alternative herbicides screened in the greenhouse, greenhouse screening showed that glyphosate had an average control rating of 22% and dicamba had an average control rating of 53% compared to an untreated check. For field studies evaluating alternative herbicides in wheat and corn, visual control ratings for glyphosate for a given field site are listed in the respective sections.

Greenhouse screening

Canola

Pre-emergent treatments for canola consisted of Sonalan and Prowl H_2O which had control ratings of 62 and 88%, respectively (Table A2.1). For post-emergent herbicides tested, Beyond, Stinger, and Aim all had less than 50% control at 11, 15, and 50 % control, respectively. The two best post-emergent treatments were Liberty and Buctril at 75 and 84% control, respectively. Even in the greenhouse under ideal conditions, kochia control was less than 80% for Liberty which highlights the problem of GR kochia control in Liberty Link canola where effective (>90%) alternative post-emergent herbicides are limited.

Soybean

For soybean alternative herbicide evaluations, seven pre-emergent herbicides and four post-emergent herbicide were included in greenhouse screenings (Table A2.2). Pursuit and Warrant had control efficacies of 8 and 32 %, respectively, control efficacy for Pursuit was low because the GR kochia used was also resistant to ALS-inhibiting herbicides. The remaining pre-emergent herbicides Sencor, Spartan, Authority MTZ, Authority First, and Boundary all provided complete kochia control. For post-emergent herbicides, Pursuit, and Raptor (ALS-inhibitors) had control ratings of 4 and 8%, respectively. Lastly, Aim had a control rating of 52% which was the highest control rating out of post-emergent herbicides. Out of the herbicides tested for GR kochia control in soybeans, multiple pre-emergent herbicides had high control efficacies, while effective post-emergent options were limited in their control of GR kochia populations.

Sugarbeet

For alternative sugarbeet herbicide evaluations, two pre-emergent herbicides and six post-emergent herbicides were included in greenhouse screening (Table A2.3). For pre-emergent herbicides, Ro-Neet and Nortron (PRE) had control efficacies of 8 and 98%, respectively. All post-emergent herbicides evaluated had control efficacies less than 30%. Nortron (POST), Ro-Neet, Stinger, Eptam, UpBeet, and Progress had control ratings of 12, 13, 15, 17, 17, and 27%, respectively. Screening of alternative herbicides showed the limited availability of alternative control options for GR kochia. Out of all products tested, only Nortron applied pre-emergent had acceptable levels of GR kochia control, which results in over-reliance on GR sugarbeets systems without many effective alternatives once GR kochia develops in these systems.

Alternative

Additional alternative herbicides were screened in the greenhouse after specific crop screening had been conducted and were grouped together (Table A2.4). Dicamba (Clarity) applied PRE at 8 fl oz/acre had 90% control compared to 53% control when the same rate was applied POST. Linex, Acuron, Sulfentrazone + Pyroxasulfone, and Authority Assist applied PRE all had control efficacies greater than 90% with 91, 99, 99, and 99% control, respectively. For post-emergent alternatives, Armezon, Edict, Autumn Super, and Solstice all had less than 50% control at 5, 12, 15, 20, and 25% control, respectively. Clarity had 53 % control, and the remaining post-emergent herbicides had greater than 75% control with Diflex, and Cobra at 78, 82 % control, respectively.

Field screening

Corn

For field screening treatments were compared to untreated check plots, and RoundUp WeatherMax had an average control rating of 82% which indicated that there was some level of glyphosate-susceptibility presence in the field where screening occurred (Table A2.5). ET, Bronate Advanced, and Liberty all had less than 50% control at 7, 28, and 28 % control, respectively. Impact, Laudis, Capreno, Callisto, and RoundUp WeatherMax all had between 50 and 90% control at 58, 63, 63, 68, and 82% control respectively (Figure A2.6). Clarity, Starane Ultra, Engenia + RoundUp WeatherMax + 2,4-D, RoundUp WeatherMax + Starane Ultra, Clarity + Full Load (adjuvant), Clarity + RoundUp WeatherMax, and Corvus + Aatrex all had greater than 90% control at 91, 92, 93, 93, 94, 95, and 97% control, respectively. Aatrex control ratings suggest that the kochia population was not triazine resistant, and that atrazine is an effective alternative control option for GR kochia if populations are susceptible. Corn has many alternative control options with acceptable control efficacies for GR kochia.

Wheat Fallow

Kochia at the field site where wheat alternatives were tested had an average control rating of 28% for RoundUp WeatherMax (Table A2.6). Besides, RoundUp WeatherMax, only Liberty had a control rating less than 50% at 37% control. 2,4-D, Balance Pro, Aim, and Sharpen had a control rating between 50 and 80% at 52, 63, 63, and 80% control respectively. Huskie, Clarity, Starane Ultra, Clarity + 2,4-D, Atrazine, and Gramoxone all had greater than 80% control at 82, 85, 89, 89, 91, and 96% control, respectively (Figure A2.7). Atrazine control efficacy suggests that the kochia population was not triazine resistant.

Fall Pre-emergent Study

For crops, early season competition with kochia can cause yield penalties if not controlled during the critical period for weed control (Knezevic et al., 2009). Therefore a study was conducted with fall-applied pre-emergent herbicides to evaluate weed control efficacies the following spring. The concept of fall-applied pre-emergent herbicides is to limit weed emergence the following spring so that crops which can tolerate residual herbicide activity can be planted with minimal weed pressure early in the growing season. Over-winter soil temperatures can reduce the rate of herbicide soil degradation, and residual soil activity can provide weed control the following spring. Out of the 18 pre-emergent herbicides applied in the fall, seven herbicides had less than 50% control the following spring (Table A2.7). Sonar, Linex, Sharpen, Dual Magnum, Callisto, Dicamba (16 fl oz/acre), and Prowl H_2O had control ratings of 15, 22, 35, 42, 42, 50, and 54%, respectively. Dicamba (32 fl oz/acre), Balance Pro, Valor SX, Atrazine, Corvus, Fierce, and Anthem had control ratings between 50-80% at 57, 62, 72, 73, 76, 77, and 77 % control, respectively. Four pre-emergent herbicides had greater than 80 % control with Authority MTZ, Pyroxasulfone, Spartan, and Anthem ATZ at 81, 83, 96, and 98% control respectively. Compared to greenhouse pre-emergent herbicide screening, fall-applied pre-emergent herbicides had more variability in their control ratings (Table A2.7). Fall-applied pre-emergent herbicide applications can be used to reduce weed competition early that following spring, but the variability of degradation over the longer time frame could result in reduced weed control efficacy compared to an early spring-applied pre-emergent herbicide application

Discussion

Effective alternative herbicides for control of GR kochia that has evolved in different cropping systems are needed to preserve the utility of glyphosate for broad spectrum weed control. Evaluations of alternative herbicides for GR kochia control showed that in general pre-emergent herbicides were highly effective in controlling GR kochia populations, where post-emergent herbicides were more variable in their control of these biotypes. Weed size can have an impact on weed control efficacies, and targeting emerging GR kochia seedlings is an effective strategy to minimize further GR kochia evolution by removing resistant individuals before they become bigger in size, and subsequently harder to control. When alternative herbicide options are available, rotating modes of action, or tank-mixing alternative modes of action can reduce the frequency of GR kochia. Once GR kochia has evolved within cropping systems, the continued use of glyphosate alone will only exacerbate the problem as GR kochia can increase exponentially under high selection pressures, and overall weed control can be reduced based on survival of a single weed species that has developed resistance.

