University of Alberta

Potential for a Pyroxasulfone and Sulfentrazone Herbicide Combination to Control Herbicide-Resistant Weeds in Field Pea, as Affected by Edaphic Factors

by

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A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of

Master of Science

in

Plant Science

Department of Agricultural, Food and Nutritional Science

Spring 2014
Edmonton, Alberta

Dedication

This thesis is dedicated first to my parents, Roman and Wanda,

for believing in me when I didn't believe in myself,

for loving me through everything, and always being

proud of me and my accomplishments.

Second, to my husband Torstein for his love and support

accepting me for who I am, and knowing just what to

do and say to keep me sane and happy.

Abstract

Herbicide resistance, particularly to Group 2 herbicides (ALS inhibitors) is increasing in incidence in Canada and is problematic in crops such as peas that rely on ALS inhibitors for weed control. Pyroxasulfone, a group 15 herbicide, and sulfentrazone, a group 14 herbicide, show potential to be used in peas for weed control. Investigations were conducted into effective rates and application timings for control of false cleavers and wild oat, effect of organic matter and soil moisture on pyroxasulfone and sulfentrazone efficacy, and the interaction of pyroxasulfone and sulfentrazone when co-applied. It was determined that effective rates vary according to location and that higher levels of soil organic matter require higher herbicide rates for equal efficacy. In addition, both fall and spring applications of pyroxasulfone are effective. Pyroxasulfone and sulfentrazone are additive when applied in combination, but broaden the weed spectrum controlled and aid in herbicide resistance management.

Acknowledgements

I would like to acknowledge Dr. Linda Hall for all her efforts and supports on my behalf. Dr. Hall has supported me tirelessly through my degree, providing me opportunities to present my data, network with incredible research scientists in the industry and helping me to launch my career in the most positive way possible. This degree would not have been possible without her help. I also need to acknowledge the support of my supervisory committee: Dr. Hugh Beckie, Dr. Edward Bork, and Mr. Eric Johnson. Your thoughts, comments, edits and questions have helped me grow immensely through the last 2.5 years and it is appreciated more than you can know.

I would also like to acknowledge the current and prior members of the weed science lab group at the University of Alberta for all their help in the field, help in classes, statistics advice and support through my degree. This experience wouldn't have been the same without you: Lisa Raatz, Judy Irving, Keith Topinka, Kim Walsh, Brendan Alexander, Ryan Low, Jagroop Kahlon, Jaime Crowe, Tim Loeppky and all other summer and support staff who got to enjoy the joys of working on my project. I'll have these memories with me forever.

Finally I'd like to acknowledge all of my family and friends for their support throughout this process. I would never have made it through without your love and support.

Funding for the projects described herein were provided by FMC Agricultural Products, Agriculture and Agri-food Canada Pulse Cluster and NSERC.

Contributions of Authors

The contributions made by the candidate and the co-authors to the completion of this work are described here. Chapter Three of this thesis was co-authored by the candidate, Dr. Linda M. Hall, Dr. Hugh J. Beckie, Mr. Eric N. Johnson, Mr. Ken L. Sapsford and Miss Lisa L. Raatz. In that chapter the candidate was responsible for conduction of trials at Ellerslie and Kinsella, AB, compilation of data, statistical analysis and writing of the manuscript. Dr. Hugh Beckie, Mr. Eric Johnson and Mr. Ken Sapsford were responsible for conducting trials at their respective locations with the help of their technical and support staff. Miss Lisa Raatz provided statistical analysis support as well as field support for the trials under conduction by the candidate. All co-authors participated in trial design. Chapter Four of the thesis was collaborated on by the same authors as above. The candidate was responsible for trial conduction at Edmonton in 2012, data compilation, statistical analysis and writing of the manuscript. Dr. Beckie, Mr. Johnson and Mr. Sapsford were responsible for trial conduction at their location with the aid of their support and technical staff, as well as design of the experiment. Miss Raatz conducted the trial at Edmonton in 2011. Technical and field support in both Chapter Three and Four was provided by Mr. Keith Topinka, Miss Lisa Raatz and Mrs. Judy Irving as well as other graduate students in the Weed Science program. The candidate was responsible for conduction of greenhouse trials in Chapter Four and trial design in collaboration with Dr. Linda Hall. Dr. Linda Hall who co-authored Chapters Three and Four was the graduate student supervisor for the candidate and worked with the candidate on the writing of all manuscripts, experimental designs and advised the candidate throughout her program.

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List of Abbreviations

ACCase	Acetyl-CoA Carboxylase
AHAS	Acetohydroxyacid Synthase
ai	Active ingredient
ALS	Acetolactate Synthase
ANOVA	Analysis of Variance
ED_{50}	Effective dose that reduces plant biomass by 50%
ha	Hectare
IMI	Imidazolinone herbicide
IWM	Integrated Weed Management
kg	Kilogram
L	Litre
m	metre
N	Nitrogen
POST	Post-plant emergence herbicide application
PPI	Pre-planting herbicide application requiring incorporation
PPO	Protoporphyrinogen Oxidase
PRE	Pre-seeding or Pre-emergence herbicide application

Proto	Protoporphyrin IX
Protogen	Protoporphyrinogen IX
Protox	Protoporphyrinogen Oxidase
PTB	Pyrimidinyl(thio)benzoate herbicide
SU	Sulfonylurea herbicide
TP	Triazolopyrimidine herbicide
VLCFA	Very Long Chain Fatty Acid
VLCFAE	Very Long Chain Fatty Acid Elongase
WDG	Water dispersible granule

Chapter One: Introduction

1.1. Background

Canada is the top exporter of field pea (*Pisum sativum* L.) worldwide (FAOSTAT 2011), with the bulk of peas produced in the Prairie provinces. Including field pea in the crop rotation provides fertility benefits (Beckie and Brandt 1997; Beckie et al. 1997), soil tilth benefits (Grant and Lafond 1993), and interrupts disease cycles (Kirkegaard et al. 2008). Field pea is a relatively non-competitive crop and early season weed control is critical to attain yield potential (Harker 2001). Predominant herbicides in field pea are group 2 herbicides (acetolactate synthase (ALS) inhibitors) imazamox and imazethapyr. Use of these herbicides provide effective control of weeds and an efficient application timing.

Herbicide-resistant weeds occupy 29% of agricultural farmland in Canada, a number that continues to increase (Beckie et al. 2013). ALS inhibitors have the highest incidence of resistance world-wide with 143 species resistant to date and still increasing (Heap 2014). Although considered a high risk group for evolution of resistance (Beckie 2006; Tranel and Wright 2002), they are frequently used in Western Canada due to high efficacy levels, good crop tolerance, and limited herbicide options in crops such as field pea. There are 25 species resistant to ALS inhibitors in Canada including wild oat (*Avena fatua*), false cleavers (*Galium spurium*), kochia (*Kochia scoparia*), and chickweed (*Stellaria media*). These weeds are significant pests on the Canadian prairies (Leeson et al. 2005), and can be detrimental to crop yield in field pea if not controlled. Herbicide-resistant weeds can be managed through non-herbicidal tactics to give crops a competitive advantage or through the use of alternate herbicides and herbicide techniques (Beckie 2006).

Pyroxasulfone is a new group 15 herbicide (Very Long Chain Fatty Acid Elongase (VLCFAE) inhibitor) (Tanetani et al. 2011; Tanetani et al. 2009) currently registered for use in wheat and triticale in Australia, and corn and soybean in the United States and Canada. Inhibition of VLCFAE prevents the production of new Very Long Chain Fatty Acids (VLCFA), >18 carbon molecules, which are components of cell membranes, plant cuticles and pollen and also function as energy storage for seeds (Babczinski et al. 2012). Use of VLCFAE-inhibitor herbicides is limited in western Canada. Pyroxasulfone has been identified as a potential new herbicide option in peas, which have previously demonstrated tolerance (Walsh et al. 2011).

Sulfentrazone is a group 14 herbicide (Protoporphyrinogen Oxidase (PPO) inhibitor) currently registered in western Canada on chickpea, sunflower, flax and pea (FMC Corporation 2014). It is registered for control of kochia, lamb's quarters (*Chenopodium album*), redroot pigweed (*Amaranthus retroflexus*), and wild buckwheat (*Polygonum convolvulus*). PPO inhibitors cause the formation of toxic oxygen radicals in the cytoplasm of cells, which leads to lipid peroxidation, membrane leakage and eventually cell death (Dayan and Duke 1997; Orr and Hess 1981). Although sulfentrazone is already registered on peas, there is interest in registration of a pyroxasulfone and sulfentrazone combination herbicide.

Pyroxasulfone and sulfentrazone are both pre-seeding or pre-emergence (PRE) herbicides applied directly to the soil without incorporation. As soil-applied herbicides, their activity is dependent on soil moisture to enter a soil water solution phase that is available for plant uptake (Walker 1971). In addition, soil characteristics such as pH and organic matter have been shown to affect herbicide efficacy (Blumhorst et al. 1990; Rahman et al. 1978). Herbicide physico-chemical properties affect the degree to which soil properties influence their efficacy. In particular, a herbicide's water solubility can affect uptake and binding to soil organic matter (Rahman et al. 1978).

For pyroxasulfone and sulfentrazone to be used in combination in Canada to control herbicide-resistant weeds in field pea, investigation into their potential interactions is required. In addition, the degree to which soil parameters affects efficacy must be investigated to determine the need to consider these parameters for registration or for guidance to growers and agronomists. As a result the following research objectives were set for this thesis.

1.2. Research Objectives

1.2.1. Determine effective rates of pyroxasulfone for control of cleavers and wild oat in peas in Western Canada.

As pyroxasulfone has not been extensively tested on western Canadian soils, it is necessary to determine rates that will be effective to control of problem weeds such as cleavers and wild oat. Environmental factors may affect weed efficacy, and tolerance of peas. This research objective was investigated in Chapter 3 and the following hypotheses were made:

- Rates for use in western Canada will be similar to effective rates in other countries

- Effective rates will vary between locations due to soil parameter and environmental differences
- Peas will exhibit tolerance to pyroxasulfone.

1.2.2. Determine if there is an advantage to fall or spring applications of pyroxasulfone

PRE application timing of pyroxasulfone leaves several windows for applications: post-harvest, PRE-seeding and PRE-emergence. Application of pyroxasulfone post harvest may be more efficient for producers. In addition, post-harvest applications may control winter annual weeds and early emerging spring annuals. However, although pyroxasulfone is a residual product, the product may degrade through the late fall and early spring seasons to an extent that weed control is not effective throughout the growing season. Spring applications of pyroxasulfone improve the probability of season-long control as well as the ability to assess soil moisture at the time of application and prevent non-effective applications. Determination of a weed control advantage to fall or spring applications of pyroxasulfone is discussed in Chapter 3. During that investigation the following hypotheses were made.

- Fall applications of pyroxasulfone will be more efficacious than spring where winter annuals are predominant.
- Spring applications will result in better season-long control than fall applications.

1.2.3. Determine whether soil organic matter affects pyroxasulfone and sulfentrazone efficacy.

Soil-applied herbicide efficacy is influenced by soil organic matter through adsorption of the herbicide to soil colloids (Blumhorst et al. 1990; Rahman et al. 1978). Sulfentrazone registered rates differ by soil texture (FMC Corporation 2014), not soil organic matter, but as a-soil applied herbicide organic matter still likely plays a part in its efficacy. In addition, other studies have indicated that pyroxasulfone efficacy varies by organic matter content (Westra 2012). The extent to which soil organic matter affects herbicide efficacy could potentially influence registration rates and soil zones of recommended use. The effect of soil organic matter on herbicide efficacy is discuss in Chapters 3 and 4 where the following hypotheses are made:

- Soils with higher organic matter will require higher herbicide rates than lower organic matter soils for similar efficacy
- Pyroxasulfone efficacy will be more influenced by OM than sulfentrazone efficacy due to higher levels of adsorption to soil colloids.

1.2.4. Determine efficacy and interaction of pyroxasulfone and sulfentrazone when co-applied.

Pyroxasulfone will be marketed in combination with sulfentrazone for use in peas in western Canada. When herbicides are co-applied, there is a potential for them to interact within the plant in terms of the effects that they exert. Interactions can lead to enhanced levels of control (synergy), reduced levels of control (antagonism), or a neutral effect on weed control termed additivity. It is important to know the nature of herbicide interactions when being marketed together, as the knowledge can affect what herbicides are mixed, and the proportions that they are mixed. The efficacy and interactions of pyroxasulfone and sulfentrazone are investigated in Chapter 4 under the following hypotheses:

- Pyroxasulfone and sulfentrazone modes of action are unlinked and therefore the herbicides are unlikely to interact synergistically or antagonistically in terms of their effects within targeted weeds.

1.2.5. Investigate the influence of soil moisture on pyroxasulfone and sulfentrazone efficacy.

As soil-applied herbicides, pyroxasulfone and sulfentrazone require soil moisture for entry into the soil solution, desorption from soil colloids and uptake by plants (Walker 1971). In addition, sufficient moisture must come early enough in the season that herbicides can be efficacious on appropriately staged plants (Walker 1971); lack of early season precipitation allows plants to grow large enough to overcome herbicidal effects. The level of influence soil moisture has on the herbicides' efficacy determines the risk to producers when applying them, as precipitation events are unpredictable. Knowledge of the effect of soil moisture on pyroxasulfone and sulfentrazone efficacy is desirable prior to marketing of the combination, and was investigated in Chapter 4. During the investigation the following hypothesis was made:

- Pyroxasulfone and sulfentrazone will be more efficacious in high soil moisture environments and have little activity under drought conditions.

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Chapter Two: Literature Review

2.1. Pea cultivation in Canada

Field pea (*Pisum sativum* L.) was grown on over 1.3 million ha in Canada in 2013, with the largest crop being 1.5 million hectares in 2009 (Statistics Canada 2013). Canada is the largest producer and exporter for dry pea worldwide (FAOSTAT 2011). Saskatchewan produces 72% of the crop (Saskatchewan Pulse Growers 2013) with the balance grown primarily in Alberta and Manitoba.

Pea is a valuable crop, due to crop value, market opportunities, crop diversification, and benefits to subsequent crops. Pea provides a nitrogen (N) residual effect, the amount of N required by crops grown on non-legume soils to achieve equal yield as those grown on legume soils, of 15-25 kg N ha^{-1} for every 1000 kg of pea seed used (Beckie and Brandt 1997; Beckie et al. 1997). Field pea interrupts disease cycles and weed populations (Kirkegaard et al. 2008). Field pea in rotation can moderate effects of tillage on soil bulk density and moisture penetration resistance, as well as reduce compaction due to tillage (Grant and Lafond 1993).

Weed control in pea relies heavily on herbicides, in direct-seeded systems. Peas are less competitive with weeds than barley or canola (Harker 2001), increasing seeding rates is not economically justified, and therefore herbicides are required to maintain yields. Early removal of weeds is critical, as potential yield begins to decrease one week after pea emergence due to weed competition (Harker et al. 2001). Acetolactate synthase (ALS) -inhibiting herbicides, imazamox and imazethapyr, are the most common herbicides used in peas due to crop tolerance, convenient post-emergence application timings, and control of many grass and broadleaf weeds. However, many broadleaf and grass weeds have been selected for ALS-inhibitor resistance (see below) and cannot be controlled by using ALS-inhibitor herbicides alone. Other options for control of ALS-inhibitor resistant weeds include group 3/K1 (mitotic inhibitors) pre-seeding (PRE) applied, ethalfluralin and trifluralin, a PRE-applied group 14/E (protoporphyrinogen oxidase (PPO) inhibitor), sulfentrazone, and post-emergent (POST) applications of photosystem-II inhibitors, metribuzin (group 5/C1) and bentazon (group 6/C3). To enhance herbicide resistance management, herbicide mixtures with multiple modes of action such as imazamox/bentazone (Viper), a group 2/ALS inhibitor/group 6/Photosystem II inhibitor, are being introduced. In tank-mixed products, ALS-inhibitor resistant weeds may be controlled by the alternative active ingredient. Novel herbicides are being tested for efficacy and crop tolerance to increase weed management options and provide alternative herbicides for group 2-resistant weeds (see below).