Based on the evaluation of alternative herbicides in these studies, it is apparent that in certain cropping systems there are more effective alternative herbicides available based on crop tolerance. In these evaluations, the relative order of available alternative herbicides from greatest to least was corn, wheat, soybeans, canola, and sugarbeets. For GR kochia management, these evaluations highlight the importance of using crop rotations as a part of integrated weed management for control of GR kochia. If a grower is planning on rotating to sugarbeets or canola where alternative options are limited, utilizing crops with more available alternative modes of action the year prior can help limit the return of GR kochia seed into the soil seed bank. The best way to achieve effective weed control in crops with limited alternative herbicides is to limit the number of GR kochia present at the start of the year. Knowing there are greater numbers of alternative modes of action in certain crops can be used to reduce GR kochia evolution over a multi-year crop rotation.

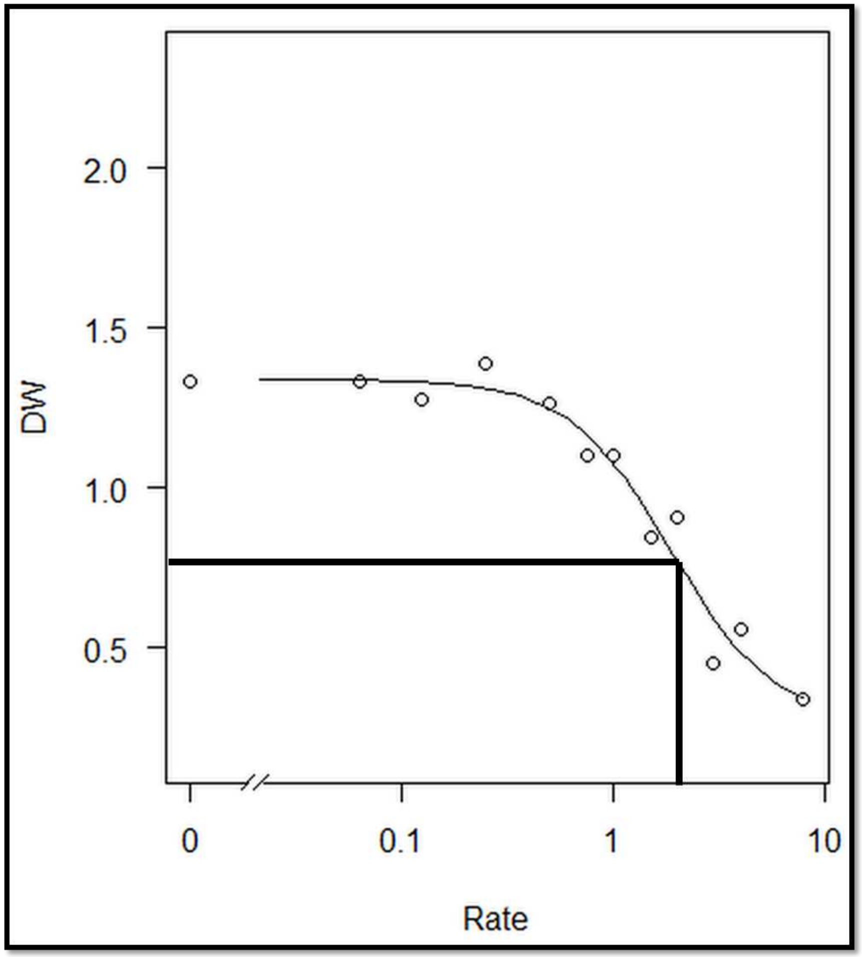

Figure A2.1 Dose response curve for GR kochia accession used in greenhouse screening of alternative herbicides. Y-axis is plant dry weight in grams, and the x-axis is glyphosate rate in lb ae/acre. Figure was made in R utilizing the DRC package. GR_{50} value is 2.03 lb ae $acre^{-1}$ with SE of 0.45 and is approximated on the graph with black lines.

Table A2.1 Visual control ratings for alternative herbicide control options for GR kochia in canola. Herbicide and respective adjuvant (if used) use rates.

										% Control	
Trt.	Field/G.H.	PRE/POST	Crop	Type	Treatment	Active Ingredient(s)	Form Type	Rate	Rate Unit	Avg.	SE
1	GH	PRE	Canola	HERB	Prowl H2O	Pendimethalin	SL	1	LB AI/A	88	6.5
2	GH	PRE	Canola	HERB	Sonalan	Ethafluralin	SL	2	PT/A	61.7	7.3
3	GH	POST	Canola	HERB	Stinger	clopyralid	SL	0.5	PT/A	15	2.9
4	GH	POST	Canola	HERB	Aim	Carfentrazone ethyl	SL	1	FL OZ/A	50	2.9
5	GH	POST	Canola	HERB	Beyond	Imazamox	SL	4	FL OZ/A	11	4.9
6	GH	POST	Canola	HERB	Liberty	Glufosinate	SL	22	FL OZ/A	75	2.9
6	GH	POST	Canola	ADJ	AMS		WG	17	LB AI/A		
7	GH	POST	Canola	HERB	Buctril	Bromoxynil	SL	1	PT/A	84.3	0.7

Table A2.2 Visual control ratings for alternative herbicide control options for GR kochia in soybeans. Herbicide and respective adjuvant (if used) use rates.

										% Control	
Trt.	Field/G.H	PRE/POST	Crop	Type	Treatment	Active Ingredient(s)	Form Type	Rate	Rate Unit	Avg.	SE
1	GH	PRE	Soybean	HERB	Sencor	metriuzin	WG	0.83	LB/A	99.0	0.0
2	GH	PRE	Soybean	HERB	Spartan	sulfentrazone	SL	10	FL OZ/A	99.0	0.0
3	GH	PRE	Soybean	HERB	Authority MTZ	sulfentrazone and metribuzin	DF	16	OZ WT/A	99.0	0.0
4	GH	PRE	Soybean	HERB	Authority First	sulfentrazone and cloransulam methyl	DF	6.45	OZ WT/A	99.0	0.0
5	GH	PRE	Soybean	HERB	Warrant	acetochlor	SL	1.7	QT/A	31.7	10.9
6	GH	PRE	Soybean	HERB	Boundary	s-metolachlor and metribuzin	SL	2.1	PT/A	99.0	0.0
7	GH	PRE	Soybean	HERB	Pursuit	imazethapyr	SL	4	FL OZ/A	8.3	6.0
8	GH	POST	Soybean	HERB	Pursuit	imazethapyr	SL	4	FL OZ/A	4.0	1.0
9	GH	POST	Soybean	HERB	Raptor	imazamox	SL	5	FL OZ/A	8.3	1.7
10	GH	POST	Soybean	HERB	Aim	Carfentrazone ethyl	SL	1.6	FL OZ/A	51.7	6.0

Table A2.3 Visual control ratings for alternative herbicide control options for GR kochia in sugarbeet. Graphs display means and standard error of means. Herbicide and respective adjuvant (if used) use rates are listed in Table 1.

										% Control	
Trt.	Field/G.H	PRE/POST	Crop	Type	Treatment	Active Ingredient(s)	Form Type	Rate	Rate Unit	Avg.	SE
1	GH	POST	Sugarbeet	HERB	Eptam	EPTC	SL	3	LB AI/A	17	6
2	GH	POST	Sugarbeet	HERB	Upbeet	Triflusulfuron-methyl	WG	0.5	OZ AI/A	17	7
2	GH	POST	Sugarbeet	ADJ	NIS		SL	0.25	% V/V		
3	GH	POST	Sugarbeet	HERB	Stinger	Clopyralid	SL	0.66	PT/A	15	3
4	GH	POST	Sugarbeet	HERB	Progress	Phenmedipham + Desmedipham + Ethofumesate	SL	3.25	PT/A	27	7
5	GH	POST	Sugarbeet	HERB	Nortron	Ethofumesate	SL	0.5	PT/A	12	3
6	GH	POST	Sugarbeet	HERB	Ro-neet	cycloate	SL	4	PT/A	13	6
7	GH	PRE	Sugarbeet	HERB	Ro-Neet	cycloate	SL	4	LB AI/A	37	30
8	GH	PRE	Sugarbeet	HERB	Nortron	Ethofumesate	SL	3	PT/A	38	0

Table A2.4 Alternative control herbicides for GR kochia that were screened after crop screening had occurred. Herbicide and respective adjuvant (if used) use rates.

										% Control	
Trt.	Field/G.H	PRE/POST	Crop	Type	Treatment	Active Ingredient(s)	Form Type	Rate	Rate Unit	Avg.	SE
1	GH	POST	ALL	HERB	RoundUp Weather Max	glyphosate	SL	22	FL OZ/A	22	4
1	GH	POST	ALL	ADJ	AMS		WG	17	LB AI/100GAL		
1	GH	POST	Alternative	HERB	Edict 2SC	Pyraflufen ethyl	SL	2	FL OZ/A	12	4
2	GH	POST	Alternative	HERB	Solstice	Fluthiacet methyl	SL	2.5	FL OZ/A	20	8
3	GH	POST	Alternative	HERB	Diflex	dicamba	SL	16	FL OZ/A	78	2
3	GH	POST	Alternative	ADJ	AMS		WG	2.5	LB AI/A		
3	GH	POST	Alternative	ADJ	NIS		SL	0.25	% V/V		
4	GH	POST	Alternative	HERB	Autumn Super	Thiencarbazone-methyl	WG	0.5	OZ WT/A	15	3
5	GH	POST	Alternative	HERB	Armezon	Topramazone	SL	0.5	FL OZ/A	5	0
6	GH	POST	Alternative	HERB	Cobra	Lactofen	SL	12.5	FL OZ/A	82	2
6	GH	POST	Alternative	ADJ	COC		SL	0.5	% V/V		
6	GH	POST	Alternative	ADJ	AMS		WG	2	LB/A		
7	GH	POST	Alternative	HERB	Clarity	Dicamba	SL	8	FL OZ/A	53	4
7	GH	POST	Alternative	ADJ	NIS		SL	0.25	% V/V		
8	GH	PRE	Alternative	HERB	Acuron	s-metolachlor, Atrazine, Mesotrione, Bicyclopyrone	SL	2.5	QT/A	99	0
9	GH	PRE	Alternative	HERB	Sulfentrazone+ Pyroxasulfone	Sulfentrazone + Pyroxasulfone	SL	7	FL OZ/A	99	0
10	GH	PRE	Alternative	HERB	Linex	Linuron	SL	1.5	PT/A	91	4
11	GH	PRE	Alternative	HERB	Authority Assist	Sulfentrazone + imazethapyr	SL	10	FL OZ/A	99	0
12	GH	PRE	Alternative	HERB	Clarity	dicamba	SL	8	FL OZ/A	90	30

Table A2.5 Visual control ratings for alternative herbicide control options for GR kochia in corn. Visual evaluations were recorded from field plots 15 days after herbicide treatment. Herbicide and respective adjuvant (if used) use rates.

										% Control	
Trt.	Field/G.H	PRE/POST	Crop	Type	Treatment	Active Ingredient(s)	Form Type	Rate	Rate Unit	Avg.	SE
1	Field	POST	Corn	HERB	Laudis	Tembotrione	SL	0.082	LB AI/A	63	2
1	Field	POST	Corn	ADJ	MSO		SL	1	% V/V		
2	Field	POST	Corn	HERB	Bronate Advanced	Bromoxynil	SL	0.375	LB AI/A	28	2
3	Field	POST	Corn	HERB	Starane Ultra	Fluroxypyr	SL	0.4	PT/A	92	1
3	Field	POST	Corn	ADJ	MSO		SL	1.5	QT/A		
4	Field	POST	Corn	HERB	Clarity	Dicamba	SL	8	FL OZ/A	91	1
4	Field	POST	Corn	ADJ	NIS		SL	1.5	PT/100 GAL		
5	Field	POST	Corn	HERB	Impact	Topramazone	SL	0.75	FL OZ/A	58	2
5	Field	POST	Corn	ADJ	MSO		SL	1	% V/V		
6	Field	POST	Corn	HERB	Capreno	Thiencarbazone-methyl + Tembotrione	SL	3	FL OZ/A	63	2
6	Field	POST	Corn	ADJ	COC		SL	1.5	% V/V		
7	Field	POST	Corn	HERB	E.T.	Pyraflufen ethyl	SL	2	FL OZ/A	7	2
7	Field	POST	Corn	ADJ	NIS		SL	0.25	% V/V		
8	Field	POST	Corn	HERB	Corvus	Thiencarbazone + Isoxaflutole	SL	3.3	FL OZ/A	97	0
8	Field	POST	Corn	HERB	Aatrex	Atrazine	SL	4	PT/A		
8	Field	POST	Corn	ADJ	COC		SL	1	QT/A		
9	Field	POST	Corn	HERB	Callisto	Mesotrione	SL	3	FL OZ/A	68	2
9	Field	POST	Corn	ADJ	MSO		SL	1	% V/V		
10	Field	POST	Corn	HERB	Roundup WeatherMax	Glyphosate	SL	22	FL OZ/A	82	4
10	Field	POST	Corn	ADJ	Full Load		SL	0.5	% V/V		

Trt.	Field/G.H	PRE/POST	Crop	Type	Treatment	Active Ingredient(s)	Form Type	Rate	Rate Unit	Avg.	SE
11	Field	POST	Corn	HERB	Clarity	Dicamba	SL	8	FL OZ/A	95	0
11	Field	POST	Corn	HERB	Roundup WeatherMax	Glyphosate	SL	22	FL OZ/A		
11	Field	POST	Corn	ADJ	Full Load		SL	0.5	% V/V		
12	Field	POST	Corn	HERB	Engenia	Dicamba	SL	10	FL OZ/A	93	0
12	Field	POST	Corn	HERB	Clean amine	2,4-D	SL	0.66	PT/A		
12	Field	POST	Corn	HERB	Roundup WeatherMax	Glyphosate	SL	22	FL OZ/A		
12	Field	POST	Corn	ADJ	Full Load		SL	0.5	% V/V		
13	Field	POST	Corn	HERB	Roundup WeatherMax	Glyphosate	SL	22	FL OZ/A	93	0
13	Field	POST	Corn	HERB	Starane Ultra	Fluroxypyr	SL	0.4	PT/A		
13	Field	POST	Corn	ADJ	Full Load		SL	0.5	% V/V		
14	Field	POST	Corn	HERB	Rely 280	Glufosinate	SL	29	FL OZ/A		
15	Field	POST	Corn	HERB	Clarity	Dicamba	SL	10	FL OZ/A	28	10
15	Field	POST	Corn	ADJ	Full Load		SL	0.5	% V/V		

Figure A2.7 Visual control ratings for alternative herbicide control options for GR kochia in corn. All treatments above (Besides RoundUp WeatherMax) were tank mixed with RoundUp WeatherMax applied at 22 fl oz/acre. Visual evaluations were recorded from field plots 17 days after herbicide treatment. Herbicide and respective adjuvant (if used) use rates.