2.2. Herbicide Resistance

Herbicide resistance is defined by the Weed Science Society of America (WSSA) as “the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type” (Weed Science Society of America 2011). As of January 2014, 232 species have been confirmed to exhibit herbicide resistance (Heap 2014), and cases of herbicide resistance continue to be reported. There are six known mechanisms of resistance: site of action mutations, enhanced metabolic degradation, gene amplification of the target site, changes in herbicide translocation, altered herbicide uptake, sequestration of the herbicide away from the target site (Powles and Yu 2010). These mechanisms confer variable levels of resistance: site of action mutations generally confer high levels of resistance, where resistant plants survive high rates of herbicides in comparison to the susceptible biotype with little or no intermediate expression (Weed Science Society of America 2011). Resistant plants with mechanisms conferring low level resistance will have injury that ranges from minimal to severe in comparison with the susceptible biotype, with the majority having intermediate injury (Weed Science Society of America 2011). Levels of resistance are quantified using R/S ratios, derived by comparing the herbicide rates required for a 50% reduction in growth (ED_{50}) for resistant and susceptible populations. ED_{50} values are experimentally derived after application of a range of herbicide rates to resistant and susceptible populations. The relationship between dependent variable (biomass, biomass as percentage of the nontreated check, or visual ratings) and herbicide rate is established using nonlinear regression, often fitting the log-logistic model, which provides an estimate of the ED_{50}.

Plants can also exhibit cross-resistance, resistance to more than one herbicide or group due to one mechanism, or multiple-resistance, resistance to more than one herbicide group due to two or more mechanisms. In addition, they can exhibit polygenic resistance, resistance conferred by several genes, each of which confers low-level resistance and are accumulated through outcrossing. Understanding selection of resistance can aid producers and weed scientists in management and reduction of the rate of selection.

2.2.1. Selection for Herbicide Resistance

Herbicides are applied in agricultural fields to large, genetically variable weed populations, and many are highly effective. Some weed biotypes, as a result of genetic variation, have mutations that allow them to survive herbicide applications. Repeated application of the same herbicide or mode of action allows resistant plants to survive and propagate. Progeny inherit the resistant genotype, increasing the frequency of resistant plants in a population. Mutations that confer a high level of resistance are selected by both high and low rates of herbicides, but effective herbicides and high rates select more quickly.

Polygenic resistance is selected for by low herbicide rates over multiple generations (Neve and Powles 2005), allowing multiple low level resistance genes to be accumulated. Polygenic resistance is much more common in allogamous species.

2.2.2. Rate of Evolution of Herbicide Resistance

The rate of selection for herbicide resistance is dependent on the characteristics of the herbicide, and the weed, producer management tactics and the weed population dynamics. Because of the long time periods involved, the low initial mutation frequency and the large numbers of individuals in a population that are selected, much of our understanding of herbicide resistant selection was developed through modelling (Diggle et al. 2003; Jasieniuk et al. 1996; Maxwell et al. 1990). In addition, modelling allows us to make predictive hypotheses and respond in a proactive, rather than reactive, manner (Manalil et al. 2012; Werth et al. 2012). Factors affecting rate of selection for resistance are listed in Table 2-1, adapted from Powles and Yu (2010).

2.3. Resistance to ALS inhibitors

ALS-inhibitor herbicides, or Group 2 (B) herbicides (Mallory-Smith and Retzinger 2003) were first introduced in 1982 when chlorsulfuron was released (Tranel and Wright 2002). The first case of Group 2 herbicide resistance was confirmed in prickly lettuce (*Lactuca serriola)* in 1990 in the United States (Mallory-Smith et al. 1990). To date there are 143 confirmed ALS herbicide-resistant species, the highest number of species resistant to any herbicide group (Heap 2014). ALS-inhibitor resistance has a large impact in Western Canada due to the frequency of group 2 product use, as well as limited alternate options in crops such as peas.

2.3.1. ALS-Inhibitor Herbicides

Group 2 herbicides inhibit the acetolactate synthase (ALS) enzyme, also known as acetohydroxyacid synthase (AHAS), the first common enzyme in the synthesis pathway of branched-chain amino acids valine, leucine and isoleucine (Chaleff and Mauvais 1984). Group 2 herbicides are non-competitive inhibitors of the ALS enzyme; they bind to the enzyme in a separate location from the substrates and block entrance of the substrates to the catalytic site (Powles and Yu 2010). Herbicides that affect this enzyme include the sulfonylurea (SU), imidazolinone (IMI), triazolopyrimidine (TP), pyrimidinyl(thio)benzoate (PTB), and sulfonylaminocarbonyltriazolinone chemical classes (Whaley et al. 2007). ALS-inhibitor herbicides are used frequently, over a large area, and repeatedly (Beckie et al. 2008) due to low toxicity, selectivity and their ability to control many broadleaf and grass weeds. They are effective at low rates relative to other herbicide groups, and many have soil residual activity. They are a high risk group for selection of resistant weeds (Beckie 2006; Tranel and Wright 2002).

2.3.2. Site of Action Resistance

Site of action mutations, substitutions of amino acids in the target enzyme limiting herbicide binding, is the most common mechanism of conferring ALS inhibitor herbicide-resistance in broadleaf weeds. ALS-inhibitor herbicides are non-competitive with substrates pyruvate and α-ketobutyrate. Mutations conferring herbicide resistance can limit herbicide binding without affecting the binding of the substrates. Typically, single amino acid substitutions confer a high level of resistance (Whaley et al. 2007). There have been eight amino acid locations identified where substitutions confer resistance in plants (Beckie and Tardif 2012), although an additional ten have been discovered in other organisms (Li et al. 2012; Whaley et al. 2007). ALS resistance mutations can cause an increase, decrease or no change in enzyme functionality due to the mutation or sensitivity changes to feed back regulation from branched chain amino acid production (Powles and Yu 2010).

Site of action mutations confer resistance and cross-resistance to herbicide structural groups, depending on the specific mutation (Tranel and Wright 2002). Cross-resistance has been reported between 1) SU and TP resistant, 2) IMI and PTB resistant, and 3) broad cross-resistance among ALS inhibitors (Tranel and Wright 2002). In addition, negative cross-resistance, where ALS-inhibitor resistant biotypes are more susceptible to other herbicide modes of action, has been reported in kochia (*Kochia scoparia)* to Group 14 (PPO inhibitor) carfentrazone and Group 27 (hydroxyphenylpyruvate dioxygenase inhibitors) pyrasulfotole and mesotrione, as a result of a Tryptophan-574 amino acid substitution (Beckie et al. 2012a). Allogamous species can inherit more than one ALS mutation conferring resistance (Powles and Yu 2010). Substitutions for the Proline-197 amino acid (as numbered on the Arabidopsis ALS gene) are the most common (Powles and Yu 2010). ALS site of action mutations confer limited or no fitness penalties to the plant (Li et al. 2012; Yu et al. 2010). Noted exceptions are the Tryptophan-574-Leucine mutation in Powell amaranth (*Amaranthus powellii)* which had a resistance cost of 67% (Tardif et al. 2006) and a seed yield reduction, as well as Glycine-654-Glutamate in IMI-resistant rice crops (Vila-Aiub et al.

2009). The type and severity of these functionality and fitness effects depends on the individual mutation. Research regarding fitness effects of herbicide resistance mutations is limited due to complex requirements for validity of the experiment such as isogenic lines, life cycle effects and growing plants in a competitive environment (Vila-Aiub et al. 2009).

Genetically, resistant ALS alleles are semi-dominant over susceptible alleles with species variation in the degree of dominance (Tranel and Wright 2002). Gene flow of ALS-inhibitor resistance mutations occurs via pollen and seed, and mutations are therefore likely to be passed on to progeny (Tranel and Wright 2002).

2.3.3. Metabolic Resistance

Metabolic resistance occurs through a relatively more rapid breakdown of the herbicide to its inactive byproducts when compared to susceptible biotypes (Cotterman and Saari 1992). Herbicide metabolism is enzyme-mediated by enzymes including cytochrome P450 monooxygenases (Hall et al. 1994; Preston 2004), a superfamily of enzymes which are involved in hydroxylation and dealkylation of herbicides in plants (Powles and Yu 2010). Metabolic resistance is conferred through induced expression or upregulation of the genes encoding P450 enzymes. The enzymes may detoxify herbicides from multiple modes of action, including novel herbicides and herbicides never applied to the weed population (Powles and Yu 2010). The first case of ALS-inhibitor metabolic resistance was in Annual ryegrass (*Lolium rigidum)* also resistant to Group 1 or ACCase inhibitors in 1986 (Heap and Knight 1986), prior to the discovery of Group 2 site of action resistance. Resistance was selected for by ACCase inhibitor applications with cross-resistance conferred by the metabolism of ALS-inhibitor herbicides (Heap and Knight 1986). The ACCase inhibitor/ALS inhibitor cross-resistance is a common metabolic resistance pattern (Preston 2004). The specific upregulated P450 enzyme varies by herbicide group, and multiple enzymes may be upregulated in cases of multiple-resistance (Preston et al. 1996). Metabolic resistance is believed to be primarily due to partially dominant alleles of nuclear-encoded genes, although there are exceptions (Preston 2004). P450-mediated metabolic resistance is predominantly found in grassy species, although it has been confirmed in broadleaf species such as wild mustard (*Sinapis arvensis*) in Canada (Powles and Yu 2010; Veldhuis et al. 2000). Reports of metabolic resistance may be underestimated as site of action resistance is typically investigated first (Powles and Yu 2010). If found, further resistance mechanisms are seldom investigated (Powles and Yu 2010). *Lolium rigidum* resistant to ALS inhibitors through cytochrome P450 metabolism has been shown to exhibit a fitness penalty related to the resistance mechanism (Vila-Aiub et al. 2009). However, this observation has not been repeated.

2.3.4. False Cleavers Resistance to ALS-Inhibitor Herbicides

False cleavers (*Galium spurium*), hereafter referred to as cleavers, have increased in abundance since the 1970s and is the 9th most abundant weed on the Canadian prairies (Leeson et al. 2005). An annual weed of the Rubiaceae family, it is a common and competitive pest in wheat, canola and pea fields (Leeson et al. 2005). Cleavers have curved, hook-like spines on stems and bur-like seeds adapted for seed dispersal. The semi-prostrate, climbing stems can cause crop lodging and harvesting difficulties, and seeds are difficult to remove from crop seed like canola after (Malik and Vanden Born 1988). Cleavers can produce up to 3500 seeds per plant (Malik and Vanden Born 1988), leading to high seed bank inputs if not controlled.

ALS-inhibitor resistant false cleavers were first reported in Alberta in 1998 (Hall et al. 1998) and subsequently in Saskatchewan and Manitoba. A closely related species, *Galium aparine,* has exhibited ALS inhibitor resistance in China (Heap 2014; Sun et al. 2011). The initial population exhibited resistance due to a target-site mutation at the Proline-197 amino acid residue (Beckie et al. 2012b). Resistance was conferred through a dominant, nuclear encoded allele (Van Eerd et al. 2004). In addition, the initial population exhibited multiple-resistance to the auxin-like herbicide quinclorac (Hall et al. 1998; Van Eerd et al. 2004).

ALS-inhibitor resistant weeds are of particular concern in crops like peas and lentils which have limited herbicide options. Cleavers ALS-inhibitor resistance is increasing in incidence with up to 17% of fields surveyed in Alberta in 2007 exhibiting resistance (Beckie et al. 2013). A recorded increase in cleavers abundance (Leeson et al. 2005), in conjunction with increased resistance levels (Beckie et al. 2013) and continued applications of Group 2 herbicides, indicates that ALS-inhibitor resistant cleavers will be increasingly problematic in the future. Alternate control options, whether chemical, biological, or mechanical, are needed to counteract these increases.

2.3.5. Wild Oat Resistance to ALS-Inhibitor Herbicides

Wild oat is the most economically important weed in Canada, accounting for more crop yield losses and herbicide expenditures in western Canada than any other weed (Beckie et al. 2012c). It is the second most abundant weed on the Canadian prairies (Leeson et al. 2005) but the most abundant in pea crops (Leeson et al. 2005). This annual weed is found across Canada and in most temperate or semi-arid cropping areas of the world (Beckie et al. 2012c). It is a competitive weed, believed to be equally as competitive as wheat, and is particularly competitive for soil nitrogen (Beckie et al. 2012c). Wild oat produces between 20 and 1070 seeds per plant, which are viable in the seed bank for an average of 4-5 years (Beckie et al. 2012c).

ALS- inhibitor resistant wild oat was reported in Manitoba, Canada in 1994 (Heap 2014), and was subsequently reported in Alberta and Saskatchewan (Beckie et al. 1999). In a recent Prairie survey, ALS-inhibitor resistant wild oats were confirmed in 12% of fields where seeds were collected and in 8% of total surveyed fields (Beckie et al. 2013). This is an increased incidence of resistance compared to prior surveys (Beckie et al. 2013). Cross-resistant wild oat were also reported in this survey to group 1, 8 and 25 herbicides (Beckie et al. 2013). In North Dakota, a wild oat biotype resistant to imazamethabenz, a herbicide requiring metabolic activation within the plant, was resistant due to decreased activation, and increased metabolism to non-herbicidal metabolites (Nandula and Messersmith 2000). More recently, two wild oat populations in Canada became the first report of ALS target site mutations in *Avena* spp. with the discovery of 2 mutations at the Serine-653 residue of the ALS (Beckie et al. 2012d); however, the majority of resistance is likely a result of enhanced metabolism by cytochrome P450 monooxygenases (Beckie et al. 2012d). Cross-resistant weed biotypes are of concern as herbicide options are greatly limited.

2.3.6. Management of ALS-Inhibitor Resistance

Herbicide resistance can be managed using alternative herbicides and non-herbicide techniques. Herbicide groups have been classified by herbicide resistance selection risk (Beckie 2006); substituting lower risk groups for the ALS-inhibiting herbicides will delay resistance evolution. Herbicide mixtures or rotations of herbicide sites of action can effectively delay target site resistance (Beckie 2006; Beckie and Reboud 2009; Diggle et al. 2003). Multiple- or cross-resistant weeds have limited herbicide options; suspected resistant weeds should be tested to determine viable options (Beckie 2006). Non-residual herbicides generally have a lower resistance risk than residual herbicides which select for resistance over a longer time period (Beckie 2006). Herbicide-resistant crops can increase the herbicide modes of action in rotations, but can alter resistance selection (Beckie et al. 2009; Norsworthy et al. 2012). Using herbicide mixtures is common due to availability of co-formulated products and is effective only if the active ingredients are efficacious on the targeted weeds (Beckie 2006; Norsworthy et al. 2012). Herbicides must be applied at appropriate weed and crop stages and labelled rates (Norsworthy et al. 2012); herbicide rates below the label rate may increase selection for polygenic resistance (Norsworthy et al. 2012).

In addition, use of an Integrated Weed Management (IWM) system including cultural, mechanical, chemical and biological control options is beneficial. Crop rotation, particularly when forage legumes, fall crops, and competitive crops are included, can aid in management of weed populations (Blackshaw et al. 2008; O'Donovan et al. 2007; Thill et al. 1994). Using competitive crop cultivars and increased seeding rates (Blackshaw et al. 2008) as well as decreasing row spacing (Norsworthy et al. 2012; Thill et al. 1994) helps control weeds through increased competition for nutrients and light. Fertilizer placement (banding rather than broadcast operations) can improve crop competition for resources (Blackshaw et al. 2008; O'Donovan et al. 2007). Equipment sanitization and use of certified seed limits propagule dispersion of resistant weeds (Norsworthy et al. 2012; Thill et al. 1994). Management of propagule invasion from field edges, ditches and right-of-ways can aid in resistance management, however this is often complicated by the additional expenditure of resources and the unavailability of producer management in these areas (Norsworthy et al. 2012). Tillage may control resistant weeds, but would reduce the environmental gains made by no-till/reduced-till cropping systems (Thill et al. 1994). Biological control of weeds through the use of plant pathogens is an option that has been successfully used, but research and available options are limited (Charudattan 2001).

2.4. Alternative Herbicides

2.4.1. Group 15 herbicides

Group 15 or K3 herbicides include the chemical classes of the chloroacetamides, chloroacetanilides, oxyacetamides and tetrazolinones (Babczinski et al. 2012). The first herbicides in this group were introduced for use in corn and soybean in the early 1950s and 1960s (Böger et al. 2000). There are only 4 species resistant to group 15 herbicides (Heap 2014), suggesting it is a low risk group.