										% Control	
Trt.	Field/G.H	PRE/POST	Crop	Type	Treatment	Active Ingredient(s)	Form Type	Rate	Rate Unit	Avg.	SE
1	Field	POST	Wheat Fallow	HERB	Roundup WeatherMax	Glyphosate	SL	22	FL OZ/A	28	9
1	Field	POST	Wheat Fallow	ADJ	Ammonium Sulfate		SG	17	LB AI/100 GAL		
2	Field	POST	Wheat Fallow	HERB	Clarity	Dicamba	SL	10	FL OZ/A	85	3
2	Field	POST	Wheat Fallow	ADJ	NIS		SL	1	PT/100 GAL		
2	Field	POST	Wheat Fallow	ADJ	AMS		WG	2.5	LB AI/A		
2	Field	POST	Wheat Fallow	HERB	Roundup WeatherMax	Glyphosate	SL	22	FL OZ/A		
3	Field	POST	Wheat Fallow	HERB	Clean amine	2,4-D	SL	2	PT/A	52	7
3	Field	POST	Wheat Fallow	ADJ	AMS		WG	2.5	LB AI/A		
3	Field	POST	Wheat Fallow	HERB	Roundup WeatherMax	Glyphosate	SL	22	FL OZ/A		
4	Field	POST	Wheat Fallow	HERB	Starane Ultra	Fluroxypyr	SL	0.4	PT/A	89	5
4	Field	POST	Wheat Fallow	HERB	Roundup WeatherMax	Glyphosate	SL	22	FL OZ/A		
4	Field	POST	Wheat Fallow	ADJ	MSO		SL	1.5	QT/A		

Trt.	Field/G.H	PRE/POST	Crop	Type	Treatment	Active Ingredient(s)	Form Type	Rate	Rate Unit	Avg.	SE
5	Field	POST	Wheat Fallow	HERB	Roundup WeatherMax	Glyphosate	SL	22	FL OZ/A		
5	Field	POST	Wheat Fallow	HERB	Clarity	Dicamba	SL	10	FL OZ/A		
5	Field	POST	Wheat Fallow	HERB	Clean amine	2,4-D	SL	2	PT/A	89	2
5	Field	POST	Wheat Fallow	ADJ	NIS		SL	1	PT/100 GAL		
5	Field	POST	Wheat Fallow	ADJ	AMS		WG	2.5	LB AI/A		
6	Field	POST	Wheat Fallow	HERB	Balance Pro	Isoxaflutole	SL	4	FL OZ/A	63	7
6	Field	POST	Wheat Fallow	HERB	Roundup WeatherMax	Glyphosate	SL	22	FL OZ/A		
7	Field	POST	Wheat Fallow	HERB	Gramoxone	Paraquat	SL	4	PT/A		
7	Field	POST	Wheat Fallow	ADJ	NIS		SL	1	PT/100 GAL	96	1
7	Field	POST	Wheat Fallow	HERB	Roundup WeatherMax	Glyphosate	SL	22	FL OZ/A		
8	Field	POST	Wheat Fallow	HERB	Sharpen	Saflufenacil	SL	2	FL OZ/A		
8	Field	POST	Wheat Fallow	ADJ	MSO		SL	1	% V/V	80	6
8	Field	POST	Wheat Fallow	ADJ	AMS		WG	17	LB AI/100 GAL		
8	Field	POST	Wheat Fallow	HERB	Roundup WeatherMax	Glyphosate	SL	22	FL OZ/A		

Trt.	Field/G.H	PRE/POST	Crop	Type	Treatment	Active Ingredient(s)	Form Type	Rate	Rate Unit	Avg.	SE
9	Field	POST	Wheat Fallow	HERB	Aatrex	Atrazine	SL	4	PT/A	91	1
9	Field	POST	Wheat Fallow	ADJ	COC		SL	1	QT/A		
9	Field	POST	Wheat Fallow	HERB	Roundup WeatherMax	Glyphosate	SL	22	FL OZ/A		
10	Field	POST	Wheat Fallow	HERB	Huskie	Pyrasulfotole and bromoxynil	SL	11	FL OZ/A	82	6
10	Field	POST	Wheat Fallow	ADJ	AMS		WG	1	LB AI/A		
10	Field	POST	Wheat Fallow	ADJ	NIS		SL	0.5	% V/V		
10	Field	POST	Wheat Fallow	HERB	Roundup WeatherMax	Glyphosate	SL	22	FL OZ/A		
11	Field	POST	Wheat Fallow	HERB	Aim	Carfentrazone	EC	1	FL OZ/A	63	3
11	Field	POST	Wheat Fallow	ADJ	NIS		SL	0.25	% V/V		
11	Field	POST	Wheat Fallow	HERB	Roundup WeatherMax	Glyphosate	SL	22	FL OZ/A		
12	Field	POST	Wheat Fallow	HERB	Liberty	Glufosinate	SL	22	FL OZ/A	37	12
12	Field	POST	Wheat Fallow	ADJ	AMS		WG	17	LB AI/100 GAL		

Figure A2.8 Visual control ratings for fall-applied pre-emergent herbicides. Graphs display means and standard error of means. Plots were sprayed on 10-16-14 and visual control ratings were evaluated on 5-21-15. Herbicide and respective adjuvant (if used) use rates.

										% Control	
Trt.	Field/ G.H.	PRE/POST	Crop	Type	Treatment	Active Ingredient(s)	Form Type	Rate	Rate Unit	Avg.	SE
1	Field	PRE	Fall-Pre	HERB	Dual Magnum	s-Metolachlor	SL	2	PT/A	42	2
2	Field	PRE	Fall-Pre	HERB	Zidua	Pyroxasulfone	WG	2	OZ/A	83	7
3	Field	PRE	Fall-Pre	HERB	Prowl H2O	Pendimethalin	SL	4	PT/A	50	0
4	Field	PRE	Fall-Pre	HERB	Clarity	Dicamba	SL	16	FL OZ/A	47	3
5	Field	PRE	Fall-Pre	HERB	Clarity	Dicamba	SL	32	FL OZ/A	57	17
6	Field	PRE	Fall-Pre	HERB	Corvus	Thiencarbazone+ Isoxaflutole	SL	5.6	FL OZ/A	76	14
7	Field	PRE	Fall-Pre	HERB	Balance Pro	Isoxaflutole	SL	4	FL OZ/A	62	9
8	Field	PRE	Fall-Pre	HERB	Linex	Linuron	SL	1.5	PT/A	22	12
9	Field	PRE	Fall-Pre	HERB	Aatrex	Atrazine	SL	2	PT/A	73	14
10	Field	PRE	Fall-Pre	HERB	Authority MTZ	sulfentrazone and metribuzin	SL	10	OZ/A	81	16
11	Field	PRE	Fall-Pre	HERB	Valor SX	Flumioxazin	WG	4	OZ/A	72	16
12	Field	PRE	Fall-Pre	HERB	Fierce	Flumioxazin and pyroxasulfone	WG	3	OZ/A	77	9
13	Field	PRE	Fall-Pre	HERB	Spartan	Sulfentrazone	SL	10	FL OZ/A	96	3
14	Field	PRE	Fall-Pre	HERB	Sonar	Fluridone	WG	9.1	OZ/A	15	15
15	Field	PRE	Fall-Pre	HERB	Sharpen	Saflufenacil	SL	3	FL OZ/A	35	8
16	Field	PRE	Fall-Pre	HERB	Callisto	Mesotrione	SL	10	FL OZ/A	42	7
17	Field	PRE	Fall-Pre	HERB	Anthem	Pyroxasulfone and fluthiacte-methyl	SL	7.7	FL OZ/A	77	9
18	Field	PRE	Fall-Pre	HERB	Anthem ATZ	Pyroxasulfone fluthiacte-methyl, and atrazine	SL	2.5	PT/A	98	2

References

Knezevic SZ, Evans SP, Blankenship EE, Van Acker RC, Lindquist JL, (2009) Critical period for weed control: the concept and data analysis. Weed Sci 50:773-786

Norsworthy JK, Griffith GM, Scott RC, Smith KL, Oliver LR, (2008) Confirmation and Control of Glyphosate-Resistant Palmer Amaranth (Amaranthus palmeri) in Arkansas. Weed Technol 22:108-113

Powles SB, Preston C, (2006) Evolved Glyphosate Resistance in Plants: Biochemical and Genetic Basis of Resistance. Weed Technol 20:282-289

Tracy EK, Lawrence RO, (1994) Palmer Amaranth (Amaranthus palmeri) Interference in Soybeans (Glycine max). Weed Sci 42:523-527

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

Appendix 3

Laboratory based work evaluating glyphosate-resistance in kochia

Molecular biology techniques are being used in weed science to study weed genetics and for the detection and confirmation of herbicide resistance. Increasingly, basic and applied weed science students will benefit from a fundamental understanding of molecular concepts to help them communicate with laboratory based collaborators and scientists from the weed science community as a whole. Lab research with suspected glyphosate-resistant kochia was initiated to use molecular biology and laboratory techniques to evaluate glyphosate resistance in kochia. This research centered on evaluating the possible existence of glyphosate-resistance in kochia from fields with unexplained glyphosate failures.

In 2013, seed was collected from four field sites where glyphosate-resistant kochia was suspected to exist based on reduced glyphosate kochia control observed by growers (Figure A3.1). All kochia plants were collected from surviving kochia streaks, or widespread patches present in wheat fallow fields after the fields had been bulk sprayed with a labeled rate of glyphosate (Figure A3.2). From each field site, a minimum of ten individual plants were collected and transplanted into 3-gallon pots. Individual plants were covered by micro-perforated bags to force self-pollination of individual plants.

Seed from each collection site was seeded in plug flats where kochia plants grew until the kochia seedlings were 2.5 cm tall. Kochia seedlings were then transplanted and grown in flat inserts that were 3.8 cm by 3.8 cm by 5.8 cm deep (American Clay Works, Denver, CO). For each field site, three individual kochia plants were selected to perform glyphosate-resistant diagnostic assays. A known glyphosate-susceptible population was included for all assays as a negative control.

The first laboratory diagnostic test conducted was a shikimate accumulation assay. In susceptible individuals, leaf disks exposed to a discriminating dose of glyphosate will accumulate shikimate when the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) is inhibited. Glyphosate-resistant individuals however do not experience shikimate accumulation because EPSPS is not completely inhibited due to additional gene copies and subsequent expression of EPSPS which over-produces EPSPS which is inhibited by glyphosate. Three replicate leaf disks from each plant were exposed to a 100 µM glyphosate solution (discriminating dose) for the detection of shikimate accumulation. Published shikimate assay methods were followed to evaluate accumulation (Shaner et al., 2005). Shikimate accumulation was detected with all three susceptible kochia plants and for one individual collected from the Denver population (Figure A3.3). The rest of the individuals tested did not accumulate shikimate when exposed to a 100 µM glyphosate solution, which indicates that these individuals were glyphosate-resistant.

Simultaneously to leaf disk collections for shikimate accumulation assays, 100 mg of young kochia leaf tissue was collected for DNA extractions. From plant tissue DNA was extracted using a QIAamp DNA plant Mini kit and the standard protocol (QIAgen, Valencia, CA). The concentration of DNA was analyzed using a NanoDrop ND-1000 Spectrophotometer (NanoDrop products, Wilmington, DE), and working solutions (4 ng/µl) were made for subsequent studies.

To look for a polymorphism in the Proline 106 codon, a ~140 bp region of EPSPS was amplified using polymerase chain reaction (PCR). Primers were developed from preexisting EPSPS kochia sequence data (Personal communication, Todd Gaines). The forward primer sequence for the EPSPS target site was 5’-CCC ATC GTC AAT CAT ATA GTA TTA GC-3’, and the reverse primer sequence was 5'- TGA CAA TAA CTT ATG CGG AAA GCA-3'. The cycling parameters used were as follows: 15 minutes at 95°C, then a cycle for 10 seconds at 90°C, 30 seconds at 58°C, and last 30 seconds at 72°C. This cycle was performed 30 times and then the reaction was cooled to 10 C for further use.

Once amplified, the PCR products were run and excised from a 1% agarose gel, purified using a QIAgen Gel purification kit using the standard protocol, and sent to the Proteomic and Metabolomics Facility (PMF) at Colorado State University for sequencing. Sequencing of the EPSPS gene showed that there was no target site mutation at the Pro-106 codon of the corresponding EPSPS protein (Figure A3.4). This point mutation was specifically targeted because past research showed it can confirm glyphosate resistance (Wakelin and Preston, 2006). Amino acid substitutions of Proline 106 to Serine, Threonine, Alanine, Leucine have all been shown to confer glyphosate-resistant across different weed species. Based on sequencing results, target-site based resistance was ruled out as a potential mechanism of glyphosate-resistance in the tested kochia populations.