In 2000, Böger et al. reported that group 15 herbicides were inhibiting very long-chain fatty acid (VLCFA) synthesis. The elongation of fatty acids is a four-step process: a condensation reaction, two reduction reactions and a dehydratization that occur in the endoplasmic reticulum membrane in a four-enzyme elongase complex (Böger 2003). The herbicides are believed to compete with acyl-CoA (used in the fatty acid elongation) at the substrate site of the elongase enzymes (Böger et al. 2000). It is believed, based upon these results and structural examinations, that the herbicides interact with the elongase enzyme, through a nucleophilic attack on a conserved cysteine residue in the active site (Böger et al. 2000) in a time-dependent manner (Tanetani et al. 2011). While there is confidence that elongases are the enzymes being inhibited, which specific elongases has not been elucidated. Trenkamp et al. (2004) expressed known elongases from *Arabidopsis thaliana* in yeast to test inhibition of the elongases by a number of K_3 and N group herbicides, and demonstrated that the elongase inhibition varied depending on the applied herbicide, indicating potential elongase specificity.

VLCFA are components of cuticles, membranes, and plant pollen, and also function as energy storage for seeds (Babczinski et al. 2012). The lack of these fatty acids is assumed to be phytotoxic. A plasma membrane lacking VLCFA's will lose stability causing leakage and disrupting cell division and production, which will eventually lead to cell death (Böger 2003). Typically, group 15 herbicides do not inhibit germination, but growth of seedlings after emergence (Tanetani et al. 2009). Symptoms on seedlings can include leaf curling and rolling, a darker green colouring, epinasty and stunting.

Most group 15 herbicides are applied at pre-seeding or pre-emergence spray timings, but a few are applied post-emergence (Senseman 2007). As a group, the VLCFA elongase-inhibiting herbicides can be applied to a wide variety of crops including grain crops such as wheat and corn, as well as crops like lima beans, sugar cane and artichokes among many others (Senseman 2007). In Canada, they are mainly applied in corn and soybean. There are wheat-registered group 15 herbicides, but they are not commonly used in the Canadian prairies.

2.4.2. Pyroxasulfone

Pyroxasulfone [{3-{5-(difluoromethoxy)-1-methyl-3-(trifluoromethyl) pyroazol-4-ylmethylsulfonyl]-4,5-dihydro-5,5-dimethyl-1,2-oxazole}], or KIH-485, is an isoxazoline group 15/K3 herbicide that inhibits VLCFA elongases. Pyroxasulfone inhibited VLCFA accumulation in rice (Tanetani et al. 2009). Specific elongations steps inhibited by pyroxasulfone include C18:0 to C20:0, C20:0 to C22:0, C22:0 to C24:0, C24:0 to C26:0, C26:0 to C28:0, C18:1 to C20:1 as well as C20:1 to C22:1 (Tanetani et al. 2009). Barnyard millet (*Echinochloa frumentacea* Link) and Italian ryegrass (*Lolium multiflorum* Lam.) exhibited symptoms consistent with other Group 15/K3 herbicides (Tanetani et al. 2011) under pyroxasulfone application, aiding in confirmation of mode of action. Tanetani et al. (2011) also discovered that there may be multiple binding mechanisms available for pyroxasulfone; they have observed time-independent binding in addition to the time-dependent binding understood to occur for group 15 herbicides (Tanetani et al. 2011). Selectivity of pyroxasulfone is due to differential sensitivities of the VLCFA elongases between tolerant and susceptible species (Tanetani et al. 2011). Pyroxasulfone is registered on a number of crops and in a number of countries (Table 2-2).

Pyroxasulfone has a log K_{ow}, octanol-water partition co-efficient of 2.39, and an average K_{oc}, soil-organic carbon sorption co-efficient, of 113 mL g^{-1} (Westra 2012) indicating that it is relatively water soluble and has high mobility potential in soil water. It is a residual herbicide with an estimated half-life of between 8 and greater than 71 days (Mueller and Steckel 2011), however a field half-life of up to 134 days has been recorded (Westra et al. 2014). There is a strong correlation between organic matter and soil adsorption for pyroxasulfone, indicating that organic matter level and soil moisture can cause variable efficacy between years, locations, and weed species (Westra 2012). Influence of organic matter level and precipitation requirements make use of soil-applied herbicides like pyroxasulfone less predictable because efficacy can be variable between years, locations, and weed species. It has, however, been tested on organic matter content up to 80% and still conferred >90% control of common lambsquarters

(*Chenopodium album*), spiny amaranth (*Amaranthus spinosus*), and purslane (*Portulaca oleracea*) (Odero and Wright 2013).

Tolerance to pyroxasulfone varies by crop, but has been demonstrated in wheat, corn and soybean (Anonymous 2006). Winter wheat exhibited minimal injury and yield reductions at rates up to 150 g ai ha^{-1} when tested in Oregon (Hulting et al. 2012). Pea and lupin exhibited good crop tolerance when tested with pyroxasulfone in Australian crop production systems at rates up to 800 g ha^{-1} and approximately 400 g ha^{-1}, respectively (Walsh et al. 2011). Sunflower crops have also shown relatively good tolerance to PRE applications of pyroxasulfone up to 333 g ha^{-1} (Olson et al. 2011). Some sunflower injury did occur at the highest tested rates where heavy precipitation events occurred shortly after application, although yield was not affected in these cases (Olson et al. 2011). Tolerance of tested sweet corn hybrids is also sufficient to withstand pyroxasulfone applications up to 418 g ai ha^{-1}, although minimal visible injury was observed that was transient and not statistically significant (Sikkema et al. 2008b). Additional testing of sweet corn has found minimal injury at all rates with the exception of sites with coarse soil texture where injury greater than 20% began to occur at doses of 250 g ai ha^{-1} (Nurse et al. 2011). In these studies, yield was a maximum of 85% of the weed-free control, even with >90% weed control (Nurse et al. 2011). Potatoes also show good tolerance to pyroxasulfone at rates up to 150 g ha^{-1}, with only occasional minor injury and yield quantity and quality losses (Boydston et al. 2012). Pyroxasulfone at 125 g ai/ha caused unacceptable yield losses in barley as well as durum wheat and oats when tested in Ontario (Soltani et al. 2012). That study found that wheat was generally the most tolerant to pyroxasulfone applications, with barley, durum wheat and oats tending to showing increased injury (Soltani et al. 2012). Pyroxasulfone has also been tested on dry, pinto and small red Mexican bean, but had an inadequate margin of crop safety for use in these crops (Sikkema et al. 2007; Sikkema et al. 2008a; Soltani et al. 2009). Many minor crops would benefit from increased diversity due to pyroxasulfone applications, and so continued research and product registrations are ongoing.

Past studies with pyroxasulfone have shown that it provides effective control of a number of weed species. Pyroxasulfone has provided up to 90% control of both perennial ryegrass (*Lolium perenne*) and annual ryegrass (*Lolium rigidum*), including multiple-resistant biotypes of annual ryegrass with rates of 150 g ai ha^{-1} and higher (Hulting et al. 2012; Walsh et al. 2011). It also has shown excellent control (>90%) of barnyard grass (*Echinochloa crus-galli*), hairy nightshade (*Solanum sarrachoides*), redroot pigweed (*Amaranthus retroflexus*) and common lambsquarters (*Chenopodium album*) at rates of 150 g ai ha^{-1} (Boydston et al. 2012). Other grass weeds affected by pyroxasulfone include large crabgrass (*Digitaria sanguinalis*) with 90% control at around 150 g ai ha^{-1}, green foxtail (*Setaria viridis*) at rates around 125 g ai ha^{-1} and field sandbur (*Cenchrus incertus*), although rates over 300 g ai ha^{-1} were required to reach 90% control (Knezevic et al. 2009; Steele et al. 2005). Broadleaf weeds controlled by pyroxasulfone include velvetleaf (*Abultilon theophrasti*), and kochia (*Kochia scoparia*) which were controlled at >90% with a rate just above 200 g ai ha^{-1}, Palmer amaranth (*Amaranthus palmeri*) and puncturevine (*Tribulus terrestris*) with rates of 125 g ai ha^{-1}, tall waterhemp (*Amaranthus tuberculatus*) controlled over 90% with 150 g ai ha^{-1}, and wild buckwheat (*Polygonum convolvulus*) which required 250 g ai ha^{-1} for the same level of control (Geier et al. 2006; King and Garcia 2008; Knezevic et al. 2009; Steele et al. 2005). Pyroxasulfone only achieved suppression of longspine sandbur (*Cenchrus longispinus*) with rates over 330 g ai ha^{-1} (Geier et al. 2006). Herbicide efficacy on these weeds is affected by soil type, soil organic matter and soil moisture, with control levels being variable by site and year (King and Garcia 2008; Knezevic et al. 2009; Walsh et al. 2011). Determining crop tolerance and efficacy on weeds in the Canadian prairie environment, particularly those that are resistant to group 2 herbicides, is key for pyroxasulfone registration in Canada and the use of pyroxasulfone to control and delay herbicide resistance.

Busi et al. (2012) has reported the selection of multiple-resistant annual ryegrass (*Lolium rigidum)* populations cross-resistant to pyroxasulfone with low-doses within three generations. Pyroxasulfone-resistant populations were also cross-resistant to prosulfocarb and triallate (Busi and Powles 2013). This suggests that Canadian weeds such as wild oat which exhibit multiple-resistance may be at risk of developing resistance to pyroxasulfone, particularly in response to low dose selection.

2.4.3. Group 14 Herbicides

Group 14, or class E herbicides inhibit protoporphyrinogen oxidase (Protox) (PPO inhibitors) and include herbicides from the diphenylether, *N*-phenylphthalamide, oxadiazole, and triazinone chemical classes (Mallory-Smith and Retzinger 2003). Group 14 herbicide use in Canadian crops was limited to soybean and corn, until recent registration of sulfentrazone and saflufenacil in pea, lentil and chickpea (Senseman 2007).

Group 14 herbicides inhibit Protox, the final common enzyme between the chlorophyll and heme synthesis pathways (See Figure 2-1) (Dayan and Duke 1997; Duke et al. 1991). Protox is responsible for oxidizing protoporphyrinogen IX (Protogen) to protoporphyrin IX (Proto) through the sequential removal of six hydrogens (Boger and Wakabayashi 1999). This reaction takes place in the chloroplast membrane. Proto is then rapidly progressed into the heme or chlorophyll production pathway by iron or magnesium chelatase, respectively (Dayan and Duke 1997). Group 14 herbicides compete with Protogen for binding on Protox , inhibit Protox and cause an accumulation of Protogen within the chloroplast membrane (Jacobs and Jacobs 1993; Matringe et al. 1989). Excess Protogen diffuses out of the chloroplast membrane and to the cytoplasm, where it interacts with a herbicide insensitive enzyme on the plasma membrane with Protox-like activity and is converted into Proto (Lee et al. 1993). Proto is a photodynamic substance that reacts with light to form an excited state compound (Dayan and Duke 1997). Ground state or triplet oxygen, which has a reversed spin electron, can react with excited state compounds to form toxic oxygen radicals (Devine et al. 1993). Oxygen, including oxygen radicals, is lipophilic and will partition into lipid membranes where transfers of electrons and hydrogen atoms cause lipid peroxidation (Devine et al. 1993). In PPO inhibitor-treated cells, peroxidation begins with the plasma membrane causing cell leakage, loss of membrane integrities, and eventually cell death (Orr and Hess 1981).

Symptoms of group 14 herbicide applications include cupping, crinkling, bronzing, and necrosis, with most damage in rapidly growing tissues (Dayan and Duke 1997). Symptoms appear quickly, with initial appearance of water-soaked spots and colour change occurring within a few to 24 hours after application (Dayan and Duke 1997).

The group 14 herbicides are diverse in terms of physical and chemical properties. Most PPO-inhibiting herbicides are applied POST although some are applied PRE or PPI (prior to planting, and incorporated) and have residual weed control (Senseman 2007).

Only six species have confirmed PPO inhibitor resistance worldwide, and 4 of the 6 species are resistant to more than one herbicide group (Heap 2014), probably as a result of enhanced metabolic degradation.

2.4.4. Sulfentrazone

Sulfentrazone is a group 14 herbicide registered for use in western Canada as Authority by FMC. Authority is registered in chickpea, field pea, flax and sunflower with application rates ranging from 219-292 mL ha^{-1} dependent on soil texture and soil organic matter. Sulfentrazone is registered for control of kochia, lamb's quarters, redroot pigweed (*Amaranthus retroflexus*), and wild buckwheat. It is applied either PPI or PRE and requires moisture for the herbicide to desorb from soil colloids, be dissolved in the soil solution, and available for uptake by plants. It is also registered in the United States by FMC as Spartan in soybean, sugarcane, tobacco, cabbage, tomato, horse radish, strawberry, lima bean, mint and sod.

Sulfentrazone has a K_{oc} of 43mL g^{-1}, a K_d of <1 mL g^{-1}, and low to intermediate soil sorption (Senseman 2007). The adsorption of sulfentrazone decreases as soil pH increases, and is also affected by soil type, likely due to organic matter levels (Grey et al. 1997). It has a relatively long soil half-life that ranges between 121 and 302 days, depending on soil organic matter and precipitation after application as well as other abiotic factors that affect rates of microbial degradation, its primary method of breakdown (Senseman 2007). Sulfentrazone is moderately mobile in soil, has low oral toxicity and is considered slightly toxic to fish and aquatic invertebrates (Grey et al. 1997; Senseman 2007).

Additionally, numerous weeds including cleavers (*Galium aparine*), chickweed (*Stellaria media*), and various *Amaranth spp.* are registered for control with Spartan in the United States. Further research and registration of sulfentrazone on Canadian weeds may allow an enhanced niche in current crop markets.

Table 2-1 Factors affecting the rate of evolution of herbicide resistance. (Adapted from Powles and Yu, 2010)

Genetic

1. Frequency of resistance genes
2. Number of resistance genes (monogenic versus polygenic)
3. Dominance of resistance genes
4. Fitness cost of resistance genes

Biology of Weed Species

1. Autogamous vs allogamous
2. Seed production capability
3. Seed longevity in soil seedbank
4. Seed/pollen dispersal capacity

Herbicide

1. Herbicide metabolism
2. Site of action
3. Residual activity
4. Efficacy (Tranel and Wright 2002)

Producer Management

1. Herbicide dose
2. Frequency of use of herbicide group
3. Use of herbicide mixtures (Neve 2007; Tranel and Wright 2002)
4. Skills of the operator (treatment machinery, timing, environmental conditions, etc)
5. Agro-ecosystem factors (non-herbicide weed control practices, crop rotation, agronomy, etc.)

Table 2-2 Registered Uses of Pyroxasulfone

Country	Trade Name	Active Ingredients	Application Rate (g ha^{-1})	Crops
Australia	Sakura	Pyroxasulfone	118	Wheat & Triticale
United States	Zidua	Pyroxasulfone	70-280 (Crop and soil texture dependent)	Corn & Soybean
	Fierce	Pyroxasulfone & Flumioxazin	210-263	Corn & Soybean
Canada	Focus	Pyroxasulfone & Carfentrazone	100-150 Pyroxasulfone & 14-22 Carfentrazone	Corn
	Fierce	Pyroxasulfone & Flumioxazin	N/A	Pending registration on soybean

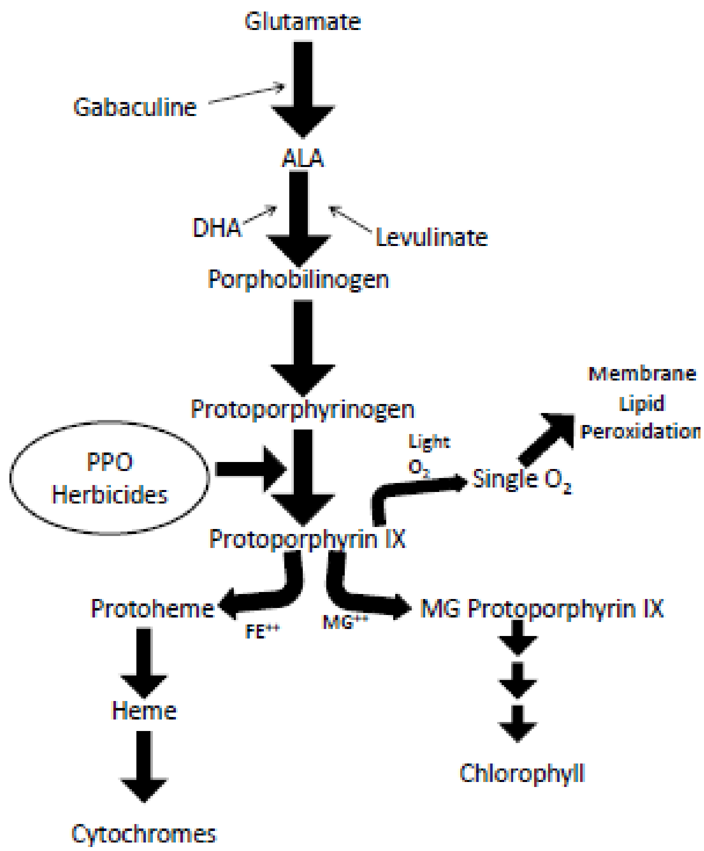

Figure 2-1 Heme and chlorophyll pathways as inhibited by protoporphyrinogen oxidase-inhibiting herbicides. Adapted from (Duke et al. 1991).