Next, quantative PCR (qPCR) was conducted with the DNA from nine replicates for each field collected population to determine the relative gene copy number of EPSPS. Acetolactate synthase (ALS) was used as a house keeping gene for comparison. qPCR for EPSPS copy number and transcript abundance contained 2.5 µl of gDNA (4 ng/ µl), 100 nL of forward and reverse primers, 10 µl of Quanta perfecta (Quanta Bio, Gaithersburg, MD) qPCR sybergreen master-mix for a total reaction volume of 12.5 µl. Samples were cycled by initially heating samples to 95°C for five minutes, followed by 40 cycles of 30 seconds at 95°C, 30 seconds at 58°C. and 30 seconds at 72°C, with a final extension at 72°C for five minutes. The fluorescent signal from Sybergreen was captured at the end of the amplification step in every cycle. Relative

EPSPS copy number was determined by using a comparative CT method to analyze relative transcript abundance (Schmittgen and Livak, 2008). ALS was used as a single copy gene that was stably expressed between resistant and susceptible plants. Quantification of EPSPS was calculated as $\Delta CT = 2ALS\ CT - EPSPS\ CT$. The three biological replicates for glyphosate-resistant and susceptible populations were run in technical triplicates, and the biological average and standard deviation were calculated for each population. Results for genomic copy number and transcript abundance are reported as the fold-increase of EPSPS relative to ALS (Figure A3.5).

Results from qPCR analysis showed that the relative EPSPS:ALS gene copy number was between 5 and 16 copies for the suspected glyphosate-resistant kochia plants tested (Figure A3.5). These results indicate that glyphosate-resistance in these tested populations was due to increased EPSPS gene copy number. The correlation of relative EPSPS gene copy number with the level of shikimate accumulation can provide two way confirmation that glyphosate-resistance was present in the suspected glyphosate-resistant kochia populations tested (Figure A3.6). Results from assays above were obtained and reported to growers that suspected the presence of glyphosate-resistant kochia in their fields. Once confirmation of glyphosate-resistant kochia was delivered to growers, proactive steps were taken to control and eliminate these resistant individuals by means of additional alternative mode of action herbicide applications or tillage. Recommendations for mode of action rotations or tank mixes were considered once fields were confirmed to contain glyphosate-resistant kochia.

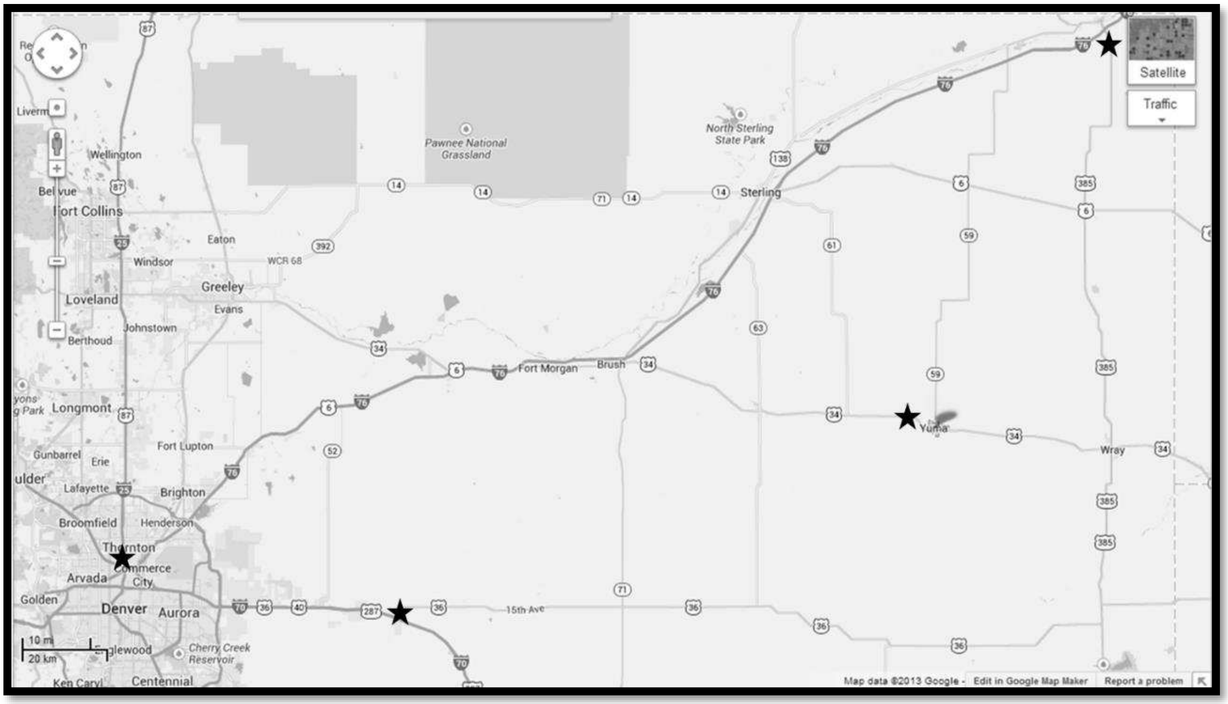

Figure A3.1 Kochia site collection map for suspected glyphosate-resistant kochia populations tested. The four sites where suspected glyphosate-resistant kochia was collected from are indicated by black stars.

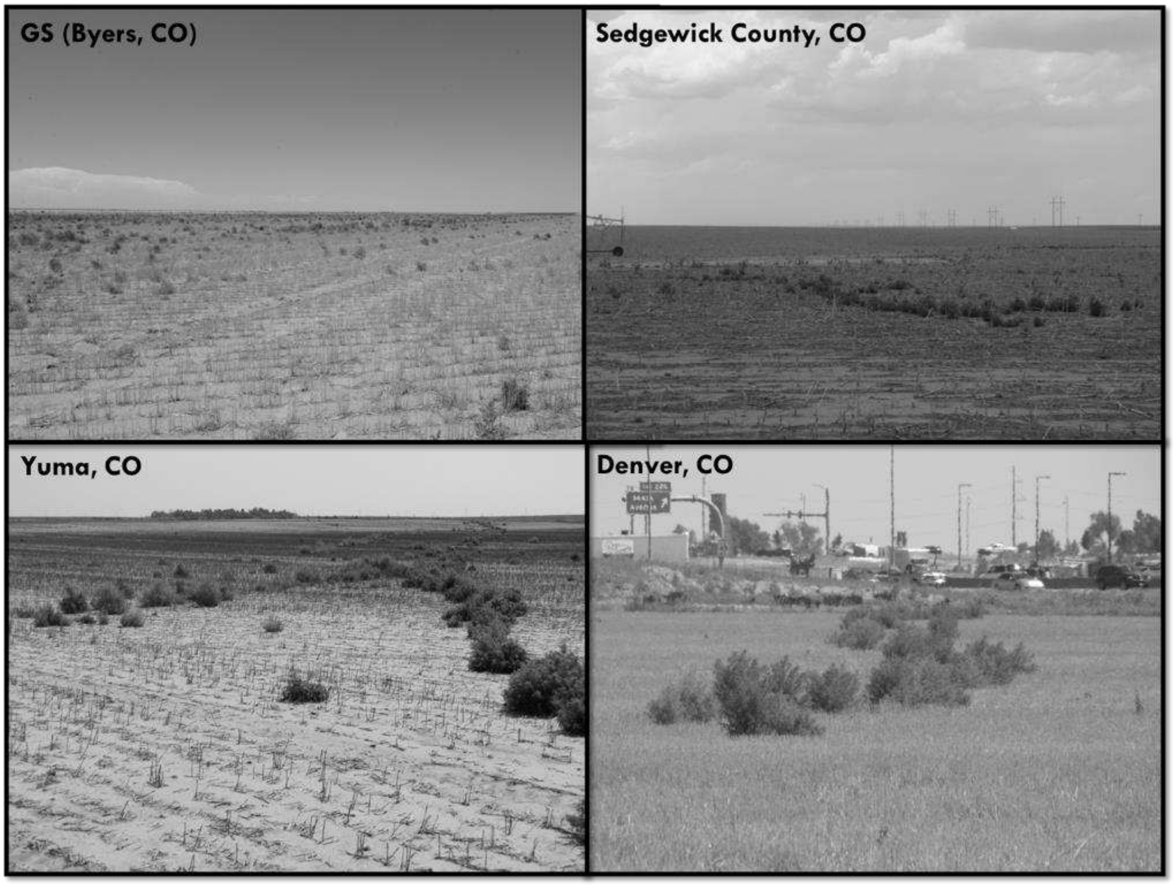

Figure A3.2 Field sites where kochia populations were collected from after growers reported the presence of suspected glyphosate-resistant kochia.

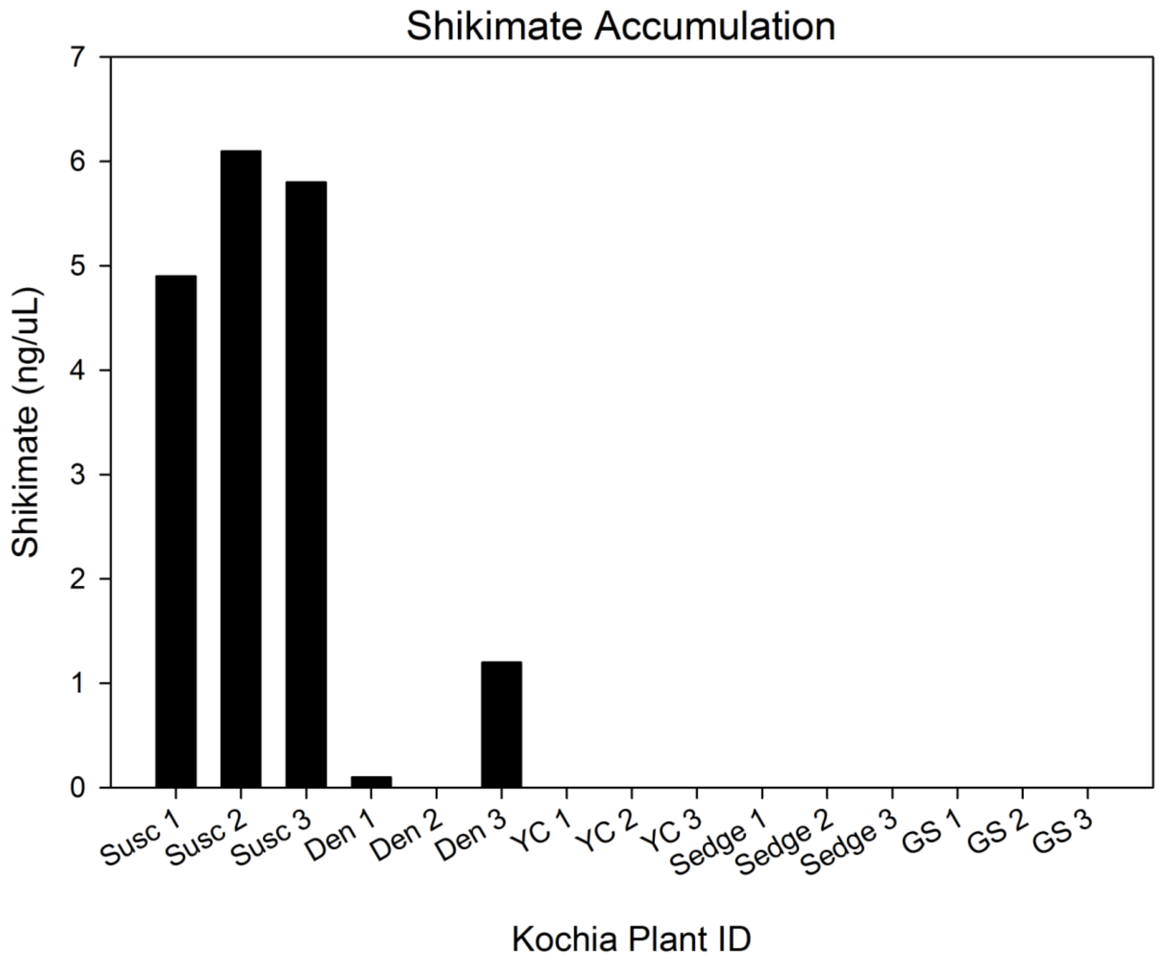

Figure A3.3 Shikimate accumulation (ng μL^{-1}) for individuals from field collected populations when incubated in 100 μM glyphosate solution.

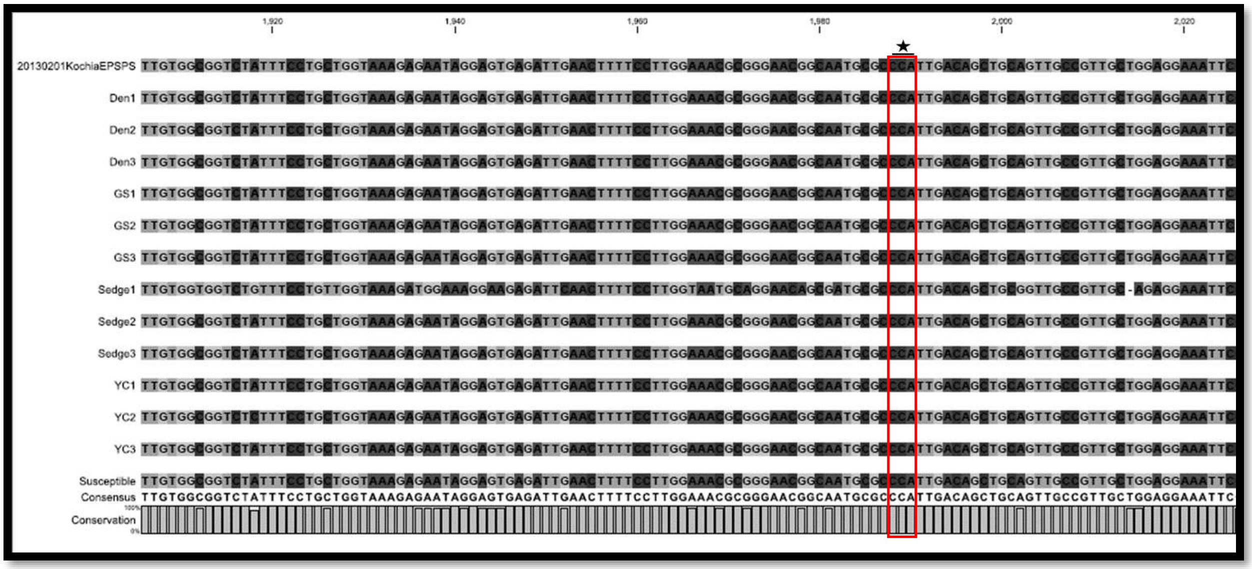

Figure A3.4 Sequencing of EPSPS protein from field populations for evaluation of target site mutations. Star and red-lined box indicate the codon for Proline 106 that is conserved across all individuals tested (no polymorphism).