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Chapter Three: Efficacy of Fall- and Spring-Applied Pyroxasulfone For Herbicide-Resistant Weeds in Field Pea[1]

3.1. Introduction

Herbicide-resistant weeds occupy an estimated 29% of agricultural farmland in the Canadian prairies (Beckie et al. 2013). In western Canada, weed resistance to acetolactate synthase (ALS) inhibitors (group 2/B) is most widespread; 19 species including wild oat (*Avena fatua* L.), kochia (*Kochia scoparia* (L.) Schrad), chickweed (*Stellaria media* L.), and cleavers (*Galium* spp.) have been identified (Heap 2013). Beckie et al. (2013) conducted a randomized survey of 1,000 fields and screened weeds with imidazolinone and sulfonylurea herbicides, two structural classes of ALS inhibitors. In Alberta fields, 12% of wild oat and 17% of cleavers sampled were ALS inhibitor-resistant. Wild oat in Alberta have also been selected for resistance to acetyl-CoA carboxylase (ACC) inhibitors (group 1/A) and thiocarbamate herbicides (group 8/Z) (Heap 2013); cleavers have also shown multiple resistance to the group-4 auxinic herbicide quinclorac (Hall et al. 1998). Resistance to these herbicides greatly reduces the herbicide options available to control these weeds in western Canadian crops.

Field pea was seeded on over 1.3 million ha of diverse agro-ecological regions with varying organic matter and precipitation in the Canadian prairies in 2012 (Statistics Canada 2012). Pea is a valuable crop and is included in crop rotations for disease and pest cycle disruptions, improved soil tilth, and nitrogen fixation (Park et al. 1999). Pea is relatively non-competitive when compared to cereal crops, and is less suited to simple integrated weed management (Harker 2001). Semi-leafless peas are typically grown in western Canada, are less competitive than the full-leafed varieties, and therefore require weed control from herbicides to limit yield

A version of this paper has been accepted for publication and is available online: Tidemann BD, Hall LM, Johnson EN, Beckie HJ, Sapsford KL, Raatz LL. 2014. Weed Technol: 28: (in press) loss from weed competition (Harker 2001). Currently, weed control in pea is typically accomplished by the ALS-inhibitor herbicides imazamox or imazethapyr, due to good crop tolerance, convenient application timings, and control of both grass and broadleaf weeds. Other options include group 3/K1 PRE-applied mitotic inhibitors (dinitroanilines), ethalfluralin and trifluralin, a PRE-applied group 14/E protoporphyrinogen oxidase (PPO) inhibitor sulfentrazone, and POST applications of photosystem-II inhibitors metribuzin (group 5/C1) and bentazon (group 6/C3). ALS inhibitor-resistant weeds threaten the viability of cropping systems like pea that relies on ALS-inhibiting herbicides for weed control. Control of resistant weeds in pea could be improved through increased herbicide options with diverse modes of action.

Pyroxasulfone is a potential herbicide for use in field pea. It is a group 15/K3 herbicide that limits production of very long chain fatty acids (VLCFA) through interference with VLCFA elongases (VLCFAE) (Tanetani et al. 2009). It is a soil-applied PRE herbicide registered for use prior to wheat (*Triticum aestivum* L.) in Australia, and for use prior to corn (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr.) in the United States. The use of VLCFAE inhibitors has selected for resistance in only four species, significantly fewer than ALS inhibitors with 131 species resistant worldwide (Heap 2013).

Like other soil-applied herbicides, pyroxasulfone efficacy and use rates can be affected by edaphic factors. Pyroxasulfone has a log K_{ow}, octanol/water partition co-efficient, of 2.39 and a moderate K_{oc}, soil/organic carbon sorption co-efficient, of 113 mL g^{-1} (Westra 2012), indicating that it is relatively water-soluble and has high mobility potential in soil water. It has an estimated half-life in soil of between 8 and >71 d (Mueller and Steckel 2011). There is a strong correlation between organic matter and soil adsorption of pyroxasulfone, indicating that organic matter content and soil moisture can cause variable efficacy between years, locations, and weed species (Westra 2012). Soil organic matter increases pyroxasulfone binding to soil colloids (Westra 2012), thus decreasing herbicide efficacy.

Tolerance to pyroxasulfone varies by crop. Wheat, corn, and soybean are tolerant to 118 g ai ha^{-1} (Anonymous 2006), while winter wheat showed minimal injury or yield reductions at rates up to 150 g ai ha^{-1} (Hulting et al. 2012). Pea and faba bean (*Vicia faba* L.) exhibited tolerance when tested with pyroxasulfone in Australian crop production systems at rates up to 800 g ai ha^{-1} (Walsh et al. 2011). Sweet corn hybrids were sufficiently able to withstand pyroxasulfone applications up to 418 g ai ha^{-1}, although minimal visible injury was observed that was transient and not statistically significant (Sikkema et al. 2008). However, on high organic matter soils, sweet corn displayed no injury at rates up to 1000 g ai ha^{-1} (Odero and Wright 2013). Potato (*Solanum tuberosum* L.) also showed tolerance to pyroxasulfone at rates up to 150 g ai ha^{-1} with minor injury, yield reduction, and quality losses (Boydston et al. 2012). Pyroxasulfone at 125 g ai ha^{-1} caused unacceptable yield losses in barley (*Hordeum vulgare* L.) as well as durum wheat and oat (*Avena sativa* L.) in Ontario (Soltani et al. 2012). They reported that of cereals tested, wheat was generally the most tolerant to pyroxasulfone, with barley, durum wheat, and oat tending to show more injury (Soltani et al. 2012). Sunflower (*Helianthus annuus* L.) has also exhibited acceptable tolerance to pyroxasulfone up to 333 g ai ha^{-1}, although injury (but not yield loss), did occur at locations with heavy precipitation events shortly after application (Olson et al. 2011). Therefore, tolerance may not only be related to rate, but also to soil moisture and organic matter content.

Pyroxasulfone has provided up to 90% control of Italian ryegrass (*Lolium perenne* ssp. *multiflorum*), with efficacy varying with differing environmental conditions (Hulting et al. 2012). Rigid ryegrass (*Lolium rigidum* Gaud.), including multiple-resistant biotypes, has also been controlled up to 90% with rates of 150 g ai ha^{-1} and higher, although efficacy was influenced by soil organic matter (Walsh et al. 2011). Pyroxasulfone has also shown excellent control (>90%) of barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.), redroot pigweed (*Amaranthus retroflexus* L.) and common lambsquarters (*Chenopodium album* L.) at rates of 150 g ai ha^{-1}, although efficacy differed between locations and years (Boydston et al. 2012). Green foxtail (*Setaria viridis* (L.) Beauv.) was also controlled (>90%) at approximately 125 g ai ha^{-1}, although efficacy was found to vary by soil type; higher rates were needed on higher organic matter soils for equivalent control (Knezevic et al. 2009). Broadleaf weeds controlled better than 90% by pyroxasulfone include kochia ,with a rate just above 200 g ai ha^{-1} , and wild buckwheat (*Polygonum convolvulus* L.), which required 250 g ai ha^{-1}, although environmental factors such as precipitation affected efficacy (King and Garcia 2008).

The objectives of this study were to (1) determine pyroxasulfone rates required to control false cleavers (hereafter referred to as cleavers) and wild oat in field pea when applied PRE in western Canada, (2) determine if soil organic matter content affect the efficacy of pyroxasulfone on cleavers and wild oat or the tolerance of field pea, and (3) compare efficacy of fall and spring applications and determine which application timing provides the greatest weed control and least crop injury.

3.2. Materials and Methods

3.2.1. Trial Location and Design

Weed efficacy and crop tolerance of pyroxasulfone were examined in five locations: Scott, Kernen Field Facility near Saskatoon, and Melfort, Saskatchewan (SK), and Kinsella and Ellerslie Research Station in Edmonton, Alberta (AB). Organic matter content ranged from 2.9% at Scott to 10.6% at Ellerslie, representing the majority of the soil organic matter range in the peaproducing areas of western Canada (Table 3-1). Soil pH ranged between 6.1 and 7.1 with the exception of soil at Scott that was more acidic with a pH of 5.0. Soil textures ranged from a sandy loam at Kinsella with 55% sand and 13.6% clay, to a clay loam at Melfort with 22% sand and 32% clay.

Split-plot size ranged from 10 m^2 at Scott to 8.4 m^2 at Ellerslie. The trial was arranged in a split-plot design with four replicates, where the main plot was application timing (fall or spring) and the split-plot was herbicide rate. Pyroxasulfone 85 WDG (FMC Agricultural Products, Philadelphia, PA, USA) was applied at 0, 50, 100, 150, 200, 300, and 400 g ai ha^{-1} directly to the soil in the fall and PRE in the spring. Fall herbicide timings were applied from October 11th to 21st, 2011. Spring herbicide timings were applied between May 12th and 18th, 2012. Prior to spring applications of pyroxasulfone, glyphosate was applied to all treatments to control winter cleavers and allow equal comparison of fall and spring applications. Visual ratings evaluated winter annual control prior to spring applications. Herbicide application equipment varied by location, although all locations used 100 L ha^{-1} of water. Applications nozzles at Ellerslie and Kinsella, AB were Teejet XR 110015 nozzles, at Scott and Kernen, SK they were flat fan Air Mix 80015 nozzles and at Melfort, SK they were Teejet 8001 nozzles.

Field pea, cultivar CDC Patrick, was seeded at approximately 180 kg ha^{-1} at all locations between May 15th, 2012 at Ellerslie and Scott to June 1st at Kernen. Seeding depths were 2, 3, 4, 5 and 5 cm at Melfort, Scott, Kernen, Ellerslie and Kinsella sites, respectively. Direct seeding occurred at all locations with residue removal operations happening in the fall at Ellerslie (rake) and Melfort (harrow), and in the spring at Scott (tooth harrow). Seeding was delayed at Kernen due to precipitation. Wild oat and cleavers were seeded just below the surface at the same time as crop seeding using separate seed runs to supplement natural populations at Ellerslie, Scott, and Kinsella using local seed sources. At Kernen, wild oat was also seeded, while cleavers were broadcast on the site and incorporated by harrowing just prior to seeding of the crop. Weeds were seeded at approximately 100 seeds m^{-2} for wild oat and 150 seeds m^{-2} for cleavers. Although wild oat and cleaver populations were not tested for ALS resistance, we assume no cross resistance between ALS resistant biotypes and pyroxasulfone, therefore allowing extrapolation of our results.

3.2.2. Data Collection

Weed and crop fresh weights were determined by harvesting aboveground material in two 0.25 m^2 randomly placed quadrats per subplot excluding outside rows to prevent data skewing due to edge effects. Fresh weights were sampled between July 6th at Ellerslie and July 20th at Kernen. Peas were between 9 nodes and early podfill, wild oats were between three leaf and flag leaf, and cleavers were between 1 whorl and 10 whorls at the time of biomass collection. The biggest range occurred between sites, with less variability within each location. Fresh weights were converted to a percent of the nontreated check.

Trials were harvested between August 21st at Scott and September 17th, 2012 at Kernen to determine crop seed yield. Plot-harvesting combines were used at each location, with harvest widths ranging from 1.8 to 2 m, for the entire length of the plot (5-7m site dependent). Crop seed samples were cleaned, and yield was expressed as a percentage of the nontreated check. Yield was not determined at Melfort because of crop mortality throughout the trial as a result of late season precipitation.

3.2.3. Statistical Analysis

For statistical analysis, the trial becomes a split-split plot design where location is main plot, timing is split plot and rate is split-split plot. This allows analysis of the location effect as well.

Analysis of Variance was conducted using Proc Mixed in SAS 9.2 (SAS Institute Inc., 2007) for weed fresh weights, where location, rate, application timing, location x rate, location x application timing, location x rate x application timing, and rate x application timing were considered fixed effects and replicate, replicate x location, and replicate x location x timing were considered to be random. Square root transformations were applied to the cleavers and wild oat weights to improve normality and homogeneity of variance. Dose-response curves were generated with the use of the *drc* package (Ritz and Streibig 2005) in the open source language R (R version 2.15.2, R Development Core Team 2012). Although other models were tested, the log-logistic three parameter model gave the best fit for this data. Data were fit to the log-logistic three parameter model (Equation 1), as suggested by Knezevic et al. (2007) where *e* is the ED_{50} (effective dose reducing biomass by 50%), the upper limit is *d*, and *b* describes the slope around *e*. Parameter reduction was performed by setting *d* to be equal for all curves. Validity of this reduction was tested through an ANOVA comparison of the two model fits, and found to be valid. ED_{50}s were generated by R as a function of the curve fitting, and statistical differences found using the SI function in *drc* and comparing to an $\alpha = 0.05$. ED_{50}s were then plotted against soil organic matter content from each location to determine significant relationships. With an apparent linear relationship, the data were fit to a linear model in R to evaluate the strength of the relationships using R^2 values (Equation 2). In Equation 2, *y* is the estimated ED_{50}, *m* is the slope of the line, *x* is the organic matter content and *b* is the intercept.

$$Y = d/[1+\exp\{b(\log x - \log e)\}] \qquad [1]$$

$$Y = mx + b \qquad [2]$$

Crop fresh weight response to pyroxasulfone rates was inconsistent at most locations, however a curvilinear response to rate was observed at Scott indicating crop injury. Because crop injury was observed only at Scott in fresh weight data, the model was reduced to exclude all other locations and ANOVA was conducted to determine whether timing was significant. Crop fresh weights at Scott were fit to a log-logistic three parameter curve as given in Equation 1, and an ED_{50} generated to determine the rate causing 50% crop injury.

Crop seed yields were also subject to ANOVA. Because of a rate by location interaction, each location was tested separately for a rate effect. Yield from the Scott location was then fitted to a log-logistic three parameter curve as given by Equation 1, and an ED_{50} estimated. Yield at Kernen was also affected by rate, however, the data did not converge when fitted to a log-logistic three parameter curve. Locations without rate effects were pooled and Least Squares Means (LSMeans) generated.

3.3. Results and Discussion

3.3.1. Growing season weather

Soil moisture was adequate at all locations at the time of treatment applications, however, Kernen and Scott received nearly 200% of the long-term average precipitation between May and August, creating abnormally wet growing conditions (Table 3-1). Average temperatures throughout the growing season were similar among locations (Figure 3-1).

3.3.2. Cleavers Control

Control of cleavers, as quantified by fresh weight, was significantly different between locations, but was not different between fall and spring application timings. There was no interaction between rate and time of application. Fall and spring application fresh weights were therefore pooled for each location. Fresh weight responses to increasing rates were used to generate dose-response curves and ED_{50} values for each location.

Cleavers were controlled at all locations, but the effective rate differed by location. Cleavers ED_{50}s ranged from 53 ± 9 g ai ha^{-1} at Scott to 395 ± 79 g ai ha^{-1} at Ellerslie (Figure 3-2A, Table 3-2). Comparison of ED_{50} values among locations indicate that values were usually different, with the exception of Melfort where variability was high due to abnormal growing conditions (Table 3-3).

3.3.3. Wild Oat Control

Wild oat control, as quantified by fresh weight, differed among locations and rates, although not between application timings. However, there was a significant interaction between location and application timing and subsequently data were analyzed separately for application timings and locations. Fresh weight responses to increasing rates were used to generate dose-response curves and ED_{50} values for both application timings at each location.

Wild oat fresh weight ED_{50} estimates based on the pyroxasulfone dose response varied between 0.54 ±2.8 g ai ha^{-1} at Scott in the fall and 410 ± 84g ai ha^{-1} in spring at Melfort (Figure 3-2B&C; Table 3-4). The response of wild oat to spring applied pyroxasulfone at Melfort is affected by a higher than expected outlier at the 300 g ai ha^{-1} rate. This outlier is unexplained but is responsible for an estimate of ED_{50} at this location that is likely higher than reality. The ED_{50} estimates at Scott were variable because the estimated ED_{50} is less than the lowest tested rate of 50 g ai ha^{-1}. ED_{50} estimates were significantly different between fall and spring pyroxasulfone applications at Kernen, Kinsella and Melfort. In Melfort and Kinsella, the fall treatments provided a significantly lower ED_{50}, indicating that the fall applications required lower rates for 50% reduction in fresh weight. Spring treatments provided a lower ED_{50} than the fall treatments at Kernen, contrary to the other locations. In these experiments, the effect of application timing may have been confounded by environmental factors.

Results indicate that while pyroxasulfone can effectively control both wild oat and cleavers, wild oat are generally better controlled than cleavers when comparing ED_{50} values at each site. Pyroxasulfone should control biotypes of these weeds that express ALS-inhibitor resistance, assuming no cross-resistance to the VLCFAE inhibitor herbicides. Application timing does not significantly impact the control of these weeds in a consistent manner, however, the highest control levels of both weeds can be expected at locations with low soil organic matter and in years with high amounts of precipitation (Eric Johnson, unpublished data).

3.3.4. Crop Tolerance and Yield

Pea injury was not observed at Ellerslie, Kinsella or Melfort. Chlorosis and stunting were observed in Kernen at the 300 and 400 g ai ha^{-1} rates of pyroxasulfone (data not shown). Visual injury at Kernen was not reflected in fresh weight measurements as pea biomass of those treated plots remained higher than those of the nontreated check, because of weed competition. Injury was also observed at Scott, where application timing was found to be non-significant and data were therefore pooled. When fit to Equation 1, an estimated 50% reduction in fresh weight occurred at 244 ± 73 g ai ha^{-1} of pyroxasulfone. In previous years, studies using rates up to 200 g ai ha^{-1} of pyroxasulfone caused no injury at either location (Eric Johnson, unpublished data). Injury is more likely due to high rainfall reducing herbicide binding to the soil and increasing herbicide activity, particularly at locations with lower organic matter content.

Pea seed yield, expressed as a percentage of the nontreated check, differed among locations, and was significantly affected by pyroxasulfone rate; there was also a rate by location interaction. Yield was not significantly affected by application timing and yields were pooled at each location. At Ellerslie and Kinsella, rate did not significantly influence yield due to a lower level of control and low weed populations, respectively. At Kernen, yield at rates greater than

150 g ai ha^{-1} were significantly greater than the nontreated check due to decreased weed competition (Figure 3-3). However, in the plots treated with 300 or 400 g ai ha^{-1}, yields decreased slightly compared to lower rates due to injury, while still remaining higher than the nontreated check (Figure 3-3). Crop yields at Scott decreased as rate increased. Yields were reduced by as much as 63% (Figure 3-3). A 50% yield reduction was estimated to occur at 340 ± 104 g ai ha^{-1} at Scott. Yield reductions are contrary to previous studies evaluating pea tolerance to pyroxasulfone at this location (unpublished data).

Yield reductions at Scott and Kernen, the two locations with the lowest organic matter content, are likely due to abnormally high amounts of precipitation during the growing season. This is supported by a technical bulletin for pyroxasulfone from Kumiai, the initial product developer, which suggests that in moisture-saturated soils, injury can be severe (Anonymous 2006). In this study, injury was not transient. Crop injury from pyroxasulfone may be expected when seasonal rainfalls are high, and herbicide becomes mobile in the soil solution, particularly those soils with low organic matter. In years with near-normal precipitation, injury from pyroxasulfone prior to field pea is not expected, as reported by Walsh et al. (2011) for Australian conditions. However, rainfall is an unpredictable factor because large amounts of precipitation can increase the chance of crop injury. Under these conditions, there is increased risk of crop damage and loss to the producer when using this product.

3.3.5. Relationship Between Soil Properties, Efficacy and Crop Tolerance

Both weed efficacy of pyroxasulfone and pea crop tolerance were affected by trial location, particularly the soil properties specific to each location. Cleavers and wild oat ED_{50} values varied by 7.4- and 746-fold, respectively. As soil organic matter content increases, the rate required to reduce cleavers biomass by 50% increases (Figure 3-4). When fitted to a linear model, an R^2 of

0.40 indicated that organic matter explained a considerable amount of variation among cleavers fresh weight ED_{50}s. Where soil organic matter is high, a higher proportion of pyroxasulfone binds to soil and is unavailable for plant uptake; more herbicide is required for similar levels of control (Westra 2012). Available soil moisture also modifies the herbicide partitioning between soil colloid and solution. Melfort, where soil was saturated, showed an increase in cleavers efficacy relative to Ellerslie with similar organic matter content. Alternatively, weeds and crops growing in wet soil may be under stress and more susceptible to herbicide effects. Soil texture can affect herbicide availability and leaching potential. Kinsella, while similar in organic matter content to Kernen, had a higher proportion of sand (55%), possibly providing better drainage and/or herbicide removal by leaching, as has been previously suggested (Boydston et al. 2012).

Soil properties also affected wild oat efficacy. A similar relationship between fresh weight reduction and soil organic matter is apparent for wild oat, although there is less variation in estimated ED_{50} values in comparison to cleavers (Figure 3-5). The linear model fits more closely to the observed relationship with an R^2 of 0.69. This indicates a significant influence of organic matter on pyroxasulfone efficacy against wild oat. Higher organic matter locations required higher rates for an equal level of wild oat control. Soils with higher organic matter tended to show less efficacy of pyroxasulfone on wild oat, as was predicted based on previous soil binding observations (Westra 2012). The rate recommended to producers will likely vary based on organic matter, similar to other registered soil applied herbicides.

3.4. Conclusion

Pyroxasulfone may be used to control cleavers and wild oat in field pea. Rates of application will vary by site organic matter and there is potential for injury under certain environmental conditions. Only four VLCFAE inhibitor-resistant weeds have currently been identified worldwide (Heap 2013), however, recent studies have shown that rigid ryegrass resistant to other herbicide groups quickly develops resistance to pyroxasulfone as well (Busi et al. 2012). Weeds such as wild oat already resistant to ALS inhibitors may evolve resistance to pyroxasulfone and other VLCFAE inhibitors given recurrent selection with these herbicides. Pyroxasulfone will not solve the problem of herbicide-resistant weeds in field pea, but can be used in a diverse, integrated weed management program to assist in their management.

Table 3-1 Soil properties and precipitation data for trial locations.

Location	Soil OM	Soil PH	Accumulated from May-Aug	Long term Average (LTA)	% of LTA	Soil Classification	Soil Texture		
	-%-		----------------mm	--------------	--%--		---------%---------		
							Sand	Silt	Clay
Scott	2.9	5.0	384	200	192	Orthic Dark Brown Chernozem	37	46	17
Kernen	4.3	7.1	414	209	198	Orthic Dark Brown Loam	20	49	31
Kinsella	5.5	6.4	288	249	116	Orthic Black Chernozem	55	31	14
Melfort	10.5	6.1	333	241	138	Orthic Black Chernozem	22	46	32
Ellerslie	10.6	6.7	314	298	105	Eluviated Black Chernozem	30	46	24

Table 3-2 Estimated parameters based on log-logistic regression analysis of cleavers biomass response to increasing rates of pyroxasulfone at each trial location.

Location	Estimated ED_{50} (SE)	Slope (SE)	Upper limit (SE)
	---g ai ha^{-1}---		√(% nontrt check)
Scott	53 (9)	3.2 (2.0)	
Kernen	154 (27)	2.3 (0.9)	
Kinsella	321 (48)	2.5 (2.6)	9.4 (0.7)
Melfort	188 (66)	0.8 (0.4)	
Ellerslie	395 (78)	2.3 (1.1)	

Table 3-3 Pair-wise comparison of locations for cleavers ED_{50} estimates. P-values less than α=0.05 indicates significant differences.

Location 1	Location 2	p-value
Ellerslie	Kernen	0.0153
Ellerslie	Kinsella	0.4320
Ellerslie	Melfort	0.1667
Ellerslie	Scott	0.0007
Kernen	Kinsella	<0.0001
Kernen	Melfort	0.5277
Kernen	Scott	0.0050
Kinsella	Melfort	0.2470
Kinsella	Scott	0.0001
Melfort	Scott	0.0522

Table 3-4 Estimated parameters based on log logistic regression analysis of wild oat biomass response to increasing rates of pyroxasulfone at each location and application timing. P-values less than α=0.05 indicate significant differences between fall and spring estimates at that location.

Location	ED_{50} (Standard Error)		p-value for Fall vs Spring comparison	Slope (Standard Error)		Upper limit (Standard Error)
	Fall	Spring		Fall	Spring	
	---g ai ha^{-1}---					√(% nontrt check)
Scott	0.54 (2.8)	4.2 (16.5)	0.3019	0.2 (0.2)	0.6 (0.7)	9.6 (0.3)
Kernen	181.0 (19.7)	77.7 (36.3)	0.0388*	3.1 (1.1)	1.3 (0.5)	
Kinsella	70.6 (36.3)	162.3 (26.7)	0.0151*	0.6 (0.3)	1.5 (0.5)	
Melfort	150.5 (34.4)	410.4 (84.1)	<0.0001*	0.9 (0.3)	35.3 (281.7)	
Ellerslie	329.0 (65.4)	270.9 (57.6)	0.5334	1.5 (0.4)	1.2 (0.4)	

*Indicates significant differences between fall and spring ED_{50} estimates

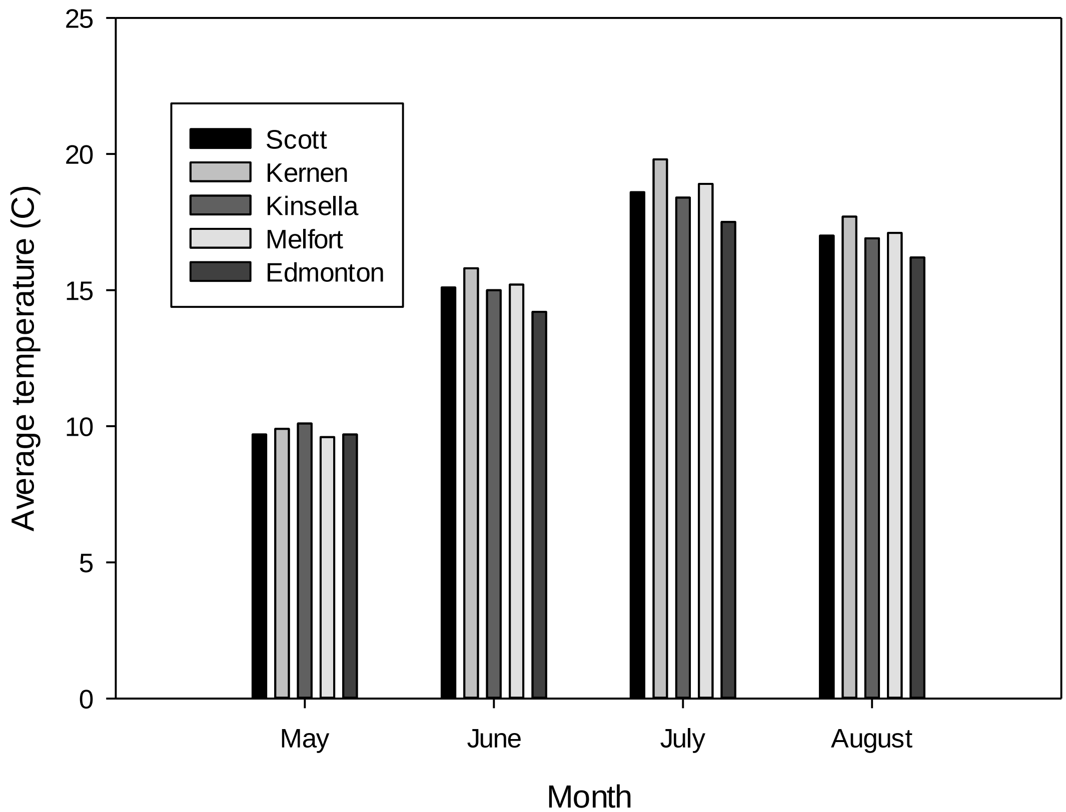

Figure 3-1 Mean monthly air temperatures during the growing season at each trial location.

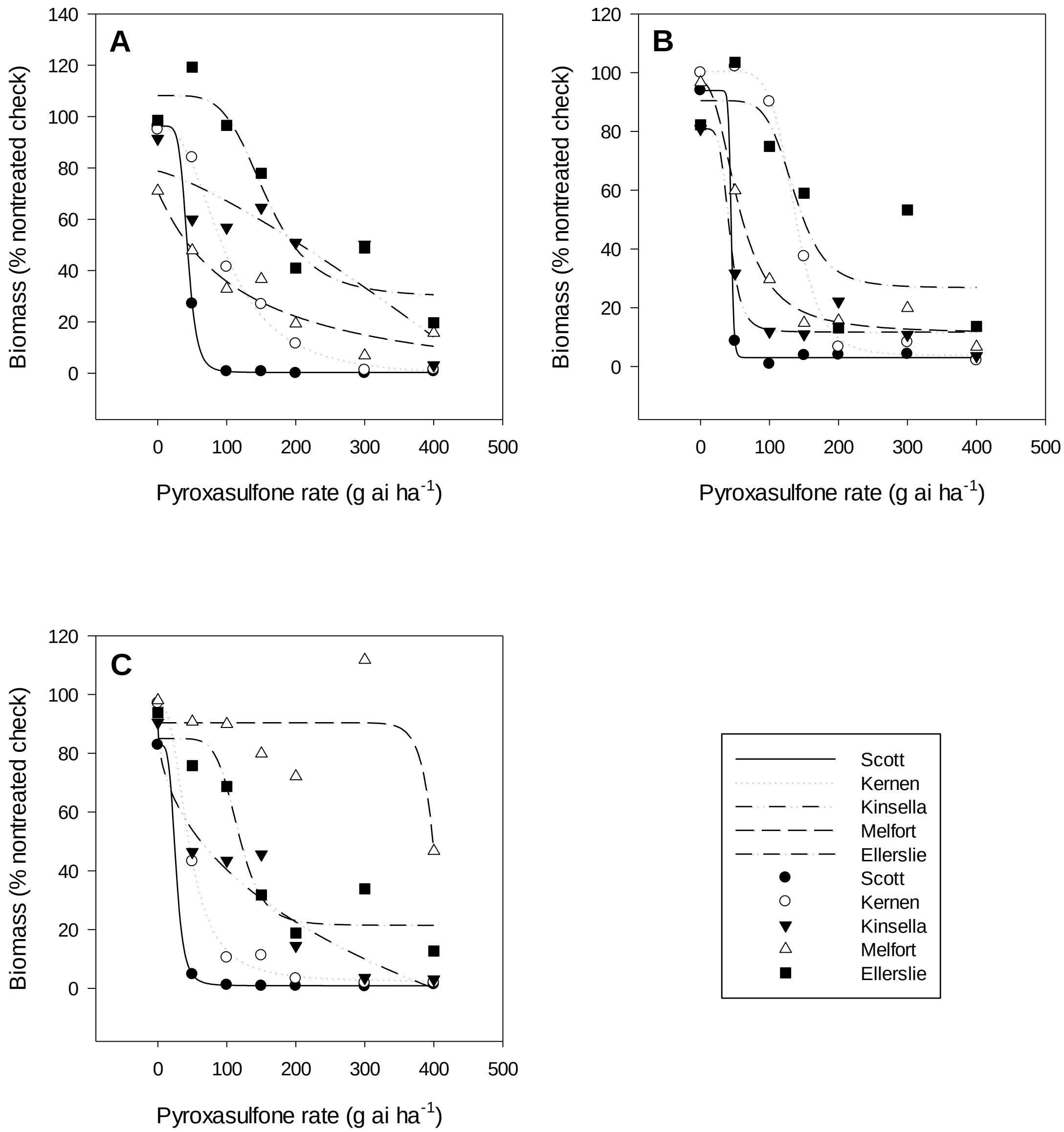

Figure 3-2 Biomass as a percent of the untreated check response to increasing pyroxasulfone rates based on non-linear regression fit to a log-logistic three parameter curve model; $Y = d/[1+\exp\{b(\log x - \log e)\}]$. Data is backtransformed from the square root transformation. **A**. Cleavers response to pyroxasulfone **B**. Wild oat response to pyroxasulfone, fall application **C**. Wild oat response to pyroxasulfone, spring application