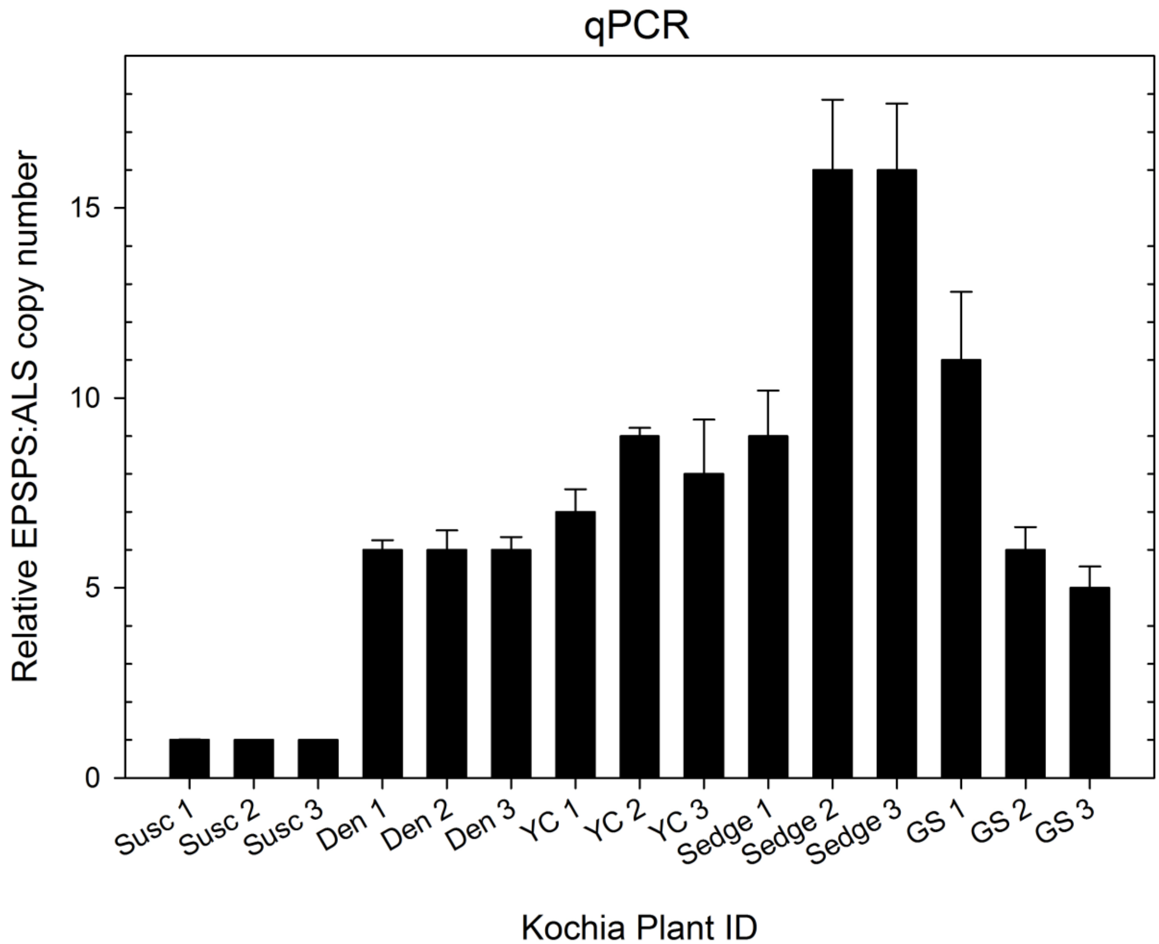

Figure A3.5 Relative EPSPS:ALS gene copy number from qPCR experiment. Bars display means from the 3 biological replicates and error bars represent standard error of the mean.

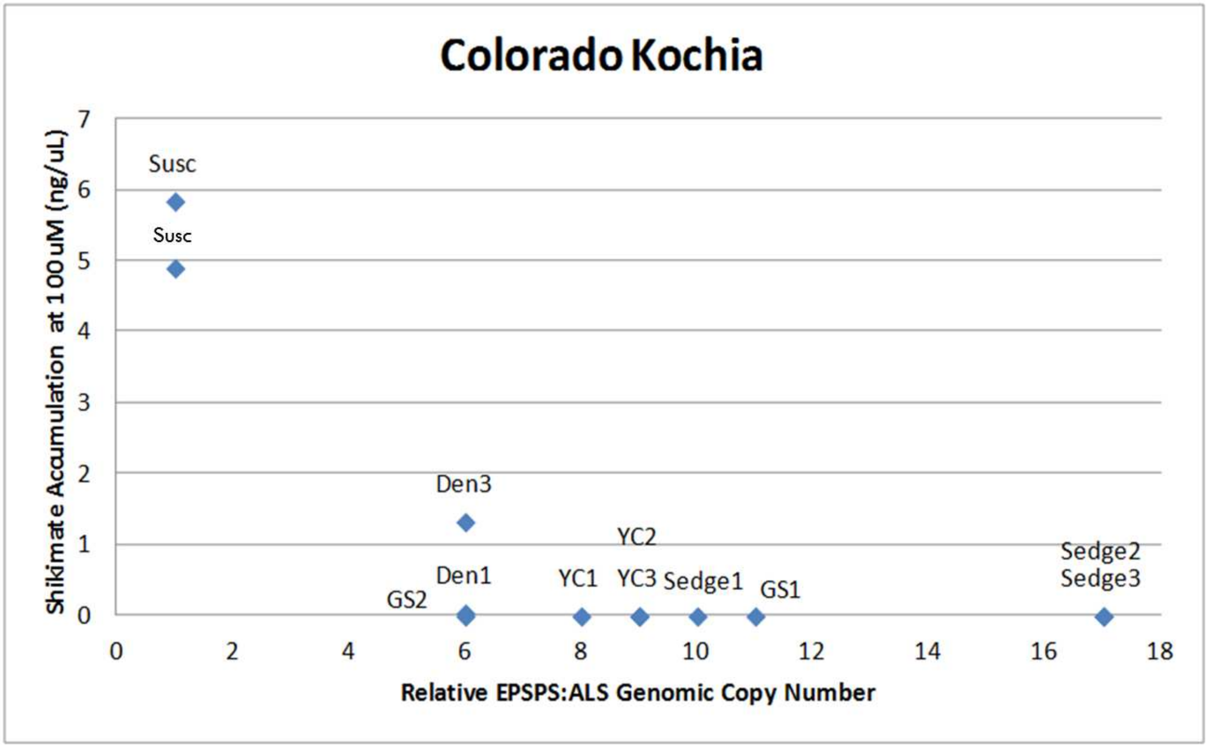

Figure A3.6 Correlation of relative EPSPS:ALS gene copy number to shikimate accumulation (ng uL^{-1}) at the 100 uM rate of glyphosate.

References

Schmittgen TD, Livak KJ, (2008) Analyzing real-time PCR data by the comparative CT method. Nature protocols 3:1101-1108

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

Wakelin AM, Preston C, (2006) A target-site mutation is present in a glyphosate-resistant Lolium rigidum population. Weed res 46:432-440