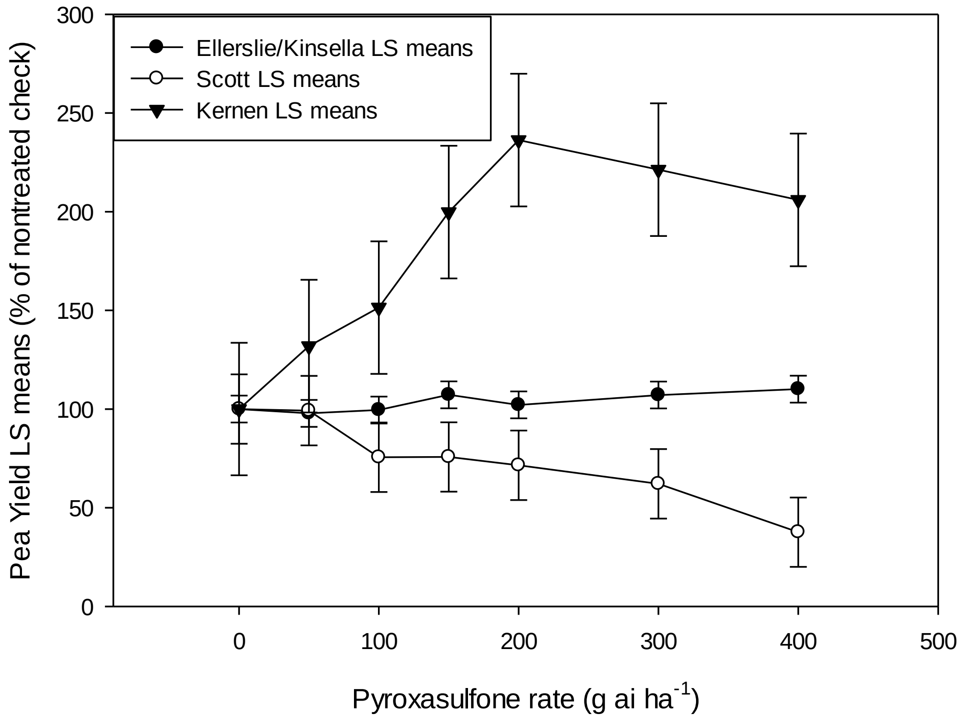

Figure 3-3 Pea yield least squares (LS) means as a percentage of the nontreated check as affected by pyroxasulfone rate at trial locations (error bars indicate SE).

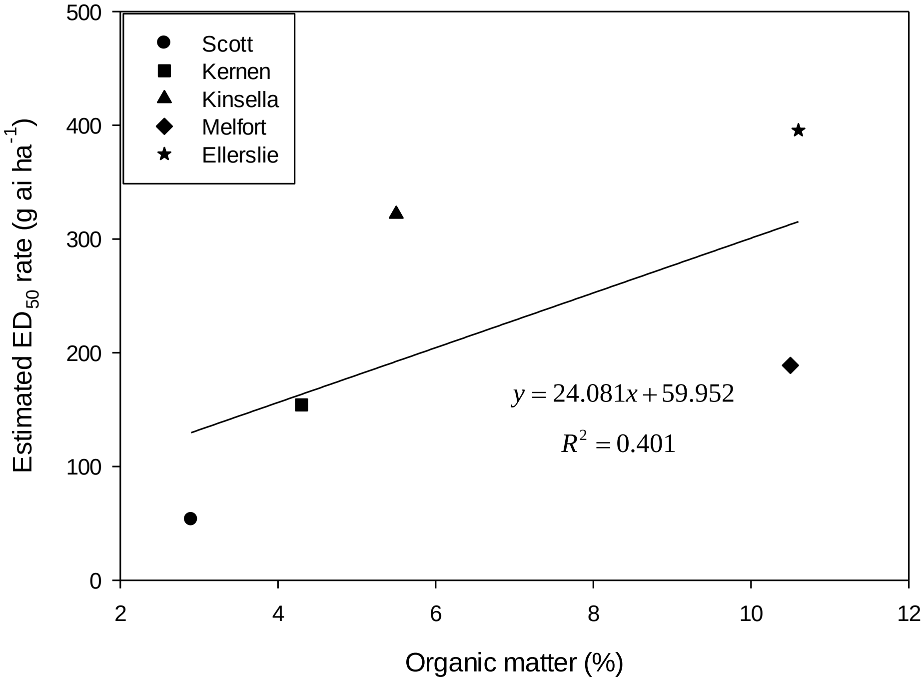

Figure 3-4 Cleavers ED_{50} values at each trial location fit to a linear model (*y=mx+b)* against soil organic matter at the respective locations.

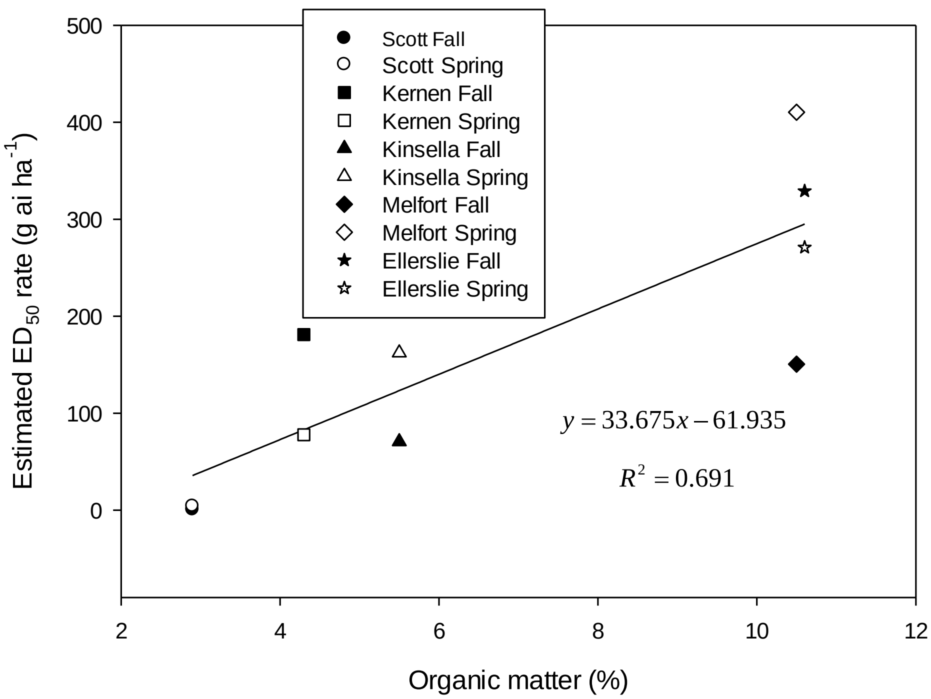

Figure 3-5 Wild oat ED_{50} values at each trial location and application timing, fit to a linear model (*y=mx+b)* against soil organic matter at the respective locations.

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Chapter Four: Efficacy of Pyroxasulfone and Sulfentrazone as Affected by Edaphic Factors, and Their Interaction When Applied to Soils of Western Canada

4.1. Introduction

Herbicide resistant weeds occupy 29% of Canadian agricultural farmland, and are increasing in incidence on the Canadian prairies (Beckie et al. 2013). In western Canada, 19 confirmed species including wild oat (*Avena fatua* L.) and false cleavers (*Galium spurium*) (hereafter referred to as cleavers) are resistant to acetolactate synthase (ALS) inhibitors (Heap 2014). Weeds resistant to ALS inhibitors are of particular concern in crops with limited herbicide options such as field pea. Pea is an important western Canadian crop that depends primarily on ALS inhibitors imazamox and imazethapyr for weed control and is not easily managed through simple integrated weed management systems. Expanded herbicide options for field pea would allow for management of ALS herbicide resistant weeds.

Sulfentrazone is a recently registered protoporphyrinogen oxidase (PPO inhibitor) for use in field pea. It is a PRE-emergence or PRE-seeding (PRE) applied herbicide that does not require incorporation. Registered rates in western Canada vary based upon soil texture, soil organic matter and pH. Pyroxasulfone is a new PRE Very Long Chain Fatty Acid Elongase (VLCFAE) inhibitor herbicide that also does not require incorporation, currently unregistered in western Canada. It is being evaluated for weed control and crop tolerance in peas (Tidemann et al. 2014), wheat and winter wheat. Pyroxasulfone is reported to have efficacy on lamb's quarters, barnyardgrass, and redroot pigweed (Boydston et al. 2012), green foxtail (Knezevic et al. 2009), wild oat, and cleavers (Tidemann et al. 2014). Pyroxasulfone efficacy is affected by organic matter and soil moisture (Westra 2012; Tidemann et al. 2014). A pyroxasulfone and sulfentrazone mixture is being investigated for use in pea in western Canada for management of herbicide resistant weeds. Combined products can broaden the weed control spectrum as well as reduce selection for herbicide resistant weeds (Beckie 2006). Herbicides applied in combination potentially interact in one of 3 ways: antagonistically, synergistically or additively. Interactions can be determined through comparison of observed plant responses with expected responses calculated through the Colby equation (Colby 1967). Comparison of means is most accurate using rates that individually exert approximately 50% control (Colby 1967).

Efficacy of soil-applied herbicides is influenced by soil properties and soil moisture through adsorbtion and desorbtion to soil colloids (Blumhorst et al. 1990; Rahman et al. 1978): higher organic matter soil has higher adsorbtive potential and requires higher herbicide rates for efficacy (Rahman 1976; Rahman et al. 1978). Soil moisture is necessary for redistribution of herbicide in soil, herbicides to be in solution, and for the plant to take up herbicide in the soil solution (Walker 1971). Soil-herbicide interactions are determined by the herbicide's physical chemical properties (Rahman et al. 1978). Octanol-water partition co-efficients, K_{ow}s, which describe herbicide solubility, are log K_{ow}=2.39 for pyroxasulfone (Westra 2012) and K_{ow}=9.8 (log Kow= 0.99) for sulfentrazone (Senseman 2007), indicating that sulfentrazone is much more soluble in water. Pyroxasulfone has an average K_{oc}, soil-organic carbon sorption co-efficient, of 113 mL g^{-1} (Westra 2012) while sulfentrazone has a K_{oc} of 43mL g^{-1}(Senseman 2007), indicating that pyroxasulfone is more highly absorbed to soil colloids than sulfentrazone, and therefore may be more affected by soil organic matter levels. Therefore, pyroxasulfone and sulfentrazone efficacy may be differentially influenced by soil properties. In order to clarify the efficacy of mixtures and the effects of edaphic factors, field and greenhouse experiments were conducted.

The objectives were (1) to determine efficacy of a pyroxasulfone and sulfentrazone co-application and determine their interactions when co-applied, (2) further investigate herbicide interactions in a controlled environment limiting confounding environmental factors, (3) determine the influence of soil moisture on pyroxasulfone and sulfentrazone efficacy and (4) determine the influence of organic matter on pyroxasulfone and sulfentrazone efficacy.

4.2. Materials and Methods

4.2.1. Field study

Trials were conducted at Scott, Kernen, and Choiceland, Saskatchewan (SK) and Edmonton, Alberta (AB) in 2011 and were repeated at Scott and Edmonton in 2012. Organic matter ranged from 2.9-12.6% between sites, along with a variance in soil texture and pH (Table 4-1). Trials were designed as a RCBD factorial of pyroxasulfone and sulfentrazone rates with 4 replicates. Pyroxasulfone 85 WDG (FMC Agricultural Products, Philadelphia, PA, USA) was applied at 0, 80, 100, 150, and 200 g ai ha^{-1} (280 g ai ha^{-1} at Edmonton 2011). Sulfentrazone 480 SC (FMC Agricultural Products, Philadelphia, PA, USA) was applied at 0, 105, 140, and 280 g ai ha^{-1}. Viper (BASF, Mississauga, ON, Canada) was applied at the label rate (70 g ai ha^{-1} imazamox, 420 g ai ha^{-1} bentazon, 2 g ai ha^{-1} UAN, and 0.25 % v/v AgSurf).

Seeding and herbicide application varied between sites (Table 4-2). Weeds, where seeded, were planted with target rates of 150 and 100 seeds m^{-2} of cleavers and wild oat, respectively. Cleavers populations at Kernen and Scott (2011 and 2012) was limited. Fertility requirements were addressed on a location basis through soil characterizations.

Visual ratings were collected using a 0-100% scale where 0% is no control and 100% is complete control. Aboveground weed and crop fresh weights were collected in two 0.25 m^2

quadrats per plot excluding outside rows to prevent skewing of data due to edge effects. Fresh weights were sampled between July 4 and August 11 in 2011 and July 3 and August 15 in 2012. Fresh weights were not collected at Kernen in 2011, and were delayed in Choiceland in 2011 and Scott 2012 due to precipitation. Fresh weights were converted to a percent of the non-treated check to allow for comparison between sites and Colby means comparison for interactions (see below). Trials were harvested between August 22 and September 15 in 2011 and on August 28 in 2012. Yields were unavailable at Kernen 2011, Choiceland 2011 and Scott 2012 due to flooding and hail damage.

Fresh weight data was tested for normality and homogeneity of variance in SAS 9.3 (SAS Institute Inc., 2007) and was not improved by transformation. Data was analyzed with a Proc Mixed ANOVA where site-year, treatment and the site-year*treatment interaction were fixed effects and rep was random. Site-years were separated due to significance, and Proc Mixed ANOVA's run with treatment as a fixed effect and replicate as random. Least squares (LS) means estimates for each herbicide treatment were used to determine Colby means for investigation of potential herbicide interactions. Colby means are calculated as (Equation 1)(Colby 1967):

$$E = X_1 Y_1 \div 100 \qquad [1]$$

where E is the expected biomass as a percent of the non-treated check when both herbicides are applied, X is biomass as a percent of the non-treated check of the weed species when treated with pyroxasulfone at rate '1', and Y is the biomass as a percent of the non-treated check of the weed species when treated with sulfentrazone at rate '1'. Expected Colby means were compared to observed means to determine the nature of interaction of pyroxasulfone and sulfentrazone. Significance was determined using 95% confidence intervals where differences were significant if larger than 3 times the standard error.

4.2.2. Greenhouse Studies

4.2.2.1. Interaction. Greenhouse investigation into pyroxasulfone and sulfentrazone interaction was conducted twice using soil collected from the Duchess Research Station in Alberta in 2011 and 2012 in each of two repetitions of this experiment, respectively (Table 4-1). Pyroxasulfone was applied at 7 and 40 g ai ha^{-1}, sulfentrazone at 7 g ai ha^{-1}, and both pyroxasulfone rates in combination with sulfentrazone, as well as a non-treated check. Treatments were tested on four species: barley, canola, cleavers and wild oat. The trial was designed as a RCBD with 4 replications.

Soil was homogenized in a mechanical soil mixer and placed in 3.5" pots. Barley, canola and wild oat were seeded at a density of 5, 5, and 6 seeds per pot in the first repeat and 10, 6, and 6 seeds per pot in the second repeat. Cleavers were seeded at a density of 6 seeds per pot, and reseeded by 10 seeds per pot in the first repeat, and seeded at 10 seeds per pot in the second repeat. Target density was five plants per pot for all species.

Herbicides were applied using a moving track cabinet chamber sprayer calibrated for 200 L ha^{-1} at 207 kPa using an Air Bubble Jet 11-015 nozzle within 24 hours of seeding. Shoots were removed after 3-4 weeks and dried in a 46^{o}C air dryer for three days minimum and weighed.

ANOVA's were conducted on untransformed data using Proc Mixed in SAS 9.3 as transformations provided no distribution improvement. Fixed factors included trial repeat, herbicide treatment and the trial repeat*herbicide treatment interaction while replicate was random. Least squares means estimates for each herbicide combination and trial repeat were derived, converted to a percent of the non-treated check and used to estimate Colby means using Equation 1 above.

4.2.2.2. Soil Moisture. Greenhouse study investigating soil moisture effects on pyroxasulfone and sulfentrazone efficacy was conducted on soil collected at Duchess 2012 (Table 4-1). The trial was designed as strip plot with herbicide and soil moisture as factors. Herbicide treatments were pyroxasulfone at 20 g ai ha^{-1}, sulfentrazone at 20 g ai ha^{-1}, and a non – treated check. Soil moisture treatments were 100% (saturated), 80% and 50% of field capacity. Species tested were similar except cleavers, which was substituted by green foxtail.

Soil was homogenized in the mechanical mixer and 3.5" pots filled with 530g of dry soil for a total weight of 550g. Water was added to saturated treatment pots and allowed to equilibrate. Soil field capacity (FC) was determined by allowing free water to drain from saturated pots and then weighing. The water needed to increase from dry to 100% FC was calculated by subtracting the saturated pot weight from the dry pot weight and multiplying by 80% and 50% respectively to determine amount of water to be added to the dry pots to reach their soil moisture levels. Pots were weighed daily and watered to soil moisture level. Leachate was captured in a petri dish under each pot to allow for re-absorbtion between watering.

Species were seeded at rates of 12 seeds per pot for barley, canola and green foxtail, and 15 seeds per pot for wild oat with a target plant density of 10 plants per pot. Herbicide applications were applied as described above. Shoot dry weights were acquired as described above.

Data were analyzed in SAS 9.3 using a Mixed model ANOVA on untransformed data where herbicide, soil moisture and the herbicide*soil moisture interaction were fixed effects and replicate, replicate*herbicide and replicate*soil moisture were random effects.

4.2.2.3. Organic Matter. Effects of organic matter on pyroxasulfone and sulfentrazone efficacy were investigated on 3 field soils. Duchess soil characterization varied between trial repeats while Kernen and Edmonton were consistent (Table 4-1). The trial was designed as a RCBD where pyroxasulfone and sulfentrazone were tested on all soils at 3 rates specific to soil organic matter plus a non-treated control (Table 4-3), with 3 replicates in each of the 2 trial repeats. Species tested were barley, canola, wild oat, and green foxtail.

Potting and seeding occurred as described above. Green foxtail was seeded at 20 and 25 seeds per pot in the first and second run, respectively. All other seeds were seeded at 15 and 20 seeds per pot in the first and second run, respectively. Targeted plant density was 10 plants per pot. Herbicide applications and sampling of dry shoot weights occurred as described above.

Data were analyzed in SAS 9.3 using a Mixed model ANOVA where trial repeat, soil type, herbicide and rate, along with their interactions were fixed effects and replicate was random. If trial repeat or any of its interactions were significant, repeats remained separate for non-linear regression, or pooled when they were non-significant. Non-linear regression was conducted using R (R vers 3.0.2, R development core team 2013) and fitting data to an exponential decay curve with 2 or 3 parameters (Equation 2 and 3, respectively) as appropriate based on lack of fit model tests and estimate fits in **R**.

$$Y = d\exp^{\frac{-x}{e}} \qquad [2]$$

$$Y = c + (d - c)\exp^{\frac{-x}{e}} \qquad [3]$$

where Y is the plant biomass, x is the herbicide rate applied, c is the biomass plateau, d is the intercept and e is the rate of decay.

4.3. Results

4.3.1. Field Study

Control of cleavers varied among locations. Visual ratings (data not shown) at Scott in 2011 indicated 100% control of cleavers with all pyroxasulfone and sulfentrazone combinations. In contrast in Edmonton in 2011, control was never higher than suppression (60% efficacy) with any herbicide rate combination. In Edmonton in 2012 cleavers were controlled (80% efficacy) by 200 g ai ha^{-1} of pyroxasulfone in combination with 280 g ai ha^{-1} of sulfentrazone, the highest rates of both herbicides.

Wild oat control also varied among locations. Visual ratings (data not shown) of wild oat control at Edmonton in 2011 was never higher than 50% control. In 2012, suppression (60% efficacy) was achieved with the herbicide combination of 100 g ai ha^{-1} of pyroxasulfone and 280 g ai ha^{-1} of sulfentrazone. In Scott in 2011 wild oats were suppressed with the herbicide combination of 200 g ai ha^{-1} of pyroxasulfone and 140 g ai ha^{-1} of sulfentrazone. In 2012, 85% control of wild oats was recorded with the 100 g ai ha^{-1} of pyroxasulfone, 140 g ai ha^{-1} of sulfentrazone combination.

For both species, herbicide combinations with higher rates were required for control at locations with higher organic matter soils. In the locations where the experiment was conducted in two years, control was better in the year with higher precipitation (Table 4-4) early in the season. At Edmonton in 2011, herbicide treatment did not affect cleavers or wild oat biomass significantly, regardless of herbicide or rate, likely due to limited precipitation and herbicide activation early in the growing season (Table 4-4).

4.3.1.1. Interaction. Least squares (LS) means estimates of plant biomass as a percent of the non-treated check and their standard error were compared to calculated Colby Means (Tables 4-5 & 4-6). Means were compared at Edmonton 2012 and Choiceland for cleavers. The interaction of pyroxasulfone and sulfentrazone on cleavers was additive in all comparisons at both sites, with no indication of antagonism or synergy. Wild oat LS means and Colby means were compared at Edmonton 2012, Choiceland and Scott (both years). Consistent with cleavers, most comparisons indicated an additive interaction while 3/48 comparisons indicated antagonism. It was previously reported that pyroxasulfone/sulfentrazone mixtures showed no antagonism although comparisons were not conducted using the Colby Method (Olson et al. 2011).

4.3.2. Greenhouse Studies

4.3.2.1. Interaction. Herbicide interactions on cleavers control were not investigated in the greenhouse due to poor emergence. For all other species, experiment repeats were significantly different. LS means of dry weights for each herbicide treatment and combination were estimated separately for each repeat and compared to Colby means (Table 4-7).

For canola, Colby means for herbicide combinations in both trials were within the 95% confidence interval of LS means indicating an additive interaction. For wild oat, Colby means were also within the confidence interval of LS mean estimated dry weights indicating an additive interaction, with one exception indicating antagonism. However, the treatment indicating antagonism had no efficacy on plant dry weight making a comparison for interaction inaccurate (Colby 1967) and the indication likely false. For barley, the second trial repeat resulted in no herbicide efficacy in any treatment so mean comparisons are not useful due to insensitivity (Colby 1967). In the first repeat, mean comparisons indicated no significant differences between LS means for dry weights and Colby means, again indicating an additive interaction. Results on all species in the greenhouse are consistent with those of the field study indicating that interactions between pyroxasulfone and sulfentrazone are additive.

4.3.2.2. Soil Moisture. Canola data were not analyzed due to poor plant emergence. Barley and wild oat biomass were significantly affected by soil moisture but not by herbicide treatment. For green foxtail, soil moisture and the interaction of soil moisture and herbicide treatment were significant, however applied herbicides had minimal to no effect on plant dry weight. The results from this experiment were not useful in determining the effect of soil moisture on pyroxasulfone efficacy. This is likely attributable to the highly basic nature of the soil (pH=8.7) being toxic to plants and confounding effects of herbicide applications. This experiment should be repeated with another low organic matter soil with a neutral pH to characterize the influence of soil moisture on pyroxasulfone and sulfentrazone efficacy.

4.3.2.3. Organic Matter. Where dry weights were significantly different between trial repeat or it’s interaction with soil organic matter or herbicide, data were analyzed separately (canola, wild oat, barley). Where there was no significant difference, data were pooled. Duchess soil was removed as a treatment because of the inhibition of growth, presumably due to high pH. Barley showed a lack of response to either herbicides, herbicides rates or soil type and were not analyzed further. Data were best fit to either a two parameter (16 of 19 regressions) or a 3 parameter exponential decay curve.

The ED_{50}s for pyroxasulfone on canola (Table 4-8) were differentially affected by soil organic matter with the highest ED_{50} rates on Edmonton soil which had the highest organic matter. Differences in the ED_{50}s between soil types was nearly 100 g ai ha^{-1} or higher, although there is no significant difference between them or within either soil type between repeats.

Sulfentrazone ED_{50}s on canola (Table 4-8) are also differentially affected by soil organic matter with the highest ED_{50}, a rate higher than tested, on Edmonton soil. Between soil types there is >100 g ai ha^{-1} difference in ED_{50}s, however, this difference is not significant. ED_{50}s are also not significantly different within soil types between repeats. Canola demonstrated the widest range of ED_{50}s to both herbicides, and, with the exception of the rate higher than tested, canola response to pyroxasulfone is more varied than to sulfentrazone, likely as a result of differences in K_{oc}s and K_{ow}s in the herbicides.

The ED_{50} values for pyroxasulfone on wild oat (Table 4-9) were higher at Edmonton, the higher organic matter soil, but while the repeats did not significantly differ with soil type, only one of the Kernen repeats was different than Edmonton soils. The ED_{50} for sulfentrazone on wild oat (Table 4-9) was less clear. Over 171 g ai ha^{-1} was required for 50% dry weight reduction at the low organic matter soil. Sulfentrazone is not registered for wild oat control (FMC Corporation 2014).

Green foxtail was easily controlled by both herbicides (Table 4-10). Pyroxasulfone ED_{50}s on green foxtail ranged from 0.02 to 0.26 g ai ha^{-1} as a result. Although the ED_{50} on Edmonton (high OM) soil is an order of ten higher than on Kernen soil they are not significantly different. The sulfentrazone ED_{50}s on green foxtail (Table 4-10) differ by over 100 g ai ha^{-1} between soil types with the higher ED_{50} on Edmonton soil, although there are no significant differences.

Overall there is a consistent trend of higher organic matter soil requiring higher rates for the same level of efficacy, although there is a lack of significance between ED_{50} estimates. There are also species-specific responses to the herbicides. Green foxtail was the most sensitive species to both herbicides with ED_{50} values ranging from 0.02 to 0.26 for pyroxasulfone and 1 to 172 for sulfentrazone, depending on organic matter. Wild oat is intermediate with ED_{50} values from 22 to 146 for pyroxasulfone and 171 to 332 for sulfentrazone, depending on the organic matter of the soil. Canola has the widest range of ED_{50}s of 5 to 332 for pyroxasulfone and 93 to greater than the highest tested rate for sulfentrazone. Differences in species' response to herbicides indicates that prediction of effective rates may vary with both site organic matter and species spectrum. This work supports observations by Westra (2012), and Tidemann et al.(2014) that pyroxasulfone efficacy is related to soil organic matter level, and hypotheses on sulfentrazone efficacy based on herbicide type and properties. Further investigations with an expanded range of tested rates, and additional soil types could quantify the effect of organic matter on efficacy of both pyroxasulfone and sulfentrazone.

4.4. Conclusions

There is no evidence for synergy or antagonism between pyroxasulfone and sulfentrazone and their interaction is additive on weeds controlled by both. Additivity has been previously reported in sunflower crops (Olson et al. 2011). However, pyroxasulfone and sulfentrazone have different weed spectrums and co-application can broaden the spectrum controlled in pea. The lack of synergy or antagonism is not unexpected as there is no obvious link in these herbicides' mode of action (VLCFAE and PPO inhibition). Their activity, however, may be linked due to their water solubility and requirement for moisture to be effective. Additional research on soil moisture effects on herbicide efficacy should be conducted to resolve the confounding effects of soil used in this study. Additionally, while the organic matter study shows the trend of higher levels of soil organic matter requiring higher rates for equal control levels, additional investigation is required to quantify the influence of organic matter on pyroxasulfone and sulfentrazone efficacy. This study provides a basis and a range within which to target rates. Use of these herbicides effectively add infrequently used modes of action to crop rotations and can aid in management of herbicide resistant weeds, however integrated weed management and responsible use of these herbicides is necessary to maintain their effectiveness in Western Canada.

Table 4-1 Soil characteristics for field and greenhouse studies.

Site/year	Organic Matter	pH	Soil Texture			Soil Class
	------%------		-----------%----------			
			Sand	Silt	Clay	
Scott 2011	3.5	6	38	40	22	Loam
Scott 2012	2.9	6	37	46	17	Clay loam
Choiceland 2011	3.3	6.8	N/A	N/A	N/A	Dark grey wooded loam
Kernen 2011	5.2	7.2	19	36	45	Silty clay loam
Edmonton 2011	12.6	6.1	23	47	30	Clay loam
Edmonton 2012	10.2	6.6	32	43	25	Loam
Duchess 2011	1.7	6.3	81	11	8	Loamy sand
Duchess 2012	3.1	8.7	68	23	9	Sandy loam
Duchess 2013A	2.8	9.2	65	21	14	Sandy loam
Duchess 2013B	2.9	7.7	69	18	13	Sandy loam
Kernen 2013	5.5	7.9	24	40	36	Clay loam
Edmonton 2013	12.3	6.5	36	31	33	Clay loam

Table 4-2 Dates and details of trial activities specified by location.

Site	PRE App. Date	In-Crop App. Date	Nozzles	Water volume	Pea Cultivar	Seeding Rate	Seeding Date	Weed population	Tillage
Scott (2011)	May 13	June 6	80-015 Airmix	100	CDC Meadow	200 kg/ha	May 13	Seeded	Spring tooth harrow
Kernan	May 11	June 28	Flatfan Airmix 015	100	CDC Golden	170 kg/ ha	May 16	Natural	Tilled
Choiceland	May 12	July 7	Flatfan Airmix 015	100	CDC Golden	170 kg/ha	May 26	Natural	N/A
Edmonton (2011)	May 18	June 13	Teejet XR110015	100	CDC Patrick	180 kg/ha	May 18	Seeded	None
Scott (2012)	May 9	June 8	110-015 Airmix	100	CDC Patrick	180 kg/ha	May 11	Seeded	Spring tooth harrow
Edmonton (2012)	May 14	June 8	Teejet XR110015	100	CDC Patrick	180 kg/ha	May 15	Seeded	Rake

Table 4-3 Greenhouse organic matter treatment list for soil type, herbicide and herbicide rate.

Treatment	Soil	Herbicide	Rate
			---g ai ha^{-1}---
1	Duchess	Pyroxasulfone	0
2			25
3			50
4			75
5	Duchess	Sulfentrazone	0
6			25
7			50
8			75
9	Kernen	Pyroxasulfone	0
10			75
11			150
12			225
13	Kernen	Sulfentrazone	0
14			75
15			150
16			225
17	Edmonton	Pyroxasulfone	0
18			200
19			300
20			400
21	Edmonton	Sulfentrazone	0
22			125
23			250
24			375

Table 4-4 Precipitation at field trial locations conducted in two sequential years.

Location	Precipitation				
	----------------mm (% of Long Term Average)----------------				
	April	May	June	July	August
Scott 2011	10.4 (41)	30.8 (86)	190.2 (300)	76.2 (107)	51.8 (120)
Scott 2012	38.4 (163)	50.6 (141)	164.6 (263)	56.4 (80)	51.4 (119)
Edmonton 2011	19.2 (73)	15.6 (31)	128.2 (147)	150.4 (158)	10.8 (15)
Edmonton 2012	44.2 (168)	37.7 (76)	72.4 (83)	104.8 (110)	79.6 (113)

Table 4-5 Field study cleavers least squares (LS) means and Colby means comparisons from plant biomass as a % of nontreated check. Means are significantly different when the difference is larger than three times the standard error.

Treatment	Choiceland 2011		Edmonton 2012	
	LS mean (SE)	Colby mean	LS mean (SE)	Colby mean
	---% of nontreated check---		--% of nontreated check--	
Pyroxasulfone 80 Sulfentrazone 105	15.2 (21.4)	1.1	45.9 (19.9)	34.3
Pyroxasulfone 80 Sulfentrazone 140	8.4 (21.4)	0	33.5 (18.5)	23.3
Pyroxasulfone 80 Sulfentrazone 280	0 (21.4)	0.2	12.1 (19.9)	12.2
Pyroxasulfone 100 Sulfentrazone 105	1.5 (21.4)	0.6	28.2 (18.5)	18.9
Pyroxasulfone 100 Sulfentrazone 140	10.1 (21.4)	0	34.4 (18.5)	12.9
Pyroxasulfone 100 Sulfentrazone 280	0 (21.4)	0.1	7.5 (18.5)	6.7
Pyroxasulfone 150 Sulfentrazone 105	20.0 (21.4)	0.8	25.1 (19.9)	3.4
Pyroxasulfone 150 Sulfentrazone 140	4.1 (21.4)	0	22.2 (19.9)	4.2
Pyroxasulfone 150 Sulfentrazone 280	1.5 (21.4)	0.2	13.4 (19.9)	3.4
Pyroxasulfone 200 Sulfentrazone 105	8.2 (21.4)	0.7	27.4 (19.9)	19.4
Pyroxasulfone 200 Sulfentrazone 140	18.5 (21.4)	0	53.7 (18.5)	13.2
Pyroxasulfone 200 Sulfentrazone 280	0 (21.4)	0.1	16.8 (18.5)	6.9

Table 4-6 Wild oat field study LS means and Colby means comparisons from plant biomass as a % of nontreated check. Means are significantly different when the difference is larger than three times the standard error.

Treatment	Choiceland		Edmonton 2012		Scott 2011		Scott 2012	
	LS mean (SE)	Colby mean	LS mean (SE)	Colby mean	LS mean (SE)	Colby mean	LS mean (SE)	Colby mean
----- g ai ha^{-1}-----	--% of nontreated check--		--% of nontreated check--		--% of nontreated check--		--% of nontreated check--	
Pyroxasulfone 80 Sulfentrazone 105	26.5 (19.1)	30.7	165.6 (21.3)	60.9*	46.6 (9.2)	51.9	5.2 (8.4)	3.3
Pyroxasulfone 80 Sulfentrazone 140	46.2 (16.8)	35.3	84.5 (18.7)	99.3	47.2 (9.2)	45.1	5.9 (8.4)	2.4
Pyroxasulfone 80 Sulfentrazone 280	67.9 (19.0)	18.4	115.9 (21.3)	66.5	39.8 (9.2)	44.4	4.8 (8.4)	1.7
Pyroxasulfone 100 Sulfentrazone 105	17.2 (19.0)	34.5	89.2 (18.7)	28.9*	60.3 (9.2)	31.6*	3.5 (8.4)	3.3
Pyroxasulfone 100 Sulfentrazone 140	27.8 (16.8)	39.7	70.2 (18.7)	47.1	45.0 (9.2)	27.5	7.4 (8.4)	2.4
Pyroxasulfone 100 Sulfentrazone 280	55.0 (16.8)	20.6	50.3 (18.7)	31.6	45.3 (9.2)	27.1	5.3 (8.4)	1.7
Pyroxasulfone 150 Sulfentrazone 105	29.6 (16.8)	35.3	57.4 (21.3)	22.5	29.5 (9.2)	34.4	0.8 (8.4)	4.3
Pyroxasulfone 150 Sulfentrazone 140	33.7 (16.8)	40.5	61.0 (21.3)	36.8	22.0 (9.2)	29.9	2.0 (8.4)	3.1
Pyroxasulfone 150 Sulfentrazone 280	56.6 (16.8)	21.1	36.2 (21.3)	24.6	37.3 (9.2)	29.4	0.5 (8.4)	2.2
Pyroxasulfone 200 Sulfentrazone 105	14.0 (16.8)	20.6	29.6 (21.3)	4.0	38.5 (9.2)	18.8	1.0 (8.4)	0.2
Pyroxasulfone 200 Sulfentrazone 140	20.8 (16.8)	23.7	36.0 (18.7)	58.4	38.3 (9.2)	16.3	15.6 (8.4)	0.2
Pyroxasulfone 200 Sulfentrazone 280	43.2 (16.8)	12.3	38.5 (18.7)	39.1	27.5 (9.2)	16.1	2.3 (8.4)	0.1

*indicates significant difference between Colby mean and LS mean.

Table 4-7 LS means and Colby means for greenhouse interaction trial based on shoot dry weights.

Species	Trial	Treatment	LS mean (SE)	Colby mean
		--g ai ha^{-1}--	---% nontreated check---	
Canola	1	P7S7	67.6 (7.4)	45.3
		P40S7	22.8 (7.4)	42.7
	2	P7S7	48.4 (18.8)	32.3
		P40S7	9.2 (18.8)	8.1
Wild Oat	1	P7S7	66.1 (11.8)	47.6
		P40S7	4.8 (11.8)	14.3
	2	P7S7	115.8 (12.8)	64.6*
		P40S7	75.4 (12.8)	51.7
Barley	1	P7S7	90.7 (8.7)	73.7
		P40S7	55.3 (8.7)	60.6

*indicates significant difference between LS and Colby means

Table 4-8 Pyroxasulfone and sulfentrazone ED_{50}s (g ai ha^{-1}) to reduce the dry weight of canola in soils from Kernen and Edmonton. Regression parameters from non-linear regression to two or three parameter Exponential Decay models are given. A p-value <0.05 indicates a significant lack of fit to the model. Significant differences between ED_{50}s are given by ABC and XYZ for pyroxasulfone and sulfentrazone respectively.

Soil	Herbicide	Trial	Model	C(SE)	D(SE)	E(SE)	p-value for lack of fit	ED_{50} (SE)	Significant Differences
								-g ai ha^{-1}-	
Kernen	Pyroxasulfone	1	EXD3	0.066 (0.007)	0.125 (0.012)	6.79 (640.9)	0.5187	5 (444)	A
Kernen		2	EXD2	--	0.114 (0.010)	274.2 (67.1)	0.9670	190 (47)	A
Edmonton		1	EXD2	--	0.079 (0.009)	413.3 (106.5)	0.7474	286 (74)	A
Edmonton		2	EXD2	--	0.102 (0.011)	479.1 (129.8)	0.9232	332 (90)	A
Kernen	Sulfentrazone	1	EXD2	--	0.130 (0.020)	134.3 (42.6)	0.5028	93 (30)	X
Kernen		2	EXD2	--	0.107 (0.013)	147.0 (38.9)	0.6642	102 (27)	X
Edmonton		1	EXD2	--	0.082 (0.020)	325.9 (184.1)	0.3656	226 (128)	X
Edmonton		2	EXD2	--	0.091 (0.007)	1308.0 (655.7)	0.6543	907 (454)	X

Table 4-9 Pyroxasulfone and sulfentrazone ED_{50}s (g ai ha^{-1}) to reduce the dry weight of wild oat in soils from Kernen and Edmonton. Regression parameters from non-linear regression to two or three parameter Exponential Decay models are given. A p-value <0.05 indicates a significant lack of fit to the model. Significant differences between ED_{50}s are given by ABC and XYZ for pyroxasulfone and sulfentrazone respectively.

Soil	Herbicide	Trial #	Model	C (SE)	D (SE)	E (SE)	p-value (lack of fit)	ED_{50}(SE)	Significant Differences
								g ai ha^{-1}	
Kernen	Pyroxasulfone	1	EXD2	--	0.115 (0.008)	31.3 (12.4)	0.0255	22 (9)	A
Kernen		2	EXD3	0.023 (0.007)	0.072 (0.008)	37.3 (30.0)	0.3929	26 (21)	AB
Edmonton		1	EXD2	--	0.072 (0.004)	131.1 (19.1)	0.6101	91 (13)	B
Edmonton		2	EXD2	--	0.053 (0.005)	210.9 (44.1)	0.2122	146 (31)	B
Kernen	Sulfentrazone	1	EXD2	--	0.092 (0.007)	479.1 (141.7)	0.6905	332 (98)	X
Kernen		2	EXD2	--	0.074 (0.009)	247.3 (74.2)	0.3247	171 (51)	X
Edmonton		1	EXD2	--	0.068 (0.009)	414.8 (156.7)	0.6439	288 (109)	X

Table 4-10 Pyroxasulfone and sulfentrazone ED_{50}s (g ai ha^{-1}) to reduce the dry weight of green foxtail in soils from Kernen and Edmonton. Regression parameters from non-linear regression to two or three parameter Exponential Decay models are given. A p-value <0.05 indicates a significant lack of fit to the model. Significant differences between ED_{50}s are given by ABC and XYZ for pyroxasulfone and sulfentrazone respectively.

Soil	Herbicide	Trial	Model	C (SE)	D (SE)	E (SE)	p-value (lack of fit)	ED_{50} (SE)	Significant Differences
								-g ai ha^{-1}-	
Kernen	Pyroxasulfone	1+2	EXD2	--	0.061 (0.003)	0.022 (10)	0.9877	0.02 (7)	A
Edmonton		1+2	EXD2	--	0.067 (0.003)	0.38 (10)	0.8455	0.26 (7)	A
Kernen	Sulfentrazone	1+2	EXD3	0.003 (0.002)	0.060 (0.004)	1.68 (10)	0.4060	1 (7)	X
Edmonton		1+2	EXD2	--	0.048 (0.007)	247.7 (83.6)	0.5643	172 (58)	X

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Chapter Five: General Discussion and Conclusions

5.1. Summary of Results

Research in this thesis supports the use of pyroxasulfone and sulfentrazone to be used in combination in western Canadian field pea. Effective rates, efficient application timings and effect of organic matter on pyroxasulfone efficacy were characterized. In addition, the interaction between pyroxasulfone and sulfentrazone when they are applied in combination was investigated. Under controlled conditions, interactions between pyroxasulfone and sulfentrazone were confirmed, and effects of soil moisture and organic matter on herbicide efficacy was studied. This research will support the registration of a pyroxasulfone and sulfentrazone combination in peas in western Canada, as well as aid in delineating registration rates and requirements for herbicide efficacy.

5.2. Results Summarized by Research Objective

5.2.1. Determine effective rates of pyroxasulfone for control of cleavers and wild oat in peas in Western Canada.

The potential for pyroxasulfone to control cleavers and wild oats, in addition to pea tolerance to pyroxasulfone was described in Chapter Three, a version of which has been accepted for publication in Weed Technology and is currently available online (Tidemann et al 2014). A split-plot trial was established in 5 locations using fall and spring applications of the following pyroxasulfone rates: 0, 50, 100, 150, 200, 300 and 400 g ai ha^{-1}. Application timing was the main plot and pyroxasulfone rate was the split-plot. Control of cleavers and wild oat was evaluated through measurement of plant fresh weights. An ANOVA and subsequent non-linear regression using a log-logistic 3 parameter model were used to determine ED_{50}s for species separately, and within wild oat for each application timing. The ED_{50}s were then compared between sites, and between time of application for wild oat. Results indicated that both species could be controlled by pyroxasulfone; however, effective rates varied between locations. Pea crop was injured at two sites: Scott and Kernen. Both of these sites were subject to nearly 200% of their normal moisture levels, compromising yield and biomass. Results indicated that under normal environmental conditions field pea will be tolerant to registered rates of pyroxasulfone while there is potential for crop injury under higher than normal precipitation levels.

5.2.2. Determine if there is a weed control advantage to fall or spring applications of pyroxasulfone

Effectiveness of fall and spring applications were investigated and compared in the trial described above and discussed in Chapter Three. Both fall and spring applications are effective for control of cleavers and wild oat. In addition, there were no consistent significant differences between the application timings. The two potential application windows for pyroxasulfone adds flexibility to the potential uses of pyroxasulfone and benefits of use for producers.

5.2.3. Determine whether soil organic matter affects pyroxasulfone and sulfentrazone efficacy.

The split-plot trial described above also allowed investigation into the effect of soil organic matter on pyroxasulfone efficacy in Chapter Three. For both species, there is an obvious trend of higher organic matter sites requiring higher pyroxasulfone rates for equal efficacy. This led to the investigation of organic matter influences under controlled environment (greenhouse) conditions discussed in Chapter Four, where three soils with four rates of each herbicide (pyroxasulfone and sulfentrazone) were tested for the level of efficacy on barley, canola, wild oat and green foxtail. Although the Duchess soil and barley were removed from analysis, the results of the trial are consistent with those from the field; higher organic matter soils require higher herbicide rates for equal levels of efficacy. This conclusion was true for both herbicides.

5.**2.4. Determine efficacy and interaction of pyroxasulfone and sulfentrazone when co-applied.**

Chapter Four describes a randomized complete block factorial trial conducted over 6 site-years to investigate the potential for herbicide interactions on wild oat and cleavers control when applied in combination. In addition, a smaller trial was conducted under greenhouse conditions to further investigate the herbicide interactions, while minimizing confounding environmental effects. Biomass means as a percent of the nontreated check were compared with expected means calculated using the Colby method (Colby 1967). Field and greenhouse studies indicated no significant synergism or antagonism, suggesting that interactions between pyroxasulfone and sulfentrazone are additive. However, while there is no synergy, co-application of pyroxasulfone and sulfentrazone broadens the weed spectrum controlled with either product alone and aids in herbicide resistance management.

5.2.5. Investigate the influence of soil moisture on pyroxasulfone and sulfentrazone efficacy.

There was an attempt to investigate the effect of soil moisture on pyroxasulfone and sulfentrazone efficacy outlined in Chapter Four. Results of this trial were not useful, and a conclusion for this research objective was not reached.

5.3. Future Research

- We have assumed a lack of cross-resistance of HR weeds in Canada to pyroxasulfone. However, cross-resistance through low dose selection has been found in annual ryegrass (*Lolium rigidum)* in Australia (Busi and Powles 2013). Triallate-resistant wild oats occupy 8% of sampled fields in the Canadian Prairies (Beckie et al. 2013). Determining whether cross-resistance exists in the triallate-resistant wild oats to pyroxasulfone is key to pyroxasulfone's potential to be used as a wild oat herbicide in Western Canada.
- As the soil moisture experiment did not yield useful results, this experiment should be repeated using a more neutral pH, low organic matter soil to quantify the level of influence soil moisture has on pyroxasulfone efficacy.
- A more in-depth study of organic matter effects on pyroxasulfone and sulfentrazone would allow the level of influence of soil organic matter to be more precisely quantified.
- Other soil characteristics such as pH should be investigated for their effect on pyroxasulfone and sulfentrazone efficacy.

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Appendix A

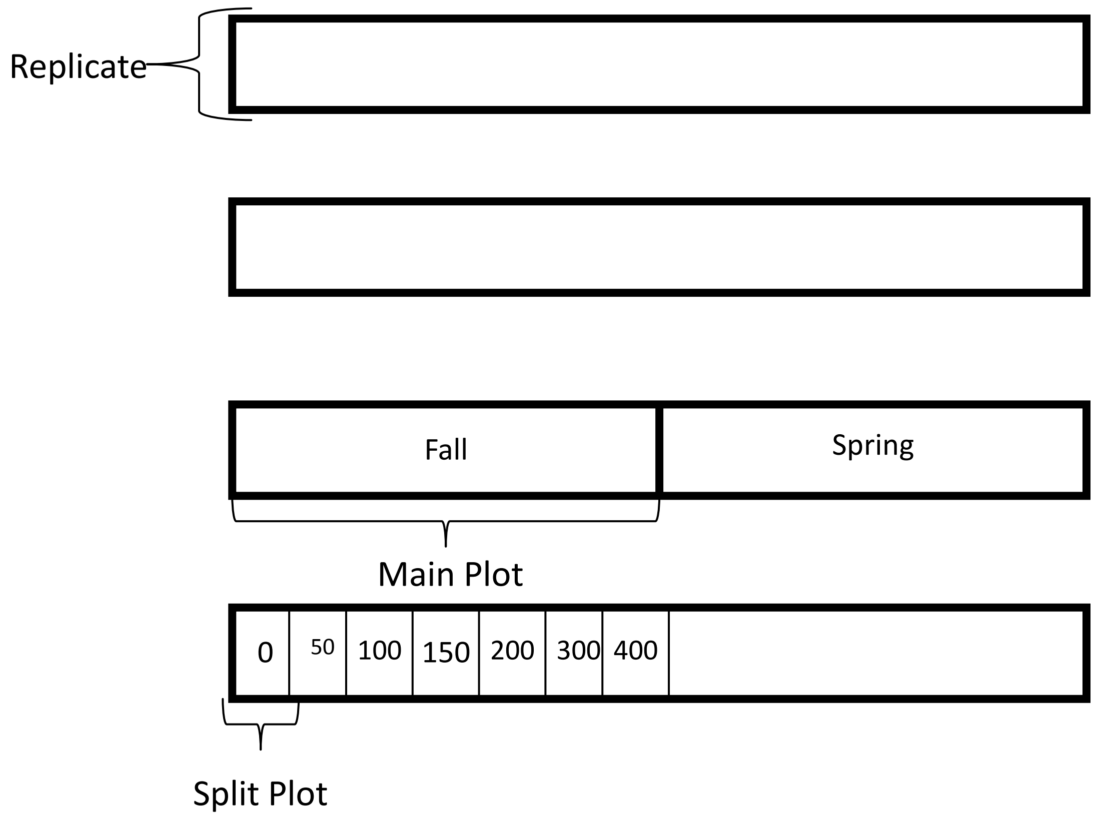

Diagram of field trial designed determine pyroxasulfone rates required to control false cleavers and wild oat in field pea comparing fall and spring applications in western Canada, described in Chapter 3, indicating replicates, main plots and a split-plot